

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/327282246>

# Microcirculation and Hyperbaric Oxygen Treatment

Chapter · August 2018

DOI: 10.5772/intechopen.75609

---

CITATIONS

0

READS

48

2 authors, including:



**Ömer Faruk Boran**

Kahramanmaraş Sutcu Imam University

26 PUBLICATIONS 6 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Effect of TAP Block for Postoperative Pain Control After Varicocele Operations [View project](#)



Comparison of Success Rates of Femoral Artery Catheterization [View project](#)

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

3,500

Open access books available

108,000

International authors and editors

1.7 M

Downloads

Our authors are among the

151

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

# Microcirculation and Hyperbaric Oxygen Treatment

---

Fethi Gul, Omer Faruk Boran and Reyhan Arslantas

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.75609>

---

## Abstract

The microcirculation is anatomical and consists of arterioles, capillaries, and venules that perform metabolic requirements and oxygen distribution to the tissues. During physiological or pathological stress, it balances between the oxygen delivery and the demand. This delicate balance can play an important role in the progression of critical illnesses and has a role in the development of organ dysfunction. Reduced microvascular perfusion is seen in many diseases, and hyperbaric oxygen treatment (HBOT) has potentially beneficial effects on the microcirculatory environment. It has been shown that HBOT improves microcirculation independent from systemic hemodynamic parameters, which is a key therapeutic target in the critically ill patient. HBOT is emerging as an adjunct to traditional surgery and antibiotic therapy for the special kinds of problematic wounds or purpura fulminans, which are caused by meningococcal sepsis. HBOT also can increase oxygen supply to the ischemic tissue to reduce the extent of irreversible tissue damage in ischemic stroke, femoral head necrosis, diabetic foot ulcer, and carbon monoxide intoxication. In this chapter, we aim to describe microcirculation with its monitoring systems and to show the effectiveness of HBOT in different clinical settings, which are related to microcirculatory dysfunction.

**Keywords:** hyperbaric oxygen, microcirculatory dysfunction, perfusion, critical illness

---

## 1. Microcirculation physiology

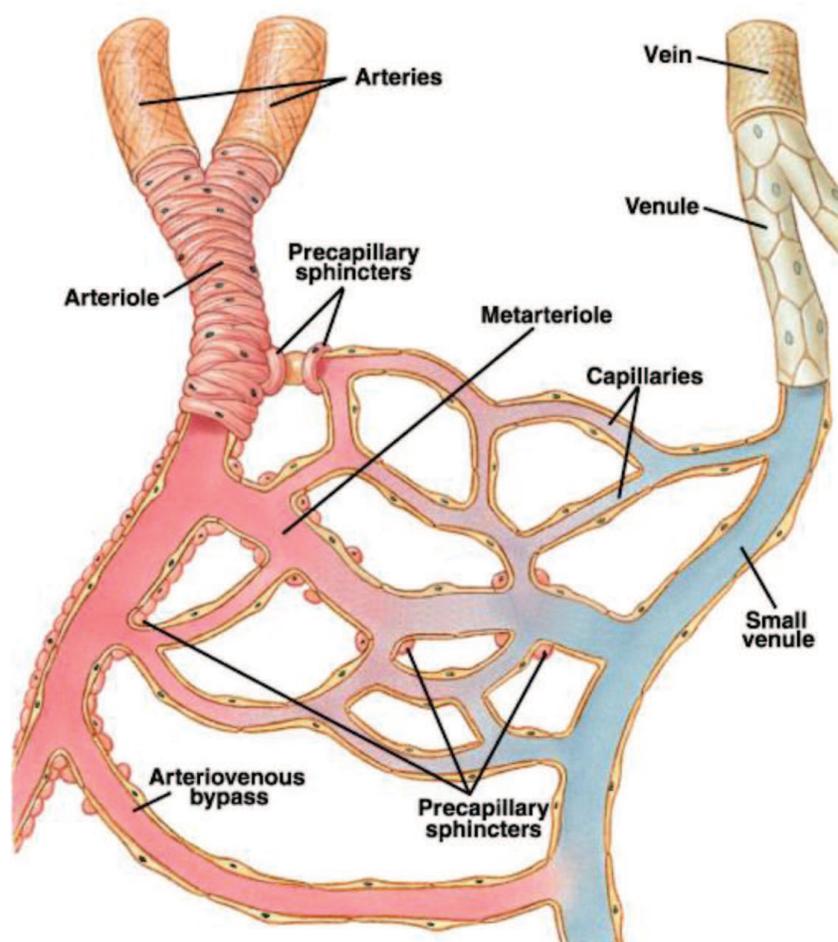
The area of the circulation system where the metabolic requirements of tissues are met is called microcirculation. In other words, this is the point at which the arterial system and venous system join.

As a result of 6–8 branches occurring in the arterial structure entering a tissue, the width of the interior lumen reduces to 10–15  $\mu\text{m}$ , and this structure is called an arteriole. The wall

structures of arterioles continue with metarterioles within the continuous surrounding smooth muscle, and capillary veins form from metarterioles. At the point where the capillaries emerge from the metarterioles, smooth muscle tissue forms a sphincter-like structure at the capillary entrance. Capillary structures continue to form venules. Venules have the larger diameter compared to arterioles though they have less muscle tissue. However, less muscle tissue causes low pressure within the venule and ensures severe contractile force (**Figure 1**).

The total wall thickness of capillary structures is  $0.5\ \mu\text{m}$ . Shaped elements in the blood can only pass through by friction along the wall of the  $9\text{-}\mu\text{m}$  lumen. There are openings of  $6\text{--}7\ \text{nm}$  width between the endothelial cells of the capillaries called fenestrations. These structures form  $1/1000$  of the total endothelial surface area but are areas where the transfer of water and water-soluble material occurs. These fenestrations may differ from organ to organ. These gaps are very narrow in the brain, while in the kidneys broad intervals ensure the necessary width for glomerular filtration of water and solutes [1].

Capillaries are not continuously open structures. The number of open capillaries varies depending on the requirements of the tissue, and here the most important stimulus is the oxygen



**Figure 1.** The area of the circulation system.

requirement of the tissue. The precapillary sphincter found at the junction of the metarterioles with capillaries plays a role in the opening of closed capillaries and closure of open capillaries, and this local control is called vasomotion [2].

There may be nearly 12 l of fluid found in the interstitial area. This cavity found between cells is kept open by collagen fibres. Proteoglycans appear to be like a brush for the interstitium. Due to proteoglycans found in the interstitial fluid, it has a gel consistency. Water and electrolytes may rapidly diffuse within the gel. Free fluid is only found in collagen fibres and at cell boundaries in the interstitial area.

The size of exchange areas for material transfer between compartments in the microcirculatory environment is directly related to the transfer amounts. Additionally, other effective factors are fixed transfer, the presence of membrane carriers, channel-dependent transport, barrier permeability, and soluble material transfer [3].

According to Fick's first law, the solute transfer from a membrane only occurs in situations with concentration differences until concentration balance is achieved [4].

$$J_s = P_d S[\Delta C]$$

where  $J_s$  is the solute flow rate,  $P_d$  is the diffusion permeability constant, and  $\Delta C$  is the concentration difference

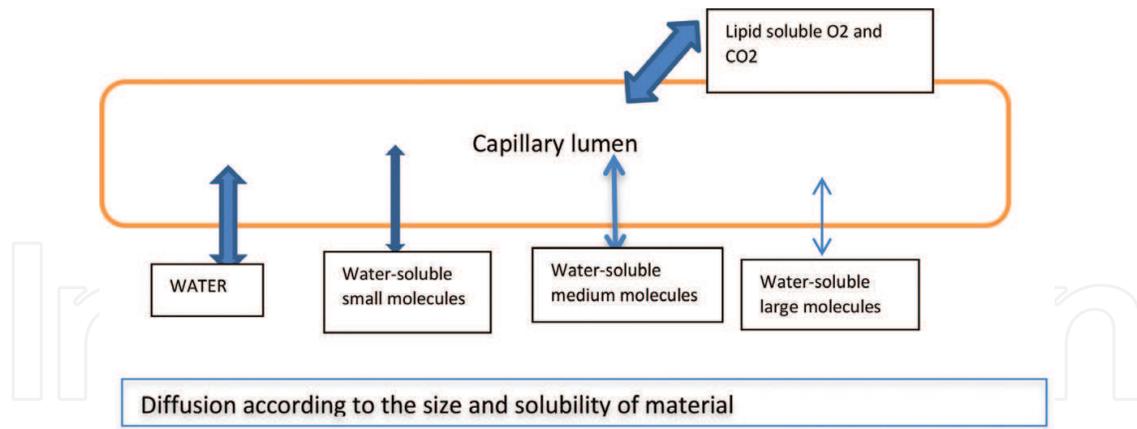
$$P_d = \frac{D_f}{\Delta x}$$

where  $D_f$  is the free diffusion constant, and  $\Delta x$  is the barrier thickness.

According to Fick's second law, the amount of diffusion is linked to the thickness of the membrane, the surface area over which diffusion occurs, molecular mass and size.

$$\frac{dn}{dt} = -DA \frac{\Delta C}{\Delta X}$$

About 97% of oxygen is bound to haemoglobin in blood and passes into tissues according to the Fick law. Oxygen presentation to tissues is dependent on the cardiac output and arterial oxygen content. Formula of arterial oxygen content is the sum of the multiplication of oxygen saturation, blood haemoglobin level and Hüfner number (amount of oxygen carried if haemoglobin is fully filled) with the multiplication of partial oxygen pressure by 0.003. There is 100 mmHg oxygen pressure at the arterial tip at the 1 atmospheric pressure while at the pressure of 3 atmospheres with 100% oxygen it increases according to the Henry law and reaches to the 2000 mmHg. Tissue oxygen pressure reaches from 55 to 500 mmHg at this point. At 1 atmosphere pressure, there is 3 ml per litre of free oxygen, and this amount reaches 60 ml where the tissues fulfil their needs without using haemoglobin-bound oxygen [5].



**Figure 2.** Diffusion according to the size and solubility of material.

The fat-soluble material does not need to pass through pores but reaches tissue directly by passing through endothelial cells. Water and water-soluble material use the pores between endothelial cells to diffuse and pass into the cell. For diffusion rate, the size of the molecule to be diffused is important (**Figure 2**).

With the Donnan effect, negatively charged proteins responsible for oncotic plasma pressure may attach to glycocalyx structures due to charge; however, they cannot bind, and plasma oncotic pressure remains high [6].

Mean values	Hydrostatic pressure	mm Hg
	Capillary	17
	Interstitial	-5.3
	Subtotal (positive = outwards)	22.3
	Osmotic pressure	
	Capillary	-28
	Interstitial	-6
	Subtotal (positive = outwards)	-22
	Total (positive = outwards)	0.3
Arterial end	Hydrostatic pressure	
	Capillary	30
	Interstitial	-5.3
	Subtotal (positive = outwards)	35.3
	Osmotic pressure	
	Capillary	-28
	Interstitial	-6
	Subtotal (positive = outwards)	-22
	Total (positive = outwards)	13.3

Venous end	Hydrostatic pressure	
	Capillary	10
	Interstitial	-5.3
	Subtotal (positive = outwards)	15.3
	Osmotic pressure	
	Capillary	-28
	Interstitial	-6
	Subtotal (positive = outwards)	-22
	Total (positive = outwards)	-6.7

**Table 1.** Pressure distribution in microcirculation.

Forces controlling fluid transfer in capillaries were defined by Starling. While capillary pressure ( $P_c$ ) and interstitial fluid oncotic pressure ( $\pi_{if}$ ) ensure water and solutes leave the vein, plasma oncotic pressure ( $P\pi$ ) and interstitial hydrostatic pressure ( $P_{if}$ ) attempt to prevent water and solute transfer in the interstitial area. In conclusion, net filtration pressure (NFP) develops as  $NFP = P_c - P_{if} - P\pi + \pi_{if}$ . The pressure distribution is shown in **Table 1**.

According to the Starling equation, there is 0.3 mmHg net outward pressure for 2 ml/min outward flow. The difference is removed from the interstitial area by the lymphatic system. Filtration occurs in the arteriole sections of the capillaries. Fluid reabsorption is clear at the venule tips [3].

Lymphatic capillaries include endothelial flaps, and these flaps prevent reverse leakage of fluid. Fluid flow is ensured by skeletal muscle contractions with flow rates in the interval 4–150 ml/hr. Interstitial fluid pressure and lymph fluid rate determine lymphatic flow.

## 2. Microcirculation regulation

In eukaryotes, transfer of oxygen and nutrition into the cell and removal of carbon dioxide and waste material occurs at the cell surface. In multicellular organisms, this event occurs in the interstitial area [3].

In humans, blood flow follows the path: left ventricle → large- and medium-diameter arteries → small arteries known as precapillary resistance arterioles and terminal arterioles → capillary beds not containing contractile elements and where oxygen and solute exchange occurs → postcapillary resistance venules and collecting veins → capacitance veins and large veins → right atrium.

Tissue oxygenation ( $DO_2$ ) is calculated as being equal to arterial oxygen saturation ( $SaO_2$ ) × blood haemoglobin level  $cHb$  × 1.39 × cardiac output (CO).

The result is that it takes 30–60 s for oxygen entering blood in the lungs to reach tissues. However, for oxygen to reach peripheral tissues, it is necessary for there to be sufficient

airway opening, normal respiratory pattern, normal alveolar gas exchange, sufficient blood haemoglobin level, and sturdy and sufficient vein structure in addition to microcirculatory blood flow supplying metabolic requirements of the tissues [7].

For metabolism and sufficient adenosine triphosphate (ATP) production to occur, tissues need to have sufficient blood perfusion. In situations without sufficient oxygen supply, anaerobic glycolysis increases in tissues and lactic acid release occurs. Increased lactic acid causes metabolic acidosis. Acidosis reduces cardiac contractility and increases peripheral vascular resistance and, as a result, tissue hypoxia deepens. At the same time, increasing blood potassium levels linked to developing acidosis reduce cardiac contractility and cause a reduction in the presentation of oxygen to tissues.

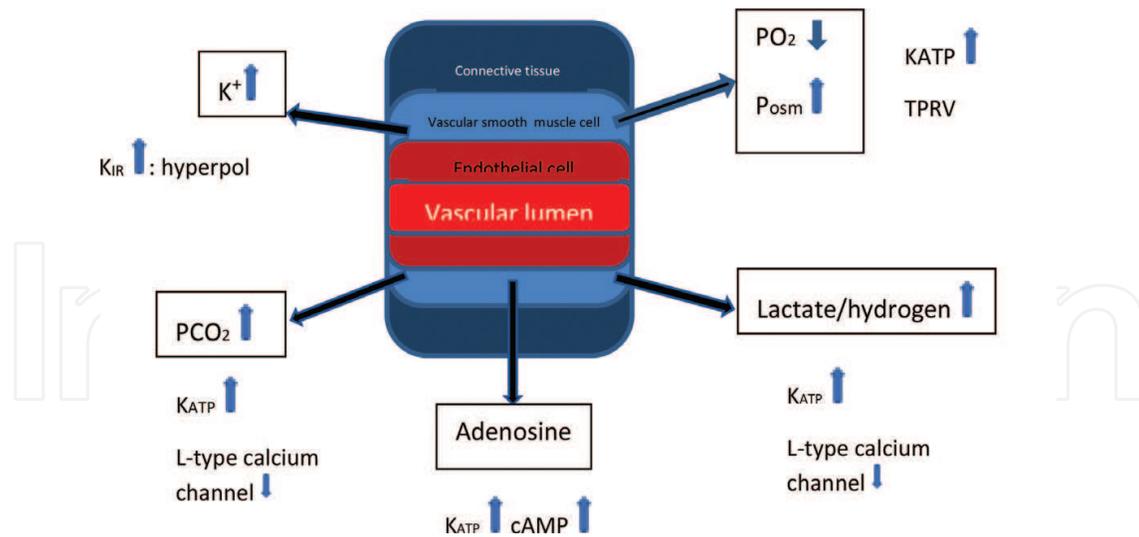
Apart from true arteriovenous shunt areas of the body, blood perfusion passes through many capillary veins, and capillary blood flow is controlled by arteriole resistance. This situation is more pronounced especially in the heart, lungs, and skeletal muscles. In situations when reduced blood flow reduces in tissues, regional vasodilatation may ensure sufficient blood perfusion of tissues and sufficient oxygenation [8].

Moving away from the arteriolar areas, smooth muscle structure begins to appear in the adventitia layer of veins. This smooth muscle structure is contracted by adrenergic stimuli and is considered to increase capillary perfusion pressure [3].

Vasoconstriction in hyperoxic situations is an attempt to keep tissue oxygenization stable and, in addition, to reduce the risk of tissue hyperoxygenation [9]. The 2.5 atmosphere pressure applied during hyperbaric oxygen treatment increases 4–5 times partial oxygen pressure in subdermal healthy and infected tissues by [10]. In a study, hyperbaric oxygen administration had reduced local blood circulation about 76.5% in pancreatitis patients, while this rate was identified as 37% with normobaric hyperoxygenation [11]. Hyperoxic situations increase oxygen amount, which dissolves in plasma. The free oxygen amount is an important point in the development of oxygen diffusion. There is a moderate level of oxygen saturation values measured in the postcapillary section of microcirculation. This effect may be explained by the vasoconstriction which occurs with the hyperbaric oxygen administration.

During inflammation increased leukocyte adhesion occurring in venules and resistance increase to venous blood flow develops and venous pressure increases. At the same time, an increase occurs in the length of venules. An attempt is made to increase oxygen presentation to tissues [12, 13]. Also during inflammation, mediators like vascular endothelial growth factor (VEGF) are released to the environment and have a venodilatatory effect.

Arterioles and terminal arterioles cause changes in local blood perfusion resistance and may directly change the blood flow amount to tissues. In situations with vasodilatation of arterioles and terminal arterioles, there is an opening of closed capillaries and lengthening of capillaries. This situation causes an increase in the surface necessary for material transfer. However, in situations with venular contraction, there is a hydrostatic pressure increase in capillary beds and diffusion pressure increases. Capacitance and large veins change cardiac output above the heart's full volume and affect microcirculation (**Figure 3**).



**Figure 3.** Local vasodilatation with tissue metabolites.  $K_{ATP}$ , ATP-dependent potassium ion channel;  $K_{IR}$ , inward-rectifying potassium ion channel that gives rise to hyperpolarization; TRPV, transitory receptor-mediated potential; cAMP, cyclic adenosine monophosphate.

As described by Bayliss, in situations with increased blood pressure, an attempt is made to keep blood flow to vital organs like the brain, heart, kidneys, liver, and carotid bodies fixed via developing vasoconstriction [14]. This adaptation is processed in reverse in hypotensive situations. In this development, the tension-sensitive sodium and calcium channels play a role.

### 2.1. Factors affecting arteriole resistance

- Autonomous nervous system (cholinergic, adrenergic, non-cholinergic, non-adrenergic system)
- Vasoactive humoral and tissue factors (angiotensin II, bradykinin, vasopressin, catecholamines, natriuretic peptides, etc.)
- Local metabolic changes (partial oxygen pressure [PO<sub>2</sub>], partial carbon dioxide pressure [PCO<sub>2</sub>], pH, osmotic pressure [Posm], potassium [K<sup>+</sup>] concentration, metabolic material like adenosine)

In endothelial tissue, the friction caused by blood triggers nitric oxide (NO) release. In situations with vasodilatation in NO terminal arterioles, increasing NO release develops. Friction-linked vasodilatation is responsible for mechanoconduction of the glycocalyx structure covering the endothelial surface and plays a role in blood flow regulation in inflammation, ischemia and other pathological situations [15].

Vasodilators like NO and prostaglandin I<sub>2</sub> are found in the whole vascular system, especially the terminal arterioles. Other vasodilator effect agonists include serotonin, histamine, adenosine triphosphate (ATP), adenosine diphosphate (ADP), bradykinin, acetylcholine, thrombin, and endothelin.

NO formed by the nitric oxide synthase enzyme found within endothelial cells is effective through paracrine routes. NO within the cell commonly shows the effect by ensuring calcium entry into the cell via guanosine monophosphate and protein kinase activation. In addition to vasodilatation, NO has anti-thrombogenic effects by reducing tissue factor expression, platelet aggregation, and adhesion molecule expression like VCAM. At the same time, proliferation in venous smooth muscles is reduced and it limits neointimal hyperplasia development in vein walls [16].

As a result of material like adrenalin, thrombin, and angiotensin II binding to receptors found in abluminal vascular smooth muscle cells, calcium entry into the cell is activated and due to the inositol triphosphate (IP<sub>3</sub>) pathway, endothelin released into the subendothelial interstitial areas causes the better-known vasoconstriction effect [17]. In situations where vasodilator materials pass through endothelial cells with increased permeability outside of the vein, the vasodilator effect reverses and may cause vasoconstriction.

Specific receptor expression available in endothelial and smooth muscle tissue found in the vein structures of organs affect agonist concentration and provide vasoactive material to luminal and abluminal vein structures affecting the concentration or dilatation response [3].

In 1922, August Krogh et al. first defined vasodilatation occurring due to the chemical stimulus. In 1971, Siggins and Weitsen showed that vasodilatation was caused by cholinergic stimulation. Khayutin et al. in 1991 showed that vasodilatation occurred due to myelin nerve stimulus. In 1959, Hilton showed that communication present between the smooth muscle cells found in the vascular walls was effective on vasodilatation.

Gap junctions are structures formed by two half channels completing each other with nearly 9 nm size, allowing transfer between cells of ions and molecules with weight lower than 1 kDa isolated from the extracellular environment and linking two neighbouring cells. Just as gap junctions form between the same type of cells (endothelium-endothelium, smooth muscle-smooth muscle, etc.), they may also form between different cells (myoendothelial gap junctions). The channels allow transfer between cells of electrical stimuli and calcium [18].

Each half of gap junctions found in vein structures contain six connexins (Cx) proteins (Cx39, Cx40, Cx43, Cx45) [19]. Each connexin protein has effects with different clinical results [20].

In development-linked vasodilatation, each agonist has a different effect mechanism, with the best-described effect mechanism belonging to the agonist acetylcholine. When acetylcholine binds to a G protein-dependent muscarinic receptor, inositol triphosphate phosphorylation develops causing calcium release from endothelial endoplasmic reticulum [21, 22]. Simultaneously, calcium-dependent potassium channels open leading to hyperpolarization development in the cell [23]. After this flow, vasodilatation develops causing closure of voltage-dependent calcium channels within endothelial and vein smooth muscle cells [24]. At the same time, ion flows are communicated between cells via gap junctions with vasodilatation spreading distal and proximal to the point of acetylcholine binding [2].

Pericytes found in venules have regulatory effects on proliferation, differentiation and contractility of endothelial cells. During new vein creation, in situations where the endothelial layer is developing with tube shape contacts pericytes, endothelial growth and proliferation are suppressed, and maturation of the endothelial basal membrane structure is ensured [25, 26].

In situations with low oxygen levels or pH, ATP molecules released from erythrocytes found in microcirculation cause vasodilatation while haemoglobin molecules occurring with erythrocyte fragmentation bind to NO causing the development of NO-dependent vasodilatation.

During hyperoxia the reactive oxygen radicals prevent nitric oxide-mediated vasodilatation; on the other hand, vasoconstrictor effect results in low tissue blood flow [27]. In addition, synthesis of the vasodilator prostaglandin is reduced while synthesis of the vasoconstrictor prostaglandin (and endothelin-1) is increased [28].

### **3. Methods to assess microcirculation flow**

The microcirculation performs the necessary oxygen distribution to meet the basic metabolic requirements of the tissues. Anatomically, it consists of arterioles, capillaries and venules. In situations where physiological or pathological stress develops, the balance between oxygen delivery and demand becomes very important. This delicate balance can play an important role in the progression of critical illnesses. In daily clinical practice, monitoring the microcirculation is essential for detection of potential organ dysfunctions. The ideal technique would allow quantification of vascular recruitment and the magnitude, heterogeneity, responsiveness, and efficiency of oxygen transfer to the tissues. There are a variety of methods and parameters available to evaluate the microcirculatory state of a patient.

### **4. Assessment of microcirculation**

The assessment of these factors can be analysed in five parameters: (i) total microvascular density (TVD), (ii) perfused microvascular density (PVD), (iii) proportion of perfused microvessels (PPV), (iv) microvascular flow index (MFI) and (v) heterogeneity of microvascular flow index. The first two reflect density, (iii) and (iv) reflect the flow, and (v) represents the heterogeneity of flow. Microcirculatory blood flow can be detected at the bedside by videomicroscopic techniques, and by laser Doppler flowmetry, near-infrared spectroscopy (NIRS), tissue reflectance spectrophotometry, or by tonometry. These techniques provide simple and quantitative data. Biochemical parameters that show microcirculation are reliable and include lactate, tissue CO<sub>2</sub> content and venous oxygen saturation.

### **5. Direct methods**

#### **5.1. Measuring microcirculatory perfusion**

Video microscopic techniques involve highly sensitive video microscopes that allow direct measurement of capillary density, perfusion and flow dynamics. Video microscopic techniques have shown a correlation between increased mortality in ICU patients [29]. These techniques, which included orthogonal polarisation spectral (OPS), sidestream dark-field (SDF)

imaging and incident dark-field (IDF) imaging (CytoCam) provide in vivo visualisation of the microcirculation. OPS and SDF imaging have become clinically useless due to the large size, motion and pressure artefacts, operator-dependent output, and the need for offline analysis, which takes time to produce data.

## 6. Indirect methods

### 6.1. Measuring elements of tissue oxygenation

There are quite some methodologies available for estimating or measuring tissue oxygenation at the different levels.

Central venous oxygen saturation ( $ScvO_2$ ) and mixed venous oxygen saturation ( $SvO_2$ ).

They provide knowledge of the patient's oxygen delivery, oxygen consumption, and cardiac output.  $ScvO_2 > 70\%$  or  $SvO_2 > 65\%$  is recommended for critical patients [30].  $SvO_2$  is an adaptive variable depending on four elementary-regulated components: the real  $O_2$  consumption,  $SaO_2$ , haemoglobin and cardiac output. Consequently,  $SvO_2$  (or its surrogate  $ScvO_2$ ) is widely fluctuating. Thus, when normal, those parameters cannot rule out any impairment in tissue oxygenation related to an impaired microcirculation [31].

### 6.2. Near-infrared spectroscopy (NIRS)

NIRS has been developed as a non-invasive diagnostic tool for measurement of regional haemoglobin oxygen saturation in a particular organ. NIRS is mainly used in the evaluation of cerebral oxygenation in cardiac, non-cardiac surgery and traumatic intensive care patients. The NIRS signal is restricted to vessels that have a diameter of less than 1 mm, and this technique is not suitable for states of heterogeneous blood flow [32]. Tissue  $O_2$  saturation ( $StO_2$ ) mostly describes the saturation of all vessels, while total tissue haemoglobin (HbT) and the tissue haemoglobin index (THI) indicate the amount of blood present in the tested region. Brain venous blood is primarily responsible for a decrease in brain saturation and predominantly results from a local increase in oxygen extraction. During haemorrhage the arterial component preserved while venous part decreases markedly. On the other hand, in trauma patients,  $StO_2$  is altered only in the severe cases and in other forms show low sensitivity [33]. NIRS-derived dynamic measurements (vaso-occlusive test, VOT) demonstrated profound alterations in microvascular reactivity. The NIRS technique is done for a brief episode of forearm ischemia induced by transient inflation of a cuff determines changes in  $StO_2$ . Some indices can be measured during this test, but the most important is the ascending slope reflecting microvascular reactivity. The severity of these alterations in microvascular reactivity is associated with organ dysfunction and mortality [34].

### 6.3. Tissue $CO_2$ (gastric tonometry, veno-arterial $CO_2$ gradient, transcutaneous $CO_2$ )

Tissue  $PCO_2$  ( $PtCO_2$ ) has been measured in the stomach, sublingual area, and earlobe. Tissue  $PCO_2$  has three major elements:  $VCO_2$ ,  $PaCO_2$ , and tissue blood flow. Normally, an increase

in tissue metabolism ( $V_{CO_2}$ ) increases tissue perfusion and decreases  $PCO_2$ . When  $PaCO_2$  is constant, and  $PtCO_2$  is increased, there is an inadequate relationship between metabolism and tissue perfusion. Normal tissue-arterial  $CO_2$  gradient ( $PCO_2$  gap)  $<7$  mmHg. Elevated  $PCO_2$  gaps may signify either flow stagnation or tissue hypoxia [35]. Importantly, there is an inverse relationship between microvascular perfusion and the  $PCO_2$  gap.  $PtCO_2$  consequently represents a good assessment of tissue perfusion.

Microcirculatory alterations in critically ill patients may play a role in the development of organ dysfunction. Video microscopic techniques and tissue  $PCO_2$  measurements can be used to evaluate microvascular perfusion. But, microcirculation monitoring is not yet part of routine clinical practice.

#### **6.4. Lactate**

Lactate in the human body is a metabolic product of anaerobic glycolysis, produced from the reduction of pyruvate by the enzyme lactate dehydrogenase, and reflects inadequate oxygen delivery. Normally, a total amount of 1500 mmol of lactate is produced daily in adult, and blood lactate levels are sustained less than 2 mmol/L. However, in the state of hypoperfusion and hypoxia, pyruvate rapidly accumulate, and its metabolism is shifted almost entirely to lactate production. The tissue hypoxia is a cause of lactate elevation and characterised by supply-dependent oxygen consumption [36]. Single measurement of lactate can only serve as a risk-stratification biomarker. Lactate clearance with its association with clinical outcome should be used during treatment to make it more clinically useful [37].

#### **6.5. Microcirculation and hyperbaric oxygen therapy**

Hyperbaric oxygen therapy (HBOT) is a clinical treatment in which a patient breathes pure oxygen for a limited period of time at an increased pressure. This therapy has been suggested to improve oxygen supply to tissues and therefore improves microcirculation [38]. HBOT in patients with diabetic foot ulcer was associated with a greater reduction in the ulcer wound area than standard therapy and significantly improves the ulcers in a short term [39]. Also, HBOT which is applied in acute ischemic stroke, femoral head necrosis, and carbon monoxide intoxication aims to increase oxygen supply to the ischemic tissue and to reduce the extent of irreversible tissue damage [40–42].

### **7. Microcirculation and disease**

#### **7.1. Microcirculatory dysfunction**

Microcirculation plays a critical role in the physiological process such as oxygen supply to tissues and nutritional exchange and has a key role in modulation of inflammation and coagulation. These functions are mainly controlled by endothelial cells, which produce biologic signals to regulate local blood flow, cell adhesion, permeability, and coagulation activation. The most important function of the microcirculation is the regulation of flow within the different organs [43].

Microcirculation, in general, provides sufficient oxygen distribution to meet the oxygen demands of every cell within an organ. A regulatory mechanism with several signalling pathways allows microcirculatory flow to occur independently of changes in systemic blood pressure; this mechanism is called autoregulation. Perfusion of capillaries increases due to the vasodilation of terminal arterioles, which causes a decrease in the number of recruited capillaries. These capillaries are mainly under local control, which is possible because endothelial cells sense metabolic and physical signals, and respond to them by modulating arteriolar smooth muscle cell tone. In this process, nitric oxide (NO), which is produced by endothelial cells, is one of the components in the exertion of this process [44].

In the pathophysiology of sepsis and septic shock, the microcirculation is the basic region of the circulatory system, in which main components are arterioles, venules, shunts, and capillaries. The structures in the microcirculation system are capable of contraction, except capillaries. Capillaries are made of endothelial cells alone, without contractile structures. Microcirculatory dysfunction may arise as a result of several factors such as endothelial dysfunction, leukocyte-endothelium interactions, coagulation and inflammatory disorders, and functional shunting that may occur during sepsis which can affect capillaries to change hemodynamics [43, 44].

Microcirculatory changes during sepsis include various mechanisms such as redistribution of blood flow from the skin and the splanchnic area to brain or heart, or with endothelial activation and injury which results with the loss of the glycocalyx around endothelium. Increased microvascular permeability with capillary damage then causes oedema formation and hypovolemia. And also, secondary capillary plugging comes out with decreased RBC deformability and increased leukocyte adhesion to the endothelial surface. On the other hand, production of reactive oxygen species (ROS) disturbs microcirculatory structures, cellular interactions, and haemostasis. Overall, these alterations contribute to a reduction in decreased functional capillary density, the progress of various abnormalities in microcirculatory blood flow, and the loss of main vasoregulation in most vascular beds. These alterations terminate with a disrupted regulation of local oxygen delivery, which turns into the fast initiation of tissue hypoxia [44, 47].

Proinflammatory cytokines become dominant in the early stages of sepsis to eliminate the pathogen. However, proinflammatory responses in sepsis generate an intense response that impairs the microcirculation. In this stage, most of the cellular components of the microcirculation, such as endothelial cells, smooth muscle cells, platelets, leukocytes, and red blood cells are affected. Increased number of interrupted capillaries results with microcirculatory dysfunction which appears to have prognostic significance in sepsis, as the severity of initial microcirculatory imbalance in the early resuscitation phase of therapy [45, 46].

In sepsis, NO synthase (iNOS) which heterogeneously expressed in various vascular beds, causes pathological shunts. Therefore, iNOS-deficient areas become hypoperfused. Furthermore, increased production of reactive oxygen species interferes with NO formation by endothelial NOS and with formed NO, reducing its concentration. Also, during sepsis, red blood cells lose their ability to release vasodilators in the presence of hypoxia that impairs

physiological regulatory mechanism of microcirculation. In that case, red blood cells become less deformable and more easily aggregate with endothelial cells during sepsis [47, 49].

During sepsis, the percentage of activated neutrophils with increased adhesion molecules generate reactive oxygen species and inflammatory soluble factors that directly disrupt microcirculatory structures, such as the endothelial glycocalyx. Oxidative stress causes changes in endothelial glycocalyx structure. Permanent glycocalyx degradation leads to loss of integrity of adherens junctions and increased paracellular permeability with the following break of endothelial barrier function, which results in fluid leakage from the intravascular space and causes tissue oedema. Afterwards, accumulation of water in tissues leads to tissue hypoxia.

In sepsis, endothelial glycocalyx degradation is associated with sepsis-related clinical conditions such as acute lung injury and cardiovascular dysfunction as a consequence of microcirculation dysfunction. Also, glycocalyx degradation contributes to the enhanced expression of adhesion molecules with increased leukocyte trafficking and shift towards the procoagulant state. Prothrombotic effect further contributes the adhesion of red blood cells, leukocytes, and platelets to the vascular endothelium, which causes vascular microthrombosis. The activation of coagulation pathways results in capillary obstruction by fibrin clots secondary to disseminated intravascular coagulation [44, 47].

## 7.2. Treatment

The microcirculation is important for the normal delivery of oxygen to vital organs. The type and phase of critical illness define the degree of microcirculatory resuscitation required. It is important to recruit the microcirculation due to heterogeneous nature of the microvascular alterations. Fluid resuscitation and vasoactive agents are one of the main therapies for the hemodynamic resuscitation that aims to restore the circulating volume, and increasing the cardiac output and arterial blood pressure in shock patients and play a critical role with the goal of improving tissue perfusion. Individual responses to vasopressors may change, but the microvascular response to vasopressors appears to be dependent on the basal condition of the microcirculation [50]. Fluid resuscitation improves microcirculatory blood flow. The mechanisms by which fluids may improve the microcirculation are not well understood but may be related to restoring circulatory volume which increases perfusion pressure and causes local vasodilation, or modulates interactions between the endothelium and circulating cells to decrease microvascular blood viscosity [46, 48, 49].

Hypertonic saline (HTS) is a potential solution to cure the microcirculation of traumatic haemorrhagic shock. HTS improves intestinal perfusion associated with arteriolar vasodilation of distal premucosal arterioles, decreases endothelial oedema and prevents leukocyte adhesion to postcapillary venules. To restore capillary density, a new approach to fluid resuscitation is based on the fluid with high viscosity to increase plasma viscosity and NO production causing microcirculatory vasodilation with resulting capillary recruitment. At the same time, vasopressor agents may maintain tissue perfusion in the presence of life-threatening hypotension in haemorrhagic shock, even if fluid dilatation is in progress and hypovolemia has not yet been corrected [49].

The effects of red blood cell transfusions also seem to be quite variable. One of the other commonly used therapy is transfusions of red blood cells (RBC), which is used in critically ill patients to restore oxygen-carrying capacity. But, transfusion decisions are based on serum haemoglobin levels, and it is difficult to notice because normal microvascular haematocrit is much lower than systemic values. Although the beneficial effect of RBC transfusions over microcirculatory parameters in septic patients is still not clear, RBC transfusions are effective in improving tissue oxygen transport by promoting RBC delivery to the microcirculation [50].

Fluid resuscitation in combination with vasoactive and inotropic support is effective in improving the microcirculation. Recruitment of the microcirculation can be achieved with combination therapies. An anti-inflammatory agent or specific iNOS inhibitor can reduce pathological shunting and improve blood flow to recruit weak microcirculatory units. The effects can be seen in the early phase of sepsis, within 24 h of diagnosis, but if cardiac output is increased, no improvement will be made after 48 h [51].

The use of steroids in sepsis may provide a clinical benefit in modulating the systemic inflammatory response. It can preserve the endothelial glycocalyx and attenuate rolling of leucocytes to the endothelium, may improve endothelial function and thereby ameliorate the distributive defect [52].

Statins, which are cholesterol-lowering agents also have pleiotropic effects and have an anti-inflammatory and anti-oxidant activity during sepsis. They increase levels of eNOS, with the down-regulation of iNOS, so this regulation of NOS increases NO levels, restoring the autoregulatory functions [53].

Vasodilator substances may have a role to restore the microcirculation and decrease the effect of excessive vasoconstriction which causes decreased vascular density and stopped-flow capillaries. In a research, it was reported that nitroglycerin administration rapidly improved the microcirculation [54].

In haemorrhagic shock, reducing the blood flow to the microvascular units may prevent hypoxia. This downregulation of cellular metabolism is called conformance or hibernation. However, increase in lactate level during the acute phase of haemorrhagic shock indicates the limits of this adaptative metabolic downregulation. The critical factor in microvascular regulation to meet oxygen supply is the local regulation of arteriolar tone. Many mechanisms contribute to the local regulation of arteriolar tone, which includes response to myogenic response, shear-dependent response, and tissue metabolic response. During haemorrhagic shock, the decrease in  $DO_2$  reduces the generation of adenosine 5' triphosphate (ATP) and adenosine 5' diphosphate (ADP) which results in the accumulation of ADP and its degradation products.  $CO_2$  is a strong vasodilator, which accumulates when there is an increase in cellular metabolism or reduced clearance of  $CO_2$  during tissue hypoperfusion. In haemorrhagic shock, the therapeutic precedence is to stop the bleeding and to prevent the increase of bleeding. Fluid resuscitation may promote coagulopathy by diluting coagulation factors [55, 56].

Reduced microvascular perfusion is a characteristic feature of sepsis and implicated in organ dysfunction with multiple organ failure. Hyperbaric oxygen (HBO) has potentially beneficial effects for the microcirculation improvement in sepsis. It was shown that HBOT improves

microcirculation independently from systemic hemodynamic parameters which is a key therapeutic target in septic shock patients. [57]. Some of well-documented HBOT mechanisms in sepsis treatment are attenuation of inflammatory mediators and free radicals, reduction of bacterial proliferation, inhibition of lipopolysaccharide-induced acute lung injury and enabling neutrophils to kill bacteria with oxygen dependent mechanisms. It was also proposed that HBOT can delay onset of sepsis and may boost the effect of antibiotics [58].

Meningococcal sepsis can cause purpura fulminans which occurs by the formation of thrombi haemorrhages and occlusion of dermal vessels and often a life-threatening condition [59]. Clinically, it was shown that HBOT is effective in meningococcal sepsis which is complicated with the necrotic tissue [60]. HBOT with its immunostimulatory, neovascularity, and bactericidal effect can be adjunct treatment for non-healing ulcers and problematic wounds.

## 8. Conclusion

In conclusion, microcirculation plays a critical role in the physiological process such as oxygen supply to vital organs, nutritional exchange and modulation of inflammation. The most important function of the microcirculation is the regulation of flow within the different organs. Microcirculatory changes include various mechanisms such as redistribution of blood flow from the skin and the splanchnic area to brain or heart, or with endothelial activation and injury which results with the loss of the glycocalyx around endothelium. The assessment of the microcirculation enables us for the early detection of possible deterioration and potential organ dysfunctions. Microcirculatory blood flow can be detected at the bedside by different techniques which are simple and gives quantitative data. Fluid resuscitation and vasoactive agents are one of the main therapies for the hemodynamic resuscitation that aims to restore the circulating volume, and increasing the cardiac output and arterial blood pressure and play a critical role with the goal of improving tissue perfusion. HBOT inhibits replicating, spreading of anaerobic and some other bacteria and is a clinical treatment adjunct to traditional surgery and antibiotic therapy for the deadly serious necrotizing infection. Also, HBOT applied in acute ischemic stroke, femoral head necrosis and carbon monoxide intoxication aim to increase oxygen supply to the ischemic tissue and to reduce the extent of irreversible tissue damage.

### Author details

Fethi Gul<sup>1\*</sup>, Omer Faruk Boran<sup>2</sup> and Reyhan Arslantas<sup>3</sup>

\*Address all correspondence to: gulfethi@gmail.com

1 Marmara University, Department of Anesthesiology and Reanimation, Istanbul, Turkey

2 Sutcu Imam University, Department of Anesteheiology and Reanimation, Kahramanmaras, Turkey

3 Health Sciences University, Kartal Dr. Lutfi Kırdar Training and Research Hospital, Anesthesiology and Reanimation Clinics, Istanbul, Turkey

## References

- [1] Guyton and Hall Textbook of Medical Physiology 11th Edition: section 16
- [2] Steven S. Regulation of blood flow in the microcirculation. *Microcirculation*. 2005;**12**:33-45
- [3] Jacob et al. Regulation of blood flow and volume exchange across the microcirculation. *Critical Care*. 2016;**20**:319
- [4] Scallan J, Huxley VH, Korthuis RJ. *Capillary Fluid Exchange: Regulation, Functions, and Pathology*. Morgan & Claypool Life Sciences. 1st Edition: Section 1.2. 2010
- [5] Gill AL, Bell CNA. Hyperbaric oxygen: Its uses, mechanisms of action and outcomes. *QJM: An International Journal of Medicine*. 2004;**97**(7):385-395
- [6] RM B, Koeppen BM, Stanton BA. *Physiology*. 4th ed. Vol. 1998. pp. 217-221
- [7] Chawla LS, Ince C, Chappell D, Gan TJ, Kellum JA, Mythen M. Vascular content, tone, integrity, and haemodynamics for guiding fluid therapy: A conceptual approach. *British Journal of Anaesthesia*. 2014;**113**:748-755
- [8] Secomb TW. Theoretical models for regulation of blood flow. *Microcirculation*. 2008;**15**:765-775
- [9] Stirban A, Lentrod S. Functional changes in microcirculation during hyperbaric and normobaric oxygen therapy. *Undersea and Hyperbaric Medical Society*. 2009;**36**:5-9
- [10] Korhonen K, Kuttilla K, Niinikoski J. Tissue gas tensions in patients with necrotising fasciitis and healthy controls during treatment with hyperbaric oxygen: A clinical study. *The European Journal of Surgery*. 2000;**166**:530-534
- [11] Cuthbertson CM, Su KH, Muralidharan V, Millar I, Malcontenti-Wilson C, Christophi C. Hyperbaric oxygen improves capillary morphology in severe acute pancreatitis. *Pancreas*. 2008;**36**:70-75
- [12] Habazettl H, Kupatt C, Zahler S, Becker BF, Messmer K. Selectins and beta 2-integrins mediate post-ischaemic venular adhesion of polymorphonuclear leukocytes, but not capillary plugging, in isolated hearts. *Pflügers Archiv*. 1999;**438**:479-485
- [13] Ezaki T, Baluk P, Thurston G, La Barbara A, Woo C, McDonald DM. Time course of endothelial cell proliferation and microvascular remodeling in chronic inflammation. *The American Journal of Pathology*. 2001;**158**:2043-2055
- [14] Bayliss WM. On the local reactions of the arterial wall to changes of internal pressure. *The Journal of Physiology*. 1902;**28**(3):220-231
- [15] Becker BF, Chappell D, Jacob M. Endothelial glycocalyx and coronary vascular permeability: The fringe benefit. *Basic Research in Cardiology*. 2010;**105**:687-701
- [16] Somani A et al. The dynamic regulation of microcirculatory conduit function: Features relevant to transfusion medicine. *Transfusion and Apheresis Science*. 2010;**431**:61-68

- [17] Davenport AP, Hyndman KA, Dhaun N, Southan C, Kohan DE, Pollock JS, et al. Endothelin. *Pharmacological Reviews*. 2016;**68**:357-418
- [18] Bagher P, Segal SS. Regulation of blood flow in the microcirculation: Role of conducted vasodilation. *Acta Physiologica*. 2011;**202**:271-284
- [19] Little TL, Beyer EC, Duling BR. Connexin 43 and connexin 40 gap junctional proteins are present in arteriolar smooth muscle and endothelium in vivo. *The American Journal of Physiology*. 1995a;**268**:729-739
- [20] Figueroa XFDBR. Dissection of two Cx37- independent conducted vasodilator mechanisms by deletion of Cx40: Electrotonic versus regenerative conduction. *American Journal of Physiology. Heart and Circulatory Physiology*. 2008;**295**:2001-2007
- [21] Danthuluri NR, Cybulsky MI, Brock TA. Ach-induced calcium transients in primary cultures of rabbit aortic endothelial cells. *American Journal of Physiology. Heart and Circulatory Physiology*. 1988;**255**:1549-1553
- [22] Himmel HM, Whorton AR, Strauss HC. Intracellular calcium, currents, and stimulus-response coupling in endothelial cells. *Hypertension*. 1993;**21**:112-127
- [23] Busse R, Fichtner H, Luckhoff A, Kohlhardt M. Hyperpolarization and increased free calcium in acetylcholine- stimulated endothelial cells. *American Journal of Physiology. Heart and Circulatory Physiology*. 1988;**255**:965-969
- [24] Ledoux J, Werner ME, Brayden JE, Nelson MT. Calcium-activated potassium channels and the regulation of vascular tone. *Physiology*. 2006;**21**:69-78
- [25] Orledge A, D'Amore PA. Inhibition of capillary endothelial cell growth by pericytes and smooth muscle cells. *The Journal of Cell Biology*. 1987;**105**:1455-1462
- [26] Sato Y, Rifkin DB. Inhibition of endothelial cell movement by pericytes and smooth muscle cells: Activation of a latent transforming growth factor-beta 1-like molecule by plasmin during co-culture. *The Journal of Cell Biology*. 1989;**109**:309-315
- [27] Katusic ZS, Vanhoutte PM. Superoxide anion is an endothelium-derived contracting factor. *The American Journal of Physiology*. 1989;**257**:33-37
- [28] Lund V, Kentala E, Scheinin H, Klossner J, Koskinen P, Jalonen J. Effect of hyperbaric conditions on plasma stress hormone levels and endothelin-1. *Undersea & Hyperbaric Medicine*. 1999;**26**:87-92
- [29] Vellinga NA, Boerma EC, Koopmans M, et al. International study on microcirculatory shock occurrence in acutely ill patients. *Critical Care Medicine*. 2015;**43**:48-56
- [30] Squara P. Central venous oxygenation: When physiology explains apparent discrepancies. *Critical Care*. 2014;**18**(6):579
- [31] Pope JV, Jones AE, Gaieski DF, et al. Multicenter study of central venous oxygen saturation [ScvO<sub>2</sub>] as a predictor of mortality in patients with sepsis. *Annals of Emergency Medicine*. 2010;**55**:40-46

- [32] De Backer D, Ospina-Tascon G, Salgado D, Favory R, Creteur J, Vincent JL. Monitoring the microcirculation in the critically ill patient: Current methods and future approaches. *Intensive Care Medicine* 2010;**36**:1813-1182
- [33] Crookes BA, Cohn SM, Bloch S, et al. Can near-infrared spectroscopy identify the severity of shock in trauma patients? *The Journal of Trauma*. 2005;**58**:806-813
- [34] Creteur J, Carollo T, Soldati G, et al. The prognostic value of muscle StO<sub>2</sub> in septic patients. *Intensive Care Medicine*. 2007;**33**:1549-1556
- [35] Schlichtig R, Bowles SA. Distinguishing between aerobic and anaerobic appearance of dissolved CO<sub>2</sub> in intestine during low flow. *Journal of Applied Physiology*. 1994;**76**:2443e51
- [36] Cain SM. Oxygen delivery and uptake in dogs during anemic and hypoxic hypoxia. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*. 1977;**42**:228-234
- [37] Slottosch I, Liakopoulos O, Kuhn E, Scherner M, Deppe AC, Sabashnikov A, et al. Lactate and lactate clearance as valuable tool to evaluate ECMO therapy in cardiogenic shock. *Journal of Critical Care*. 2017;**42**:35-41
- [38] Milstein DM, Helmers R, Hackmann S, Belterman CN, van Hulst RA, de Lange J. Sublingual microvascular perfusion is altered during normobaric and hyperbaric hyperoxia. *Microvascular Research*. 2016;**105**:93-102
- [39] Kranke P, Bennett MH, Martyn-St James M, Schnabel A, Debus SE, Weibel S. Hyperbaric oxygen therapy for chronic wounds. *Cochrane Database of Systematic Reviews*. 2015;**24**(6):5-6
- [40] Poli S, Veltkamp R. Oxygen therapy in acute ischemic stroke – Experimental efficacy and molecular mechanisms. *Current Molecular Medicine*. 2009;**9**(2):227-241
- [41] Li W, Ye Z, Wang W, Wang K, Li L, Zhao D. Clinical effect of hyperbaric oxygen therapy in the treatment of femoral head necrosis : A systematic review and meta-analysis. *Der Orthopäde*. 2017;**46**(5):440-446
- [42] Bleecker ML. Carbon monoxide intoxication. *Handbook of Clinical Neurology*. 2015;**131**:191-203
- [43] De Backer D, Donadello K, Sakr Y, Ospina-Tascon G, Salgado D, Scolletta S, Vincent JL. Microcirculatory alterations in patients with severe sepsis: Impact of time of assessment and relationship with outcome. *Critical Care Medicine*. 2013;**41**:791-799
- [44] Bateman RM, Jagger JE, Sharpe MD, Ellsworth ML, Mehta S, Ellis CG. Erythrocyte deformability is a nitric oxide-mediated factor in decreased capillary density during sepsis. *American Journal of Physiology. Heart and Circulatory Physiology*. 2001;**280**:2848-2856
- [45] Borutaite V, Matthias A, Harris H, Moncada S, Brown GC. Reversible inhibition of cellular respiration by nitric oxide in vascular inflammation. *American Journal of Physiology. Heart and Circulatory Physiology*. 2001;**281**:2256-2260

- [46] Constantinescu AA, Vink H, Spaan JA. Endothelial cell glycocalyx modulates immobilization of leukocytes at the endothelial surface. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2003;**23**:1541-1547
- [47] Cabrales P, Vázquez BY, Tsai AG, Intaglietta M. Microvascular and capillary perfusion following glycocalyx degradation. *Journal of Applied Physiology*. 2007;**102**:2251-2259
- [48] Cepinskas G, Wilson JX. Inflammatory response in microvascular endothelium in sepsis: Role of oxidants. *Journal of Clinical Biochemistry and Nutrition*. 2008;**42**:175-184
- [49] Doerschug KC, Delsing AS, Schmidt GA, Haynes WG. Impairments in microvascular reactivity are related to organ failure in human sepsis. *American Journal of Physiology. Heart and Circulatory Physiology*. 2007;**293**:1065-1071
- [50] Kanoore Edul VS, Dubin A, Ince C. The microcirculation as a therapeutic target in the treatment of sepsis and shock. *Seminars in Respiratory and Critical Care Medicine*. 2011;**32**:558-568
- [51] Dubin A, Pozo MO, Casabella CA, Murias G, Pálizas F, Moseinco MC, Kanoore Edul VS, Estensoro E, Ince C. Comparison of 6% hydroxyethyl starch 130/04 and saline solution for resuscitation of the microcirculation during the early goal-directed therapy of septic patients. *Journal of Critical Care*. 2010;**25**:659
- [52] Trzeciak S, Cinel I, Phillip Dellinger R, Shapiro NI, Arnold RC, Parrillo JE, Hollenberg SM. Resuscitating the microcirculation in sepsis: The central role of nitric oxide, emerging concepts for novel therapies, and challenges for clinical trials. *Academic Emergency Medicine*. 2008;**15**(5):399-413
- [53] Bhagat K, Vallance P. Inflammatory cytokines impair endothelium- dependent dilatation in human veins in vivo. *Circulation*. 1997;**96**:3042-3047
- [54] Boerma EC, Ince C. The role of vasoactive agents in the resuscitation of microvascular perfusion and tissue oxygenation in critically ill patients. *Intensive Care Medicine*. 2010;**36**:2004-2018
- [55] Boerma EC, Koopmans M, Konijn A, Kaiferova K, Bakker AJ, Van Roon EN, Buter H, Bruins N, Egbers PH, Gerritsen RT, et al. Effects of nitroglycerin on sublingual microcirculatory blood flow in patients with severe sepsis/ septic shock after a strict resuscitation protocol: A double-blind randomized placebo controlled trial. *Critical Care Medicine*. 2010;**38**:93-100
- [56] Trzeciak S, Dellinger RP, Parrillo JE, Guglielmi M, Bajaj J, Abate NL, Arnold RC, Colilla S, Zanotti S, Hollenberg SM. Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: Relationship to hemodynamics, oxygen transport, and survival. *Annals of Emergency Medicine*. 2007;**49**:88-98
- [57] Ferré F, Silva S, Ruiz J, Mari A, Mathe O, Sanchez-Verlaan P, Riu-Poulenc B, Fourcade O. Microcirculatory effect of hyperbaric oxygen therapy in septic patients. *Critical Care*. 2011;**15**(Suppl 1):284

- [58] Igor A, Michael S, Stuart M, Chi T, George H. New roles of Hyperbaric oxygen in Sepsis. Chest. 2007;**132**(4):557
- [59] Mathieu C, Olivier JL, Herve Le'cuyer, Sandrine B, Stefano M, Xavier N. Pathogenesis of Meningococemia. Cold Spring Harbor Perspectives in Medicine .2013;3:012393
- [60] Alex PB, Tom B, Banwari A. Purpura Fulminans in Sepsis. The American Journal of the Medical Sciences. 2006;**332**(6):339-345

IntechOpen

IntechOpen