

The impact of the glycocalyx on microcirculatory oxygen distribution in critical illness

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Current Opinion in Anaesthesiology 2009, 22:155–162

Purpose of review

Main problems of critical illness and sepsis are an altered oxygen distribution and microvascular dysfunction linked to tissue oedema. This review seeks to analyse the role of the endothelial glycocalyx in this context.

Recent findings

The presence of vascular leakage is typically associated with interstitial oedema, arterial hypotension, hypovolaemia and often a bad outcome in patients with systemic inflammation. Early goal-directed therapy provides significant benefits in severe sepsis and septic shock, but is mostly aimed at improving macrohaemodynamics. Recent data suggest that microcirculation also contributes significantly to the pathophysiology of critical illness. In fact, the endothelial glycocalyx plays a major role in vascular barrier competence. According to experimental evidence, it can easily be degraded in the presence of inflammation, but, theoretically also protected by several measures. Clinical studies revealed a positive correlation of the severity of sepsis and ischaemia with mortality, but also with a deterioration of the endothelial glycocalyx. Future investigation should focus on the preservation of this structure and assess microcirculatory variables to judge the success of cardiocirculatory therapy.

Summary

Deterioration of the endothelial glycocalyx initiates a breakdown of the vascular barrier in systemic inflammatory response syndrome and sepsis. Preserving this structure in critical illness might be a future therapeutical goal to improve microcirculatory oxygen distribution.

Keywords

endothelium, glycocalyx, microcirculation, oxygen distribution, sepsis, systemic inflammatory response syndrome

Curr Opin Anaesthesiol 22:155–162
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0952-7907

Introduction

Systemic inflammation is a common feature of critically ill patients [1]. Maintaining aerobic metabolism as far as possible during the complete healing process is essential to the organs energy balance. Notably, aerobic degradation of 1 mol glucose to carbon dioxide and water delivers 36 mol of adenosine-tri-phosphate (ATP), whereas the alternative anaerobic way down to lactate only provides 2 mol of ATP. Additionally, accumulation of lactate may result in metabolic acidosis and is strongly related to increased mortality in patients suffering from circulatory failure [2]. In patients with septic shock, serum lactate concentration was better related to patient survival than alternative oxygen transport parameters [3]. Nevertheless, the severity and duration of arterial hypo-

tension and inadequacy of cardiac output, as reflected by a decrease in central venous oxygen saturation, have also been related to a poor outcome. In this situation, early goal-directed therapy can improve and stabilise macrohaemodynamics achieving a balanced oxygen supply/demand relationship [4]. However, also persistent microvascular perfusion derangements play a pivotal role in the patients way to multiple organ failure and death [5].

Physiological metabolism requires an adequate oxygen delivery to organs and tissues with cardiac output and arterial oxygen content representing the macrohaemodynamical determinants. However, the combination with an adequate microcirculatory oxygen distribution and utilization is crucial. This review article elucidates the

potential role of the endothelial glycocalyx in this context.

Oxygen balance in the tissue: principal considerations

A reduction of cardiac preload, resulting in arterial hypotension based on a reduced cardiac output and vasodilatation may impair tissue perfusion and organ oxygenation. Inflammatory mediators induce nitric oxide synthase in vascular small muscle cells, resulting in massive production of nitric oxide [6]. This causes widespread vasodilatation and arterial hypotension, which may become hyporeactive to adrenergic agents in the presence of systemic inflammation. Other pathogenetic causes are dysregulation of the cortisole metabolism, relative vasopressin deficiency and activation of ATP-sensitive potassium channels. These alterations are common features in critically ill patients and foster the pathogenesis of organ dysfunction [7]. In patients with septic shock, the severity and duration of arterial hypotension (mean arterial pressure <65 mmHg) and inadequacy of cardiac output [reflected by a central venous oxygen saturation (ScvO₂) <70%] are related to a poor outcome [5]. Fluid resuscitation is essential in this context to restore blood flow [8]. However, important goals like microcirculatory flow and related tissue oxygenation both still cannot be measured directly in clinical routine. Early goal-directed therapy is, therefore, aimed at macrohaemodynamical parameters, such as central venous and arterial pressure, as well as central venous oxygen saturation. Despite this practical provides significant benefits with respect to outcome in patients with severe sepsis and septic shock [4], caution should be given not to solely aim at macrocirculatory perfusion goals alone [9^{*}]. This may not be sufficient to optimize regional distribution of blood flow to tissues in many patients, especially during maldistributive shock. Perfusion of the microcirculation is based on macrohaemodynamics, but, in addition, regulated by interplay of many pathways. All together,

they adapt to the balance between tissue oxygen transport and metabolic needs to ensure that the supply exceeds the demand. In sepsis, this process is compromised due to decreased deformability of red blood cells raising viscosity and activated neutrophils with increased aggregation due to the upregulation of adhesion molecules [10]. Above that, activation of the clotting cascade with fibrin deposition, dysfunction of vascular autoregulatory mechanisms and the secondary enhanced perfusion of arteriovenous shunts contribute to this phenomenon, leaving the microcirculation hypoxic [11]. Also, mitochondrial dysfunction contributes to tissue dysoxia. The consecutively diminished production of ATP, despite normal (or even supranormal) pO_2 values in the vicinity of mitochondria within cells is termed cytopathic hypoxia [12]. Clinically, these processes are perceived as an oxygen extraction deficit, a prominent feature of sepsis. Accordingly, despite high oxygen delivery, the extraction by the tissues might be low and the tissues could, nevertheless, suffer from severe hypoxia. In addition, neutrophil activation in the presence of respiratory burst can consume a major part of the extracted oxygen, which, therefore, cannot be utilized by the parenchymal cells [14]. Although being an important basis of treatment, intact macrohaemodynamics cannot guarantee sufficient tissue oxygenation in critical illness.

Systemic inflammatory response syndrome, sepsis, severe sepsis and septic shock: the who is who of inflammation

The systemic inflammatory response syndrome (SIRS) represents an unspecific inflammatory state, which is characterized by at least two of the symptoms listed in Table 1 [15^{**},16]. The lethality in SIRS is around 10%. Sepsis is defined as SIRS in which there is an identifiable focus of infection. If there are signs of at least two organ dysfunctions, this condition is called 'severe sepsis', whereas 'septic shock' is further complicated by

Table 1 Definitions of various stages of infection

Stage	Definition
Infection	A response to the presence of microorganisms or tissue invasion by microorganisms
Bacteremia	The presence of viable bacteria in circulating blood.
Systemic inflammatory response syndrome (SIRS)	A systemic inflammatory response to a wide variety of severe clinical insults, manifested by two or more of the following conditions: Temperature >38°C or <36°C Heart rate >90 beats/min Respiratory rate >20 breaths/min or PaCO ₂ <32 mmHg White blood cell count >12 000/mm ³ , <4000/mm ³ or >10% immature (band) forms
Sepsis	An infection plus systemic manifestations of infection (see systemic inflammatory response syndrome)
Severe sepsis	Sepsis plus sepsis-induced organ dysfunction or tissue hypoperfusion
Septic shock	Severe sepsis plus hypotension not reversed with fluid resuscitation
Multi-organ dysfunction syndrome	Presence of altered organ function in an acutely ill patient such that homeostasis cannot be maintained without intervention.

Data from [15^{**},16].

volume-refractory arterial hypotension [15^{••}]. In severe sepsis and septic shock, mortality depends on the number of affected organs [15^{••}] and approximates 80% in septic shock. Importantly, despite being the trigger, it is often not the infection itself, but the host response which is responsible for the effects detrimental on the organism [17]. Being beneficial within normal ranges to eradicate microorganisms, in SIRS this response becomes generalized and increasingly autodestructive.

The systemic inflammatory response: a physiological reaction at a pathological amount

Aim of the innate immune response to local invasion of microbes is local inflammation, enabling immune competent cells to access and combat them. Among others, it is initiated by endotoxin release from bacteria and implies synthesis of nitric oxide and the release of pro-inflammatory cytokines, such as various interleukins or tumour necrosis factor- α (TNF- α) [18]. They cause local vasodilation, an increase in vascular permeability and attraction and activation of leucocytes. The initial target is elimination and local tissue repair.

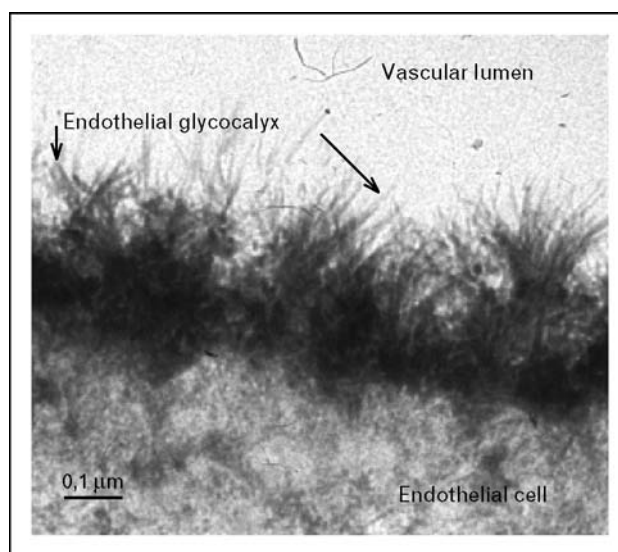
This reaction might become a problem beyond the primary damage to the organism if it causes an enrichment of inflammatory mediators. Activation of leucocytes and their corresponding adhesion molecules throughout the circulation lead to a generalized inflammation, also in primarily unaffected parts of the body. In sepsis, inflammatory cells generate reactive oxygen and nitrogen species that may induce cell injury, platelet activation, endothelial dysfunction and an increased risk for capillary plugging, microthrombus formation and microvascular shunting, mainly within postcapillary venular sites [19]. These pathophysiological mechanisms contribute to an impairment of the microcirculation. They include persistent and vasopressor-refractory vasodilation, combined with vascular leakage, leading to a loss of protein and fluid from the circulation with interstitial accumulation [20]. The resulting intravascular hypoalbuminemia may aggravate the clinical situation via a reduced colloid osmotic pressure, which, in turn, further compromises the ability to preserve intravascular volume [7]. A consecutive reduction of cardiac preload, in addition to a raise in interstitial hydrostatic pressure, results in impaired tissue perfusion, leading to organ dysfunction [7].

Accordingly, the vascular barrier is one centre of attention during SIRS and sepsis. In order to understand its fate in critical illness, we will first enlighten its physiological functions.

The intact vascular barrier

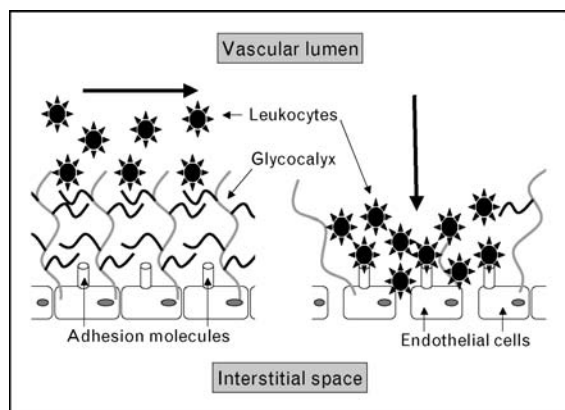
Under physiological conditions, the positive intravascular hydrostatic pressure forces blood constituents towards the interstitial space. Large molecules, such as colloids and proteins normally cannot cross the vascular barrier in relevant amounts. The traditional view, introduced by Ernest Starling in 1896 [21], suggests an interstitial colloid osmotic pressure far below the intravascular pressure. This schematically generates an inward-directed oncotic gradient across the endothelial cell line, the net result being an only low filtration rate per unit of time. Recent evidence, has suggested that a traditionally not recognized small structure might play an additional pivotal role in this context. Importantly, every healthy vascular endothelium is coated by a great variety of transmembrane and membrane-bound molecules, which together constitute the endothelial glycocalyx (Fig. 1) [22,23]. The principal proteins on the endothelial cell surface are transmembrane syndecans and membrane-bound glypicans, both containing heparan sulfate and chondroitin sulfate side chains [24[•]]. Together with bound plasma proteins, hyaluronan and solubilised glycosaminoglycans, the glycocalyx forms the endothelial surface layer with a thickness of around 1 μm [22,23,24[•]]. Therefore, the glycocalyx normally covers endothelial adhesion molecules at a dimension of approximately some tens of nanometers (Fig. 2) [22,23]. In addition, the endothelial glycocalyx represents a major functional part of the vascular barrier [25]. Furthermore, it has been

Figure 1 Electron microscopic view of the endothelial glycocalyx



Staining of the glycocalyx was based on an *in-situ* stabilization of the glycocalyx by intracoronary application of a fixative containing lanthanum and glutaraldehyde.

Figure 2 A schematic image of the intact and a degraded glycocalyx



(a) An image of the intact and a degraded glycocalyx. Nonactivated leukocytes flow in the normal blood stream above the glycocalyx and do not come in contact with endothelial adhesion molecules harboured within the glycocalyx. (b) The situation changes when the glycocalyx is degraded. Adhesion molecules are exposed, activated and interact with leukocytes, causing activation, conglomeration, adhesion and migration.

shown in a rat mesenteric microvessel model that the effective colloid osmotic pressure difference opposing hydrostatic filtration was near 70% of the luminal osmotic pressure, although the colloid concentration outside equalled that inside the lumen of the vessel [26]. It seems as though the interstitial protein concentration, in contrast to Starlings classical concept, does not play a major role in this context. The endothelial glycocalyx rather appears to act as a molecular filter, actively retaining plasma proteins [27,28], increasing the oncotic pressure within the glycocalyx and building up the functional endothelial surface layer [23]. A small space beneath, but still at the luminal side of the anatomical vessel wall, is practically protein-free [25–28]. Accordingly, transcapillary fluid loss actually seems to be limited by an oncotic pressure gradient across the endothelial glycocalyx, and not across the whole anatomical vessel wall. Therefore, Starlings classical principle needs to be modified into a current form, considering the fact that an intact endothelial glycocalyx is the main prerequisite of a working vascular barrier [29,30].

Alteration of the endothelial glycocalyx in the presence of systemic inflammatory response syndrome and sepsis

Capillary leak represents a central problem in the inflammatory response. Despite its clinical importance, however, there are no standardized criteria for its diagnosis [7], as assessment of fluid distribution is extremely

difficult. Unfortunately, there is still no technique reliably quantifying interstitial oedema in clinical practice, nor is it possible to routinely measure blood volume or the functional extracellular compartment at the bedside. Until now, definitions of the capillary leak syndrome, for example, noncardiogenic generalized oedema and haemodynamic instability [31] or generalized oedema with a body weight increase of more than 3% [32], are nonspecific.

Recent studies have shown glycocalyx shedding in patients undergoing major vascular surgery with global or regional ischaemia [33], or suffering from diabetes [34]. Also, it has been reported that increased levels of glycocalyx components, syndecan-1 and glycosaminoglycans appear in the blood of septic shock patients [35]. Moreover, the level of these components revealing a deteriorated glycocalyx correlated positively with mortality. Integrity and destruction of the endothelial glycocalyx could, therefore, be the key to understand many pathophysiological sequences for inflammatory processes. The vascular endothelium is not only one of the earliest locations of injury, potentially leading to organ dysfunction and failure in the context of inflammation [9]. The endothelial cell changes involve membrane damage, increased permeability, swelling, cell necrosis and apoptosis [36]. As introduced above, endothelial-directed recruitment and activation of leukocytes at the site of infection to eradicate pathogens is an important mechanism of the inflammatory response [19]. However, adhesion molecules are harboured within the glycocalyx [22,23]. Release of inflammatory mediators could initiate accessibility of immunocompetent cells to adhesion molecules by degrading the enveloping glycocalyx [22]. It is well recognized that exposure to TNF- α [37], oxidized lipoproteins [38] or ischaemia/reperfusion [39], all well known problems in SIRS, can reduce its thickness. TNF- α additionally activates mast cells, being rich sources of cytokines, proteases, histamine and heparanase [40]. All these mediators have previously been shown to degrade the glycocalyx [37, 39,41,42]. Shedded heparan sulfates have an additional chemotactical impact on leukocytes and increase their presence at the site of inflammation by a positive feedback mechanism [43]. Therefore, the generalized destruction of the glycocalyx could be a trigger for generalized leukocyte adhesion and permeability disorders with general oedema development.

In accordance, several studies have allocated the glycocalyx to play a major role in the context of SIRS and sepsis. TNF- α , in a hamster cremaster [42] and isolated heart [37] model, as well as lipopolysaccharide (LPS) endotoxin applied as bolus infusion into Sprague–Dawley rats [44], have been shown to cause shedding of heparan sulfate, glycosaminoglycans and syndecan-1

from the endothelial glycocalyx. In humans, a LPS injection caused shedding of hyaluronan [45^{*}], a main constituent of the endothelial surface layer. TNF- α antagonism has been shown to reduce intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) expressions, as well as leucocyte infiltration with a reduction of intramyocardial inflammation and cardiac fibrosis in a rat model. TNF- α has been shown to induce a markedly increased coronary resistance, development of myocardial oedema and pronounced coronary leak, together with enhanced vascular permeability to colloid in isolated perfused guinea pig hearts [37]. All this might not base on simple disruption of the endothelial cell line but seems to be associated with a deteriorated glycocalyx.

Microcirculatory oxygen distribution in systemic inflammatory response syndrome and sepsis

In an inflammatory state, tissue hypoxia can persist despite achievement of normal or even supranormal global oxygen delivery. Evidence is provided by high lactate levels and increased p_{CO_2} [47]. This condition 'oxygen extraction deficit' has been well documented in different animal models of shock [48,49]. Clinically, this problem can manifest with persistent acidosis, decreased urine production or progressive multiorgan failure or both [50].

As explained before, disruption of microcirculatory homeostasis is a common complication in sepsis [51]. The heterogeneity of microvascular blood flow might explain some of the alterations in oxygen extraction capabilities [47]. Using a mathematical model, Walley *et al.* [52] reported an increase in blood flow heterogeneity to be associated with an increase in critical delivery of oxygen. Humer *et al.* [53] demonstrated that gut and muscle blood flow heterogeneity increase together with impaired oxygen extraction after endotoxin administration. However, it appears questionable whether the oxygen extraction deficit should be explained only by pathologic flow heterogeneity [47]. In a clinical study infusing *Escherichia coli* LPS to healthy volunteers, a loss of endothelial glycocalyx and a decrease in capillary density was found, suggesting that degradation of the endothelial glycocalyx leads to capillary perfusion impairments, a reduction of functional capillary density and increased erythrocyte flux in the remainder of perfused capillaries [45^{*},54]. Meanwhile, it has been shown that, for the same level of arterial hypotension in mice, mucosal perfusion disorders are considerably larger in endotoxin-induced hypotension than in haemorrhagic hypotension [55]. Due to its crucial functions on the vascular barrier, degradation of the glycocalyx leads to interstitial oedema, which is strongly related to a decrease

in tissue oxygenation [56]. Therefore, limiting interstitial oedema should help to improve tissue oxygenation. It seems as though the glycocalyx plays a major role in the development of capillary leakage. In addition to its protection, however, also application of an adequate fluid therapy might be a possibility to limit fluid accumulation in the interstitial compartment.

Fluid therapy in systemic inflammatory response syndrome and sepsis: a never ending story

Main goal in the treatment of sepsis is currently to establish an adequate hydration status to achieve stable haemodynamics. Crystalloids, being free of colloid osmotic force are not retained even at the intact vascular wall. They distribute within the whole extracellular, that is, the vascular (20%) and the interstitial (80%), space for physiologic reasons [29^{**},30]. This explains, for example, the perioperative observation that crystalloid boluses have no major effect on the incidence or severity of anaesthesia-related arterial hypotension [29^{**}]. Rather, crystalloid infusion increases the interstitial hydrostatic pressure and stresses the lymphatic system. Infused isoosmotic colloids theoretically do not change, but maintain the intravascular colloid osmotic pressure and cannot cross the intact barrier in relevant amounts. Accordingly, they should primarily remain within the circulatory space by almost 100% and represent a rational option to maintain or restore cardiac preload during acute blood loss if the barrier is intact [29^{**}]. If additionally infused into a primarily normovolaemic circulation, however, colloids vanish by almost two-thirds towards the interstitial space, destroying the endothelial surface layer and causing oedema [57]. This means that colloidal volume effects are context-sensitive and that a rational perioperative fluid approach might be of need, instead of traditional overloading the circulation, hoping the kidney will compensate our mistakes [29^{**},58]. In order to maintain a primarily existing perioperative steady state, hypervolaemia should be avoided as far as possible [29^{**}].

As during systemic inflammation the circulation is not in a steady state, the primary goal is to establish it. As described extensively [35^{*},37,42,44], the vascular barrier is largely deteriorated and the theoretical considerations concerning the difference between colloids and crystalloids do not count to a large extent. Nevertheless, it would be reasonable to rely on a residual function of the vascular barrier and to apply colloids if immediate restoration of cardiac preload is intended. Also, the widespread fear of a suction of fluid towards the tissue, due to accumulation of colloid, is neither based on data, nor does it appear logical that the interstitial oncotic pressure should exceed the intravascular one, if the barrier is open.

Rather, in order to rapidly establish cardio-circulatory optimization in critically ill patients, colloids might be beneficial in comparison to crystalloids in severe sepsis and septic shock [59]. However, an improved overall outcome has not been related to this resuscitation strategy so far [60], despite it should represent a fluid-saving and, therefore, oedema-limiting concept, theoretically also improving tissue oxygenation. Anyway, based on the currently available studies it can only be recommended to apply enough, irrespective of the kind of fluid [15**].

Preservation of the glycocalyx

During a generalized inflammatory response, the integrity of the endothelial glycocalyx is already severely deteriorated, and any means of protection or preservation should be in vain. Nevertheless, various experimental and clinical studies exist in which this, at least theoretical, option has been examined. For example, Nieuwdorp *et al.* [45*] have shown in healthy volunteers that intravenous application of a soluble TNF- α blocker (etanercept, Enbrel, Wyeth, Madison, New Jersey, USA) attenuates a reduction of endothelial glycocalyx perturbation, abolishing shedding of glycocalyx constituents and reducing coagulation activation. In a study on isolated guinea pig hearts, hydrocortisone and antithrombin III were able to suppress the effects of the inflammatory cytokine TNF- α on vascular permeability, coronary leak and interstitial oedema [37]. Glucocorticoids and antithrombin are prominent in the treatment of sepsis, along with antibiotics, fluid resuscitation and vasopressors [61]. They stabilize the endothelial glycocalyx and enable it to maintain the physiological endothelial permeability barrier in the face of inflammatory challenge [37]. How far this knowledge can be transferred to the clinic has to be clarified in further studies. First, data from isolated heart animal model cannot be directly transferred into in-vivo situations. Second, both drugs were applied prior to endotoxaemia, which does not represent daily clinical practice. However, despite only being an option for the future, preservation of the glycocalyx at a very early time in the development of SIRS and sepsis might have the potential to alleviate leucocyte and platelet adhesion, thereby possibly mitigating inflammation and tissue oedema. Limiting glycocalyx shedding, possibly an important, but still unknown, action of well known drugs in the therapy of sepsis, promises to help break through one of the main pathomechanisms of critical illness.

Outlook

In septic patients, microcirculatory failure appears to be a major perturbation with prognostic significance [9*]. Severe derangements of microcirculatory flow have been

associated with lower survival in humans, possibly representing an early triggering event in the development of sepsis-induced multiorgan failure. Recently, new translational research techniques of in-vivo video microscopy for assessment of microcirculatory flow in humans have been introduced [9*]. In addition, tissue-oxygen assessment using near-infrared spectroscopy has been suggested [56]. Clinical trials need to evaluate whether a goal-directed approach aimed at the microcirculation, instead of merely focussing on macrocirculatory goals, has the potential to further improve outcome after SIRS and sepsis. Protection of the endothelial glycocalyx, however, only seems to be possible in early stages of a developing sepsis, as cytokines degrade it rapidly. The exact pathophysiological mechanisms, shedding time points, kinetics of restructuring the damaged glycocalyx and the question what role an at least partly intact glycocalyx could play, need to be examined in future studies.

Conclusion

Microcirculatory dysfunction plays a pivotal role in the pathophysiology of sepsis and represents a critical piece in the jigsaw of sepsis-induced circulatory failure. Recent evidence suggests the endothelial glycocalyx to have a major impact in this context. Detecting microvascular blood flow and tissue oxygenation may yield a more problem-based insight compared to global macrocirculatory indices. Nevertheless, future studies are needed to elucidate whether microcirculation-oriented therapies really provide a beneficial amendment of the current macrohaemodynamical approach.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 315).

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