Chapter

Organ Damage in Sepsis: Molecular Mechanisms

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Abstract

Sepsis is one of the most common reasons for hospitalisation. This condition is characterised by systemic inflammatory response to infection. International definition of sepsis mainly points out a multi-organ dysfunction caused by a deregulated host response to infection. An uncontrolled inflammatory response, often referred to as "cytokine storm", leads to an increase in oxidative stress as a result of the inhibition of cellular antioxidant systems. Oxidative stress, as well as pro-inflammatory cytokines, initiate vascular endothelial dysfunction and, in consequence, impair microcirculation. Microcirculation damage leads to adaptive modifications of cell metabolism. Moreover, mitochondrial dysfunction takes place which results in increased apoptosis and impaired autophagy. Non-coding RNA, especially miRNA and lncRNA molecules, may play an important role in the pathomechanism of sepsis. Altered expression of various ncRNAs in sepsis suggest, that these molecules can be used not only as diagnostics and prognostic markers but also as the target points in the pharmacotherapy of sepsis. The understanding of detailed molecular mechanisms leading to organ damage can contribute to the development of specific therapy methods thereby improving the prognosis of patients with sepsis.

Keywords: sepsis, cytokine storm, microcirculation, oxidative stress, RAS, ncRNA

1. Introduction

Sepsis was already known to ancient Greeks since the times of Hippocrates (460–370 BC) as a condition causing "rotting" of the body. Later, the Persian philosopher and physician Ibn Sina, or Avicenna (980–1037 AD) described for the first time sepsis as a "decay" of blood and tissues accompanied by fever. Sepsis is one of the most frequent causes of hospitalisation in Intensive Care Units. It constitutes a significant clinical problem, in many cases with a fatal outcome. Significant advances in critical care medicine have defined sepsis as an organ dysfunction syndrome. The incidence of this type of disorder is still constantly rising. Morbidity significantly correlates with the ageing of societies and presence of other comorbidities. The prognosis is worse when the mentioned correlations are strong and the patient is diagnosed too late. Although sepsis can occur in any age group, its risk is definitely greater in patients over 60 years of age. Moreover, sepsis is growing rapidly as a result of a poorly controlled, multidirectional response of the organism to an external factor, i.e. pathogen, and its further increase may be due to endogenous factors or coexisting diseases [1].

Sepsis starts with a period of hyperinflammation, in which the macrophages, monocytes, T-cells and neutrophils are activated and recruited to various organs.

The uncontrolled inflammatory response, so-called cytokine storm leads to many metabolic disorders associated with oxidative stress. The oxidative stress, but also proinflammatory cytokines, promote endothelial function disorders and, consequently, microcirculatory damages. At the final stage of cytokine storm a hypoinflammatory reaction develops, leading to multiple organ damages. These changes are underlined by mitochondrial function disorders, intensified oxidative stress and deregulation of apoptosis and autophagy processes. A neurohormonal activation, in the first place of the renin-angiotensin system (RAS), is an important element of the mechanisms leading to organ damage. Many studies have suggested that in sepsis, changes occur of the expression of various RNA molecules: long non-coding RNA (lncRNA) and microRNA (miRNA). The non-coding RNA fragments can thus play the role of molecular markers, both diagnostic, and prognostic, in the development of sepsis. The knowledge of the molecular mechanisms responsible for organ damages would enable a development of adequate and effective therapeutic methods, improving the prognosis for patients.

2. Cytokine storm in the course of sepsis

Cytokine storm is frequently described as an extremely dangerous immune response, being a positive feedback loop between cytokines and immune system cells [2]. That immune reaction provokes releasing of circulating proinflammatory cytokines, causing a direct threat to life. At the same time an increased activation occurs of the immune system cells as a result of, among other factors, action of various pathogens, autoimmune disorders, monogenic diseases or malignancies.

Proinflammatory cytokines, at the time of the beginning of the fight against pathogens by the immune system, send a signal to the lymphocytes and macrophages to start their transfer to the inflammation site. Cytokines activate also the cells to produce effector cytokines. All that cascade of interrelations frequently falls outside control, leading to hyperactivation of immune cells at a given site, damaging the organ involved in the inflammation. Remote organs also suffer damages but in the mechanism of cytokine release into the circulation, activating other immune system cells. Moreover, according to the literature data, it is believed that some inflammation mediators can be helpful for the monitoring of the inflammatory condition but also can be harmful for patient's organism [2]. That depends on the patient's health condition when the inflammation mediators are secreted into the bloodstream - whether the patient is in a physiological good condition or in a dysfunction state i.e. with upset immune system.

The course of cytokine storm itself can prognosticate the events being a consequence of the inflammatory condition but also suggests possible effects after administration of an appropriate treatment. Cytokine storm includes a number of immune system disorders with characteristic systemic signs of inflammation and multiple organ dysfunction, leading to damage in case of inadequate therapy. A plethora of factors are directly or indirectly involved in cytokine storm. The key factors include: interferon γ, interleukin-1, −6, −18, TNF-α and NFκB, but the transcription factor NFκB emerges to the foreground in view of its ability to induce expression of proinflammatory genes. NFκB and its protein inhibitor (IκB) are located in the cell cytoplasm. NFκB activation can occur due to the effect of many stimulators, such as: bacterial pathogens recognised by Toll-like receptor 4 (TLR-4) or proinflammatory cytokines recognised by their specific membrane receptors (e.g. TNF receptor) [3].

The inhibitor protein closely related to NFκB, being a heterodimer built from two subunits: p50 and p65, undergoes phosphorylation. The phosphorylation

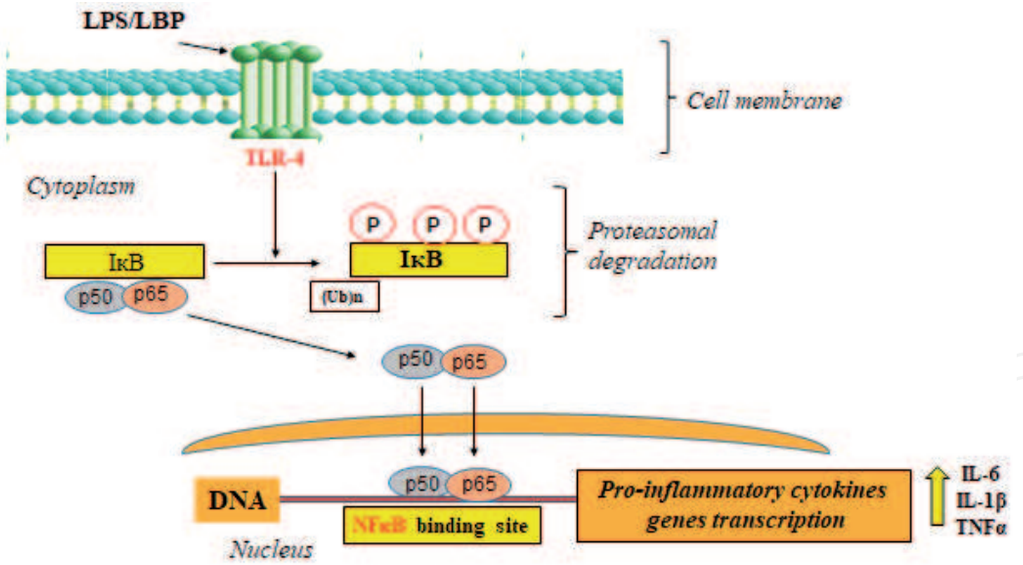

Figure 1.
NFκB activation pathway.

process is preceded by TLR-4 receptor activation by a lipopolysaccharide (LPS) complex with binding protein (LPS/LBP), leading to activation of redox-dependent kinases. Then, after ubiquitination, a degradation of the inhibitor occurs in proteasomes, as shown in **Figure 1**. At the same time, the p50:p65 dimer is released from its bond with IκB and transferred to the cell nucleus, where it regulates the expression of the genes of the proinflammatory molecules: TNFα, IL-1β, IL-6, MCP-1 (monocytic chemoattractant protein 1). The NFκB factor can also activate the expression of inducible nitric oxide synthase (iNOS) and cyclo-oxygenase-2 (COX-2). Such intensity of NFκB participation in immune system response, as well as in inflammatory reaction gives a possibility to consider that factor a candidate in the strategy of therapeutic management of multiple organ damage in sepsis [4].

3. Microcirculation and endothelial dysfunction in sepsis

Vascular endothelial cells constitute a dynamic vascular homeostasis regulator. They play not only the role of a barrier limiting the transportation of water, gases, proteins and cells between the intravascular and interstitial compartments, but also actively produce and release mediators, which regulate a wide range of physiological and pathological processes, including: vascular wall tension, angiogenesis, inflammatory condition and coagulation process. The activation and dysfunction of the endothelium are important elements of sepsis, and seem to play the key role in the sepsis phenotype as presented in **Figure 2**. During sepsis, endothelial cells become activated and dysfunctional, leading to haemostasis disturbances, increased migration of leucocytes, enhanced inflammatory condition, altered vascular wall tension and loss of the barrier function [5, 6]. The disturbed endothelial cell function can play a decisive role in microcirculation dysfunction. Microcirculatory changes in sepsis are characterised by heterogeneous perfusion of tissues due to absent or intermittent perfusion of the capillary vessels. Microcirculation heterogeneity in sepsis can disturb tissue oxygenation and lead to insufficient oxygen supply even in the presence of maintained total blood flow to organs. Such microcirculation disorders create favourable conditions for perfusion impairment, insufficient oxygen supply to tissues and then for organ failure.

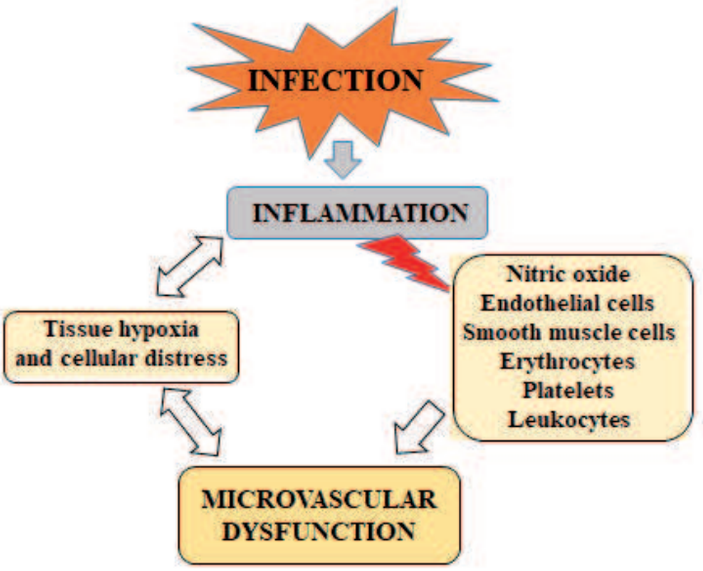

Figure 2.
Pathophysiology of microcirculatory change in sepsis.

Endothelial cells, receiving metabolic and physical signals regulate the microcirculatory flow through local releasing of vasodilating substances, particularly nitric oxide (NO), which modulate the tonus of vascular smooth muscles. NO is an activator of soluble guanylyl cyclase, the enzyme responsible for production of cGMP, a mediator of smooth muscle cell relaxation. For that reason, NO is considered the key factor of maintaining and autoregulation of homeostasis and microcirculation patency. During sepsis, the NO system is significantly disturbed – iNOS is non-homogeneously expressed in various vascular spaces, what results in pathological blood flow in the microcirculation.

Endothelial cells usually promote antithrombotic properties and prevention of thrombocyte activation and aggregation. Endothelium also participates in the major pathogenetic pathways of diseases associated with a coagulopathy in sepsis, including, in the first place, in tissue factor (TF)-mediated generation of thrombin, and in dysfunctional and impaired fibrinolysis. A natural antithrombotic protein – protein C is activated on endothelial cell surface, while thrombin binds to thrombomodulin (TM) – a transmembrane glycoprotein. In sepsis, the C protein system is weakened, possibly due to reduced synthesis and increased protein C consumption and reduced protein C activation as a consequence of reduced endothelial expression of thrombomodulin. In sepsis, an internalisation and degradation of TM occurs, leading to formation of inactive soluble fragments as illustrated in **Figure 3**. Under these

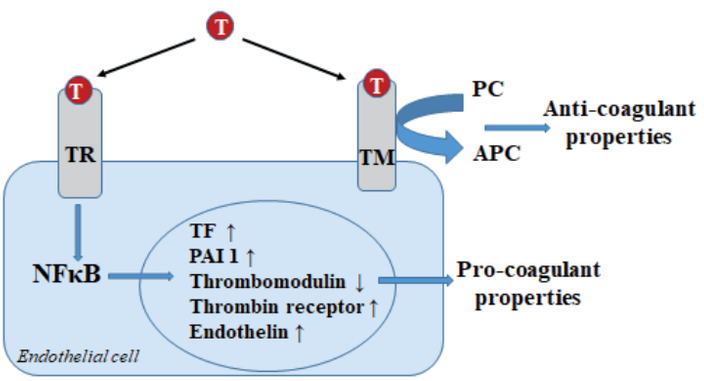

Figure 3.
Change of endothelial cell properties after thrombin receptor stimulation.

conditions the interaction between thrombin and thrombin receptor leads to a change of endothelial phenotype from antithrombotic to a prothrombotic phenotype.

In sepsis, the tissue factor can be released not only by monocytes and macrophages but also by endothelial cells. The TF pathway inhibitor, mainly expressed by endothelial cells, is functionally inhibited by reduced synthesis of glycosaminoglycans on endothelial surfaces. Furthermore, platelets, the aggregation of which leads to the development of thrombocytic thrombi, are a strong amplifier of the coagulation cascade. Thrombus formation can be additionally facilitated by the factors released from neutrophils undergoing apoptosis. The formation of microvascular thrombi can cause tissue ischaemia and multiple organ failure.

4. Renin-angiotensin system in sepsis

The renin-angiotensin system (RAS) is one of the most important hormonal mechanisms controlling haemodynamic stability through regulation of blood pressure, fluid volume and sodium-potassium balance as shown in **Figure 4**. Changes in the concentrations of molecules which form RAS contribute to arterial hypertension development. Renin is synthesised in the kidneys as inactive form and released into the bloodstream, where pro-renin is proteolytically transformed into its active form. Active renin catalyses angiotensinogen breakdown, generating angiotensin I (Ang I). Ang I is decomposed through angiotensin-converting enzyme (ACE) to angiotensin II (Ang II), the main effector in RAS (**Figure 4**). Ang I is also transformed through neutral endopeptidase (NEP) into angiotensin (1–7), another active peptide, which remains in opposition to Ang II (**Figure 4**). Angiotensin (1–7) can be also produced by Ang II splitting by angiotensin-converting enzyme 2 (ACE2), reducing thus Ang II concentration [7].

The mechanisms of RAS effect on sepsis development is presented in **Figure 5**. RAS participates in the pathogenesis of sepsis through equilibration of the modulation of the inflammation-related pathways. Through binding to AT1R, Ang II can increase the abundance of inflammatory mediators, increase vascular permeability and stimulate the expression of chemoattractants and adhesive molecules and also lead to recruitment of inflammatory cells. Moreover, the activation of the ERK 1/2, JNK, p38MAPK and NF-κB pathways is also involved in the intensification of inflammatory reaction by Ang II.

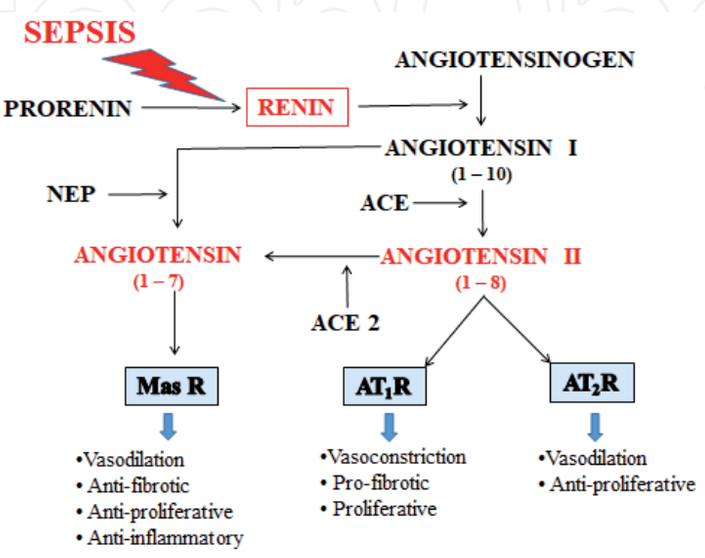

Figure 4.
Renin-angiotensin system.

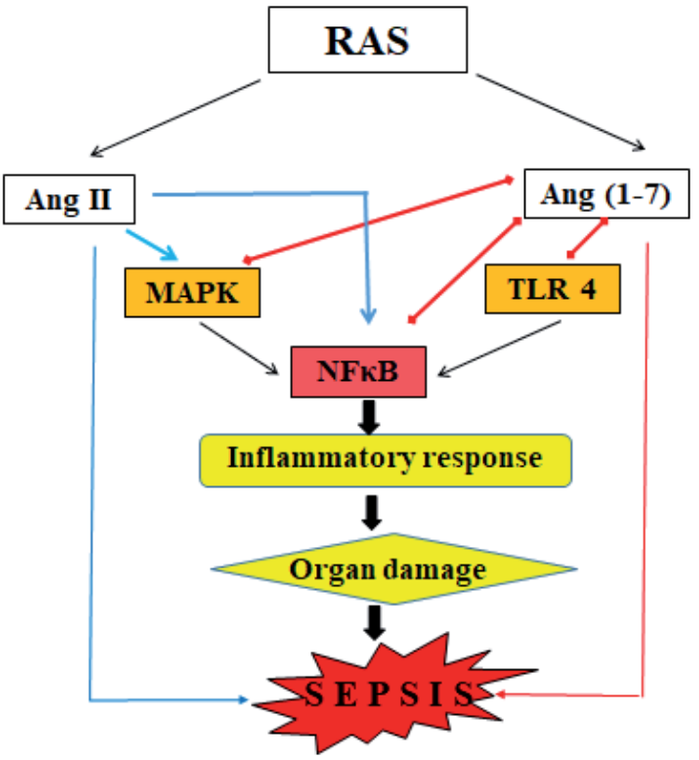

Figure 5.
The mechanisms of RAS effect on sepsis development, blue line - promote; red line - inhibit.

Ang (1–7) can inhibit the activity of these signalling pathways and thus can inhibit the inflammatory reaction in sepsis. Many studies have provided evidence that RAS interventions can alleviate sepsis and associated organ function disorders through inhibition of the above mentioned inflammation-related pathways. ACE2 exerts a protective effect against acute respiratory distress syndrome (ARDS) caused by sepsis, through inhibition of TLR4, ERK1/2, JNK and NF-κB pathways. Ang (1–7) inhibited the p38MAPK pathway in order to protect mice against sepsis-induced skeletal muscle atrophy and liver damage. AT1R blockade can exert a protective effect against sepsis-induced multiple organ damage (SIMD) through inhibition of the MAPK and NF-κB pathways. Moreover, angiotensin I-converting enzyme inhibitors (ACEIs) and sartans (angiotensin receptor blockers, ARBs) decrease the release of proinflammatory cytokines, pro-oxidants and proapoptotic factors and thus alleviate the damages caused by sepsis.

5. Oxidative stress and mitochondria in sepsis

5.1 Generation of reactive oxygen species (ROS)

The internal mitochondrial membrane is a vast, impermeable structure containing enzymatic complexes of the respiratory chain (I – IV) and ATP synthase system (complex V). The transportation of electrons through respiratory chain complexes is accompanied by translocation of protons (H^+) to the intermembrane space and development of potential difference on either side of the membrane.

That process includes development of reactive oxygen species (ROS) as a consequence of electron "escape" and mono-electron reduction of oxygen to 0_2**.** radical. That reaction occurs in the I and III complexes of the respiratory chain as presented in **Figure 6**. The developing oxygen free radicals are transferred both

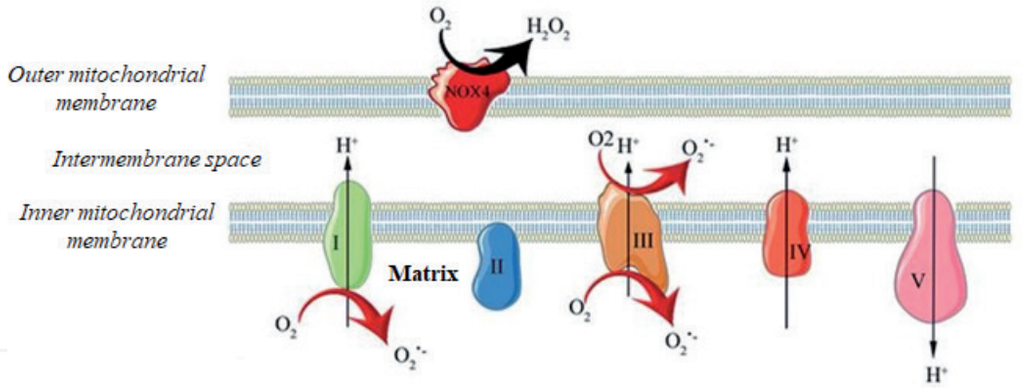

Figure 6.
ROS production in mitochondria.

to the mitochondrial matrix and intermembrane space. The outer mitochondrial membrane is the site of location of NADPH, which generates hydrogen peroxide (H_2O_2). The ROS produced in the mitochondria cause damages of the mitochondrial proteins and mitochondrial DNA (mtDNA). These damages lead to the development of a mega-channel, enabling outflow of cytochrome C into the cytosol, with consequent initiation of apoptosis process. An increased permeability of the inner mitochondrial membrane creates also a possibility of transportation of many small molecules. Mitochondrial ROS affects also many processes both under normal and pathological conditions, what as shown in **Figure 7** can modulate vital cell functions.

5.2 Generation of reactive nitrogen species (RNS)

In sepsis, besides ROS production in the mitochondria, increased synthesis of nitric oxide (NO) is also an important element of oxidative stress. NO is produced by various cells, such as: activated macrophages, neutrophils or lymphocytes. Many molecules involved in septic inflammatory process, such as: tumour necrosis factor-α (TNFα) interferon γ (IFNγ) or interleukin-1β (IL-1β) also participate in the induction of type II NO synthase (iNOS) through activation of IκB degradation and transcription of *iNOS* gene.

An adequate nitric oxide production is a prerequisite of normal vascular structure and function. Muscle cells and haematopoietic cells are an important source of NO. Numerous inflammatory mediators participate in the induction and activation of the isoform of calcium-independent nitric oxygen synthase (iNOS). In order to prevent NO overproduction during sepsis, an administration of NOS inhibitors was suggested. NOS inhibition, however, has a limited therapeutic efficacy in view of intensification of organ dysfunction, which results in a high mortality rate. This is most likely related to the double role of iNOS in sepsis [8].

In particular cases, besides NO production, iNOS catalyses also the formation of reactive nitrogen species (RNS). NO reacts with superoxide anion to form peroxynitrite anion ($ONOO^-$), which oxidises and nitrosylates various biological targets. Peroxynitrite can be a potential mediator of the cytotoxic effect of NO.

During sepsis, iNOS can become an important source of RNS as a consequence of enzyme decoupling. Decoupled iNOS is a source of superoxide anion, which is rapidly broken down by superoxide dismutase (SOD), and hydrogen peroxide (H_2O_2) is produced. Moreover, the availability of NO is reduced in view of its rapid reaction with the peroxide. During sepsis, three main factors can contribute to iNOS decoupling: suboptimal tetrahydrobiopterin (BH_4) concentration, insufficient

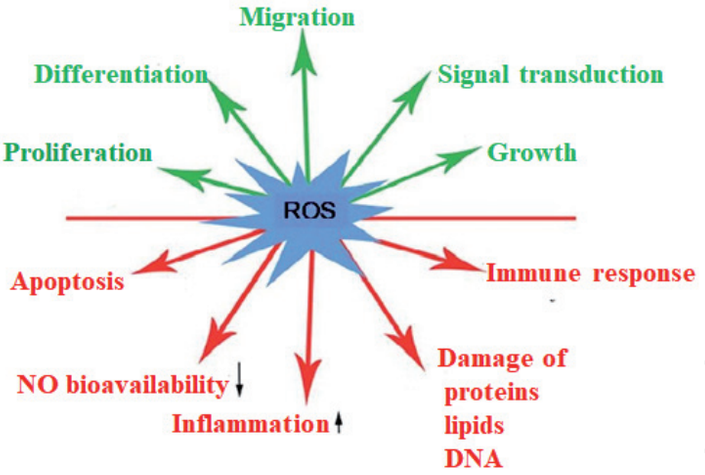

Figure 7.
The role of ROS in normal and pathological conditions.

concentration of L-arginine substrate and also increased production of asymmetric dimethylarginine (ADMA, endogenous NOS inhibitor) [8].

5.3 Cellular antioxidant systems

Cells have developed protective mechanisms counteracting mitochondrial dysfunction. The most important of them include: a system of endogenous antioxidants, dynamic changes of mitochondria and also processes of removal of damaged organelles and biogenesis of new ones. To counteract oxidative damage, mitochondria contain high concentrations of antioxidants, i.e. substances, which, when present in low concentrations, reduce the level and/or protect the substrates against their oxidative modification. ROS uptake is the function of the antioxidants. Two types of antioxidants can be distinguished: enzymatic and non-enzymatic. The important antioxidant enzymes include superoxide dismutase (SOD), catalase and glutathione peroxidise (GPx). SOD catalyses conversion of $O_2^{\cdot}$ to H_2O_2 and oxygen. In mammalian cells two forms of SOD are present: CuZnSOD (SOD 1) and MnSOD (SOD 2). SOD 1 requires presence of copper and zinc as cofactors and is mainly present in cytosol, while SOD 2 is manganese-dependent and is mainly found in mitochondria. The hydrogen peroxide produced as a result of a reaction catalysed by SOD is then detoxicated by catalase or GPx. In the reaction catalysed by catalase, water and oxygen are produced. GPx converts H_2O_2 into water in a reaction, in which glutathione (GSH) is oxidised to glutathione disulfide (GSSG) and then reduced to GSH by glutathione reductase (GR). Both GPx and GR require the presence of selenium to reach their full activity.

The important non-enzymatic antioxidants include vitamins, enzymatic cofactors and many endogenous substances. The antioxidant vitamins include vitamin A, C and E, which mainly act as compounds scavenging free radicals. As mentioned above, microelements such as manganese, copper, zinc, and selenium are important elements of the antioxidant systems. Endogenous antioxidant substances include in the first place bilirubin albumin, ferritin and melatonin.

5.4 Mitochondrial dynamics

Mitochondria are dynamic organelles undergoing constant regular cycles of fusion and breakdown. The fusion of damaged mitochondria and then asymmetric breakdown are the mechanism leading to regaining of the functionality of the basic

components. The damaged elements present in the organelles can be grouped, as a result of fusion, in one mitochondrium. Asymmetric breakdown leads to creation of functionally efficient organelles and of mitochondria, in which all damages are accumulated. These dysfunctional mitochondria are eliminated by autophagy [9].

Mitochondrial fusion and breakdown are strictly balanced processes. Both an uncontrolled fusion and breakdown can constitute an extreme threat to the cell function and lead to cell death. At present, the data on mitochondrial fusion and breakdown in critical conditions are scarce. An increased level of markers presenting the mitochondrial dynamics was found in *post mortem* liver biopsies but not *in vivo* in critically ill patients. In an animal model, in rabbits, which had a significant dysfunction of the hepatic and renal mitochondria, the level of mitochondrial fusion protein of the inner membrane (optic atrophy protein-1) was only significantly increased in the liver. A reduction was also observed of the concentration of mitofusin-2, involved in the fusion of the outer mitochondrial membrane. The breakdown markers remained unchanged in both organs. The results of those studies may suggest that the ability of the mitochondria to fuse and break down differs between the organs.

The elimination of dysfunctional mitochondria requires replenishing of their population through biogenesis. Mitochondrial biogenesis depends on nuclear and mitochondrial transcription systems. Peroxisome proliferator-activated receptor gamma-activator 1 alpha (PGC-1α) has been identified as the key element of the biogenesis process. It activates the nuclear respiratory factors 1 and 2 (NRF1, NRF2), which induce important transcription factors, such as mitochondrial transcription factor A (TFAM) and nuclear-encoded mitochondrial proteins – subunits of respiratory chain complexes. That complex transcription programme causes mtDNA replication and synthesis of new proteins indispensable for development of new mitochondria. That programme is extremely metabolically expensive, since it requires a huge energy expenditure.

In animal sepsis models increased levels were observed of hepatic markers of mitochondrial biogenesis: PGC-1α, NRF1 and TRAM, which was associated with regaining of metabolic activity and improvement of the clinical condition. No differences were found in the levels of hepatic and renal mitochondrial biogenesis markers in rabbits, which were in critical condition but survived, compared to the animals, which failed to survive the experiment. In patients surviving a sepsis, no changes in mitochondrial protein synthesis were observed *in vivo*, in spite of an increase of the mRNA level of mitochondrial transcription factors.

6. Apoptosis in sepsis

Apoptosis plays a significant role in the pathophysiology of sepsis. The role of a potential factor involved in immunosuppression and mortality in sepsis has been ascribed to lymphocyte apoptosis. An increased apoptosis of T and B cells was observed in patients dying of sepsis. The results of clinical studies have confirmed the observations, which demonstrated a significant increase of lymphocyte apoptosis in the model of cecal ligation and puncture (CLP)-induced sepsis in mice. Although death of adaptative immune system cells, limiting thus the inflammatory reaction, may be beneficial for the body, but the presence of intense apoptosis of immune cells leads to a reduced possibility of defence during invasion of pathogens. It seems that lymphocyte apoptosis, leading to immune response impairment, can predispose to septic death. That suggestion has been confirmed by the results of studies in transgenic mice with increased expression of antiapoptotic Bcl-2 protein. In the model of CLP-induced sepsis a protective effect of Bcl-2 on T-cells has

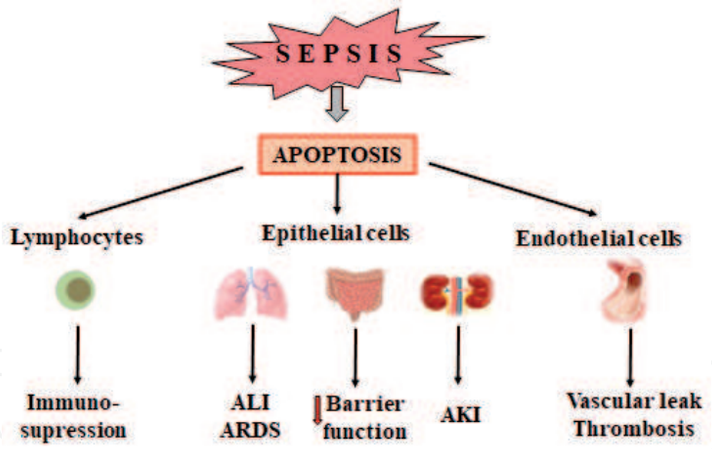

Figure 8.
Apoptosis of various cells in sepsis and its consequences for organ function.

been demonstrated, which increased the survival. That suggests that apoptosis of immune cells plays a significant role in the development of sepsis and is of decisive importance for its possible unfavourable course. The role of a apoptosis of various cells in sepsis and its consequences for organ function is presented in **Figure 8**.

In experimental sepsis models, also an intense apoptosis has been observed of interstitial cells, such as intestinal and pulmonary epithelial cells. A defect of intestinal epithelial cells can lead to a significant impairment of their barrier function and to facilitation of bacterial translocation into blood and/or lymphatic system. That results in an increased antigen presentation and massive immune response, which have a direct effect on the survival.

Apoptosis of pulmonary epithelial cells leads to the development of acute lung injury (ALI). That pathology is directly caused not only by inflammation or trauma but also by haemorrhagic shock and multibacterial sepsis. The silencing of Fas receptors on the membranes of pulmonary epithelial cells in mice with haemorrhagic shock, leads to ALI reduction through blocking of their apoptosis and, thus, inhibition of histological remodelling of the lungs.

7. mTOR and autophagy in sepsis

The mTOR pathway plays an important role in promoting sepsis progression. TLR4 activation through LPS binding increases mTOR activity. In a clinical study in 106 patients with sepsis an activation of HIF-1α and mTOR genes was observed in peripheral blood leucocytes. An injection of LPS to mice caused an activation of mTOR signalling in macrophages and led to an increase of inflammatory renal injury followed by fibrosis. An activation of mTOR pathway by LPS in rats resulted in a blood pressure reduction and heart rate acceleration and also in an increase of the level of inflammatory markers in the tissues of the kidneys, heart and blood vessels. In both mentioned animal models, an administration of rapamycin and mTOR inhibitor significantly reversed the harmful effects of LPS. The inhibition of mTOR in sepsis can be partly explained by activation of the autophagy process.

Autophagy is a degradation system, preserved in an evolutionary way in the cells, and it participates in maintaining of intracellular homeostasis. The process of autophagy includes formation of autophagosomes, fusion of autophagosome-lysosomes and development of degradation products. In sepsis, autophagy is a recognised protective adaptative mechanism limiting cell injury and apoptosis [10, 11]. Autophagy not only eliminates damaged protein aggregates and organelles, but also

eliminates bacteria and pathogens present in the cytoplasm. Some special bacteria, such as *Staphylococcus aureus,* can avoid selective autophagy through activation of cell kinases of the host.

The main role of autophagy in sepsis illustrates in **Figure 9**. Autophagy promotes pathogen elimination directly by phagocytosis, regulation of antigen presentation, activation of immune cells and secretion of type I interferon. It inhibits the immune response through degradation of inflammasomes. It prevents immunosuppression through inhibition of apoptosis and elimination of damaged mitochondria. It affects the metabolism through regulation of mitochondrial functions and participation of AMPK and mTOR in the regulation of autophagy activity. That effect is, however, limited. In the case of severe sepsis, even a significant increase of autophagy fails to reverse the overwhelming inflammatory reaction. The body of evidence gathered, suggest that autophagy dynamically changes to become insufficient and non-adaptative at later stages of sepsis. Dynamic changes of autophagy during sepsis was shown in **Figure 10**. That deficit is associated with mTOR signalling regulation, while ineffectiveness of elimination of dysfunctional organelles and toxic intracellular material causes an overwhelming accumulation of danger-associated molecular patterns (DAMPs).

Both autophagy and mTOR pathway are promoted at the initial stages of sepsis. At later sepsis stages a long-lasting drop of autophagy is observed, contributing to organ dysfunction and reduced lymphocyte count, what is important for inflammatory dysregulation, apoptosis and mitochondrial disorders. Two separate animal models demonstrated that autophagy regulation through rapamycin administration reversed the heart damage observed during sepsis. These studies have shown that mTOR is the main inhibitor of autophagy.

Autophagy has been proposed as a potential therapeutic goal, particularly during evident immunosuppression and then in the phase of sepsis, as a way of immune homeostasis restoration. It seems that autophagy promotion can be a novel

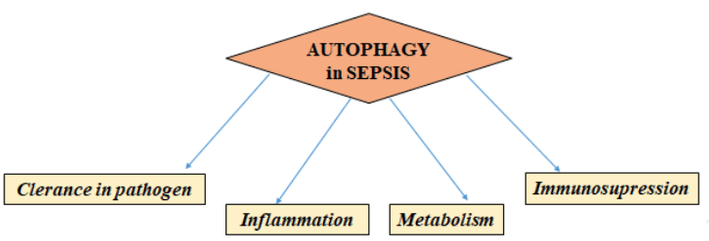

Figure 9.
The main role of autophagy in sepsis.

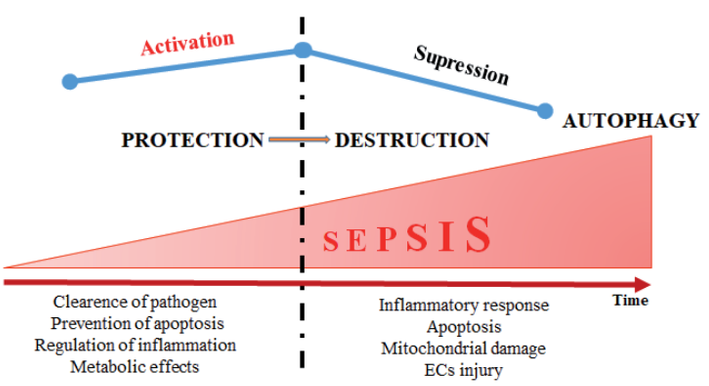

Figure 10.
Dynamic changes of autophagy during sepsis.

and effective intervention in order to reduce organ dysfunction caused by insufficient and non-adaptative autophagy during severe sepsis.

8. Non-coding RNAs in sepsis

Non-coding RNAs are responsible for the regulation of many cell signalling pathways. The molecules include long non-coding RNA (lncRNA) and microRNA (miRNA), participating in many biological processes, such as apoptosis, mitochondrial dysfunction and innate immunity.

All cells synthesise RNA molecules of 21 to 25 nucleotide length, called miRNA. The molecules of miRNA bind to complementary sequences at 3′ ends of target mRNAs and are able to post-transcriptionally regulate the expression of many genes. They can, therefore, exert protective or harmful effects in various immune system-related disorders and affect the levels of proinflammatory cytokines: TNFα and IL-1β through signalling pathways including p38 mitogen-activated protein kinase (MAPK) and MAPK 1 phosphatase (MKP-1). Many studies have demonstrated significant differences in the expression of some miRNAs in septic patients. It was suggested, therefore, that miRNAs may serve as biomarkers in the diagnostic process or risk stratification, and even can be a therapeutic target in the treatment of sepsis.

The lncRNA family includes molecules of protein-non-coding RNAs containing about 200 nucleotides and consists of over 60,000 individually catalogued members. lncRNAs have many functions and activities such as: regulation of gene expression, changing chromatin arrangement, modification of histones and alternative gene splicing, which suggest their possible role in the pathogenesis of various diseases and disorders.

8.1 MicroRNA

MicroRNA molecules play an important role in the regulation of adaptative and innate immune responses in the pathogenesis of sepsis as presented in **Table 1**. The molecules affect the development of regulatory T-cells and also T-helper cells, which are indispensable for immune response optimisation. During sepsis the immune system of the host seems to be both in the state of immunosuppression, but also, at the same time can be in a proinflammatory state [12].

In proinflammatory state, many cytokines, including TNFα, are overexpressed. miRNAs can control TNFα production both at translation and transcription levels. miR-181 has been shown to regulate TNFα synthesis through intensification of TNFα mRNA degradation. It has been also observed that a significantly reduced miR-125b expression was accompanied by a higher TNFα expression in the monocytes of newborns after LPS stimulation. Besides that, miRNAs can also regulate the TNF pathway and mediate inflammatory reactions. It has been suggested that miR-511 is a regulator of TNF receptor protein synthesis and, thus, it affects the sensitivity of cells to TNF. An effect of miR-511 is therefore possible, protecting against TNF-dependent endotoxic shock syndrome. It has been also demonstrated that the levels of other proinflammatory cytokines, such as IL-6, are significantly increased in septic patients. Experimental studies have shown that miR-146a expression is correlated with an increased IL-6 concentration in septic patients. Many studies have demonstrated that miRNA molecules are able to regulate inflammatory reactions through their effect on the Toll-like receptor 4 (TLR4) signalling pathway. The TLR4-induced signalling activates in the first place NFκB, the key transcription factor modulating the expression of proinflammatory and

miRNAs	Expression in Sepsis	Observed effects of miRNAs
miR-150	down	miR-150 levels in both leukocytes and plasma correlated with the severity of sepsis and could be used as a marker of early sepsis. Plasma ratio of levels of miR-150/IL-18 could be used for assessing the severity of sepsis
miR-31	down	miR-31 down regulation in CD4+ T cells contributes to immunosuppression in sepsis patients via promoting TH2 skewing humans T cells
miR-27a	down	miR-34a, miR-15a, and miR-27a are correlated with shock development in severe sepsis patients; they also target cell cycle regulation, apoptosis, cell layer permeability, and inflammatory pathways humans plasma
miR-25	down	A correlation between levels of miR-25 and the severity of sepsis was observed; surviving patients had higher levels of this biomarker compared with non-surviving subjects; decreased levels of miR-25 were associated with the concentrations of oxidative stress indicators in sepsis
miR-15a	up	Upregulated miR-15a down regulated the LPS induced inflammatory pathway
miR-16	up	Upregulated miR-16 down regulated the LPS induced inflammatory pathway
miR-574-5p	up	Serum level was correlated with the death of sepsis patients; the combined analysis of miR-574-5p, SOFA humans serum scores, and the sepsis stage on the day of diagnosis provided a good predictor for sepsis prognosis
miR-297	up	serum miR-297 level was higher in survivors than non-survivors among septic patients
miR-143	up	serum miR-143 levels were significantly higher in sepsis than in SIRS and healthy controls

Table 1.
Selected miRNAs involved in human sepsis.

immunoregulatory factors. Some miRNA molecules, such as miR-155, miR-125 and miR-146a play the main role in the negative modulation of the TLR4/NFκB inflammatory cascade, but also in innate immunity. The expression of miR-155 and also many other miRNA molecules depends on NFκB, since it has been shown that LPS stimulation of THP-1 monocytes induces the expression of miR-146a and miR146b. The miR146a molecule directly regulates the expression of TNF receptor-associated factor 6 (TRAF6) and IL-1 receptor-associated kinase 1 (IRAK1), which are important adaptor molecules in the TLR4 signalling pathway. miR-146a plays also a significant role in the *in vitro* tolerance of monocytes to endotoxins. That effect can be, however, reversed by miR-146a suppression. It has been recently reported that the NFκB/DICER signalling pathway inhibits TNFα synthesis through production of mature forms of miR-130 and miR-125b, which regulate TNFα mRNA. The presented miRNA effects seem particularly important in the aspect of the critical role of TLR4-induced pathway in sepsis.

8.2 lncRNAs and sepsis

The molecules of lncRNA play an important role in biological processes and their dysregulation is associated with various disorders [13]. The experimental studies presented in **Table 2**, were conducted in order to assess the correlation between sepsis and expression of lncRNA molecules. It has been demonstrated that lncRNA expression is changed in human monocytes, cardiomyocytes and renal canalicular epithelial cells during development of sepsis or after exposure to LPS.

lncRNAs	Expression in Sepsis	Observed effects of lncRNAs
NEAT1	up	Circulating lncRNA NEAT1 was related to severity, increased risk, and unfavourable prognosis in sepsis patients
ANRIL/miR-125a axis	up	lnc-ANRIL/miR-125a axis could serve as a biomarker for prognosis, severity, and inflammation in sepsis patients
ITSN1-2	up	High expression of ITSN1-2 is associated with disease severity and inflammation in sepsis patients.
TUG1	down	Decreased TUG1 expression may induce sepsis related AKI by modulating the NF-kB pathway and regulating the miR-142-3p/SIRT1 axis (*humans in vitro*)
MALAT1	down	IL-6 induced upregulation of MALAT1 in LPS treated cardiomyocytes, and MALAT1 could promote the expression of TNF-a at least partly bySAA3 in response to LPS treatment in cardiomyocytes (*mice in vitro*)
HULC	down	Upregulation of lncRNA HULC is required for the pro-inflammatory response during LPS induced sepsis (*mice in vitro*)

Table 2.
Selected lncRNAs involved in sepsis.

In spite of many studies on that topic, sepsis still remains a complex clinical condition, the pathophysiology of which has not been fully elucidated. Non-coding RNAs offer a chance for early diagnosis and monitoring of patients in Intensive Care Units.

9. Conclusions and perspectives

Uncontrolled inflammation, immune disorders, oxidative stress, apoptosis, mitochondrial damage and also endothelial function disorders play the key role in sepsis and organ dysfunction associated with it. Sepsis releases a series of signalling cascades, starting with recognition of the whole spectrum of pathogen-associated molecular patterns (PAMPs) and organ damage-associated molecular patterns (DAMPs). Each stage of the signalling pathways participating in the release of inflammatory mediators is extremely important for the course of the disease and can be a target point in the therapeutic strategy in septic patients. The general agreement present as yet, concerning the inflammatory response only mediated by the TLR4/NFκB pathway is not so obvious any longer. Administration of anti-inflammatory drugs, including corticosteroids, or antagonists of TNFα and IL-1, failed to produce the expected effects in septic patients.

The target points in the treatment of sepsis can also be the molecules of non-coding RNA (ncRNA). Recent studies have suggested, among other findings, that the miRNA-23b molecule prevents the development of myocardial dysfunction in late sepsis. It seems therefore, that research in the field of development of drugs targeted at ncRNA molecules can be the future of antiseptic pharmacotherapy.

The new SARS-CoV-2 coronaviral disease has aroused interest in viral sepsis. Although bacteria are the predominant pathogens in sepsis, a viral sepsis cannot be disregarded. According to the literature reports, the percentage of septic patients with negative results of blood cultures for bacterial pathogens reaches 42% [14, 15]. In COVID-19 patients a septic shock and multiple organ dysfunction may develop. In view of absence of specific drugs, the current therapeutic strategies include isolation of patients, controlling of infections and maintaining normal organ

functioning. The SARS-CoV-2 infection depends on the host's cell factors. Recent studies have demonstrated that COVID-19 virus uses the angiotensin 2-converting enzyme (ACE2) and serine protease TMPRSS2 to penetrate into host's cells. That suggests a possibility of administration of protease inhibitors as effective drugs blocking the transmission of the virus [16]. The studies on viral sepsis caused by COVID-19 could help to indicate also other target points for drugs, which would reduce the damages of the lungs and other organs.

Conflict of interest

The authors declare no conflict of interest.

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