

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

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

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

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Interaction of Host-Microbial Metabolism in Sepsis

Beloborodova Natalia Vladimirovna

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/68046>

Abstract

The majority of species of the human gut microbiota is not cultivated on artificial nutrient media, but they are included in the functioning of microbial metabolic conveyor. Between the numerous gut bacteria (transmitter) and billions of intracellular mitochondria (receiver), the function of signaling molecules performs aromatic metabolites. Sepsis destroys the coordinated work of the indigenous anaerobic microflora. This leads to the imbalance of aromatic microbial metabolites (AMM). We hypothesized and proved diagnostic and pathogenic significance of this. First, deficiency of the end products of microbial metabolism—lipophilic AMM (PhPA and derivatives-cinnamic and benzoic acids) in sepsis, and second, excessive accumulation in blood of intermediate products named, “sepsis-associated” AMM—both lead to the development of mitochondrial dysfunction. Particularly, the total suppressed production of mROS can manifest by “hibernate-like state” of cells and lead to MOF. The participation of aromatic metabolites in the development of septic shock can be explained by the inhibition of tyrosine hydroxylase and impaired synthesis of catecholamines. In clinical research, the high levels of “sepsis-associated” AMM (p-HPhAA, p-HPhLA, and PhLA) correlate with the severity according to APACHE II, Sepsis-related Organ Failure Assessments (SOFA) score and mortality. To improve the survival of ICU patients, requires more attention to the role of imbalance of microbial metabolites in sepsis.

Keywords: sepsis, microbiota, aromatic microbial metabolites, organ dysfunction, mitochondrial dysfunction, tyrosine, septic shock

1. Introduction

Currently, improvement of diagnostic and medical technologies in surgery and intensive care allows us to provide real treatment of patients, including previously considered incurable. However, the development of infectious complications, syndrome of multiple organ failure (MOF), and septic shock significantly worsens the results. Every year the problem of

increasing sepsis-associated mortality becomes more acute in different categories of high-risk patients (e.g., premature infants and elderly, patients with cancer, diabetes mellitus, states after injuries, strokes, surgeries, transplants, etc.). Further progress in medicine is impossible without a revision of basic ideas about the important role of metabolic disturbances of the microbiota in the pathogenesis of sepsis. Nowadays, the question of the role of the microbiota comes to the forefront. Numerous research teams conduct in-depth study using the most contemporary technologies, including metagenomics sequencing, etc. Identifying the microbial DNA and RNA, scientists try to decipher hundreds of species of microorganisms living in the human gut. It is important to note that the majority of species of the intestinal microbiota of man is not cultivated on artificial nutrient media, but is included in the functioning of multistage microbial metabolic reactor [1]. More and more data appear on crosstalk between the organism and host microbial ecology, including epigenomic and metagenomic programming with the involvement of gut microbiota [2]. There are serious reasons to believe that in order to decipher mechanisms of sepsis, it is essential to evaluate the functional component of the microbial metabolism. Integration of the metabolism of the host and its normal microbiota provides the human health sepsis means a profound failure in the general metabolic pathways of man and microbiota, often irreversible that leads to unfavorable outcome.

The absence of a unique approach to an objective assessment of vital threat of damage in the main integration points of host-microbial metabolism makes difficult an early diagnosis, prognosis, and targeted treatment of sepsis. In this chapter, we summarize knowledge available from the literature and the results of our own experience of research, the pathogenetic significance of some microbial metabolites in sepsis—at the organismal, cellular, and subcellular levels.

2. Microbial metabolism in healthy and in sepsis

The symbiosis of the host and its microbiota exists due to the large number and variety of species of bacteria (aerobic, facultative anaerobic, and strict anaerobic bacteria). The microecological system is the boundary of the inner sterile environment from the external world (all of the mucous membranes and skin), but most richly represented in the gut microbiota.

A large number of different types of microorganisms perform the biochemical functions as a multilevel “conveyor”, which involved numerous members of the microbiota. The result depends on many factors. The quality and quantity of substrate (food components), the function of the stomach, pancreas, liver, gallbladder, and bowel motility, etc. definitely influence the metabolism of microbiota. At the same time, the diversity of species with different biochemical activity provides coordinated work of the microbiota. Therefore, in normal biotransformation of any of the substrates in the intestinal lumen takes place sequentially and ends with the formation of the final metabolites.

It is important to note that many microbial metabolites have biological activity and perform important functions in the host organism. They are necessary for the normal functioning of organs and systems. For example, fecal short-chain fatty acids (SCFAs) are important

energy substrate for enterocytes. They provide functioning of the local immunological barrier, preventing bacterial translocation. SCFAs are sent directly into the cells of the intestinal epithelium and “delayed” at the level of the mucosa, therefore, normally they are not detected in the blood of healthy people. Other products of microbial metabolism permanently enter the internal environment of the organism (blood and lymph); therefore, they are called extracellular microbial metabolites or exometabolites.

In the body of a healthy person have well-functioning mechanisms to maintain the homeostasis of microbial metabolites at a constant level [3]. If microbial exometabolites enter the blood in excess amounts, they are neutralized in the liver (for example, phenol is converted into cresol), form sulfates and conjugates with amino acids (glycine and glutamine) and glucuronic acid, etc., then in the form of water-soluble compounds excreted in the urine.

MS-based metabolomics studies (using LC- and GH-MS) on germ-free (gnotobiotic) mammals showed that many classes of low molecular weight compounds (free or conjugate) in blood are of microbial origin [4]. For example, many phenolic metabolites, such as phenyl sulfate, *p*-kresol sulfate, conjugate of phenyl propionic, and cinnamic acids (phenylpropionylglycine and cinnamoylglycine) observed only in normal (conventional) animals. Concentrations of other phenolic compounds were many times higher in the blood of normal animals compared to germ-free animals, *p*-value 10^{-8} to 10^{-9} , such as phenyl benzoic conjugate (hippuric) and phenyl acetic conjugate (phenylacetyl glycine).

The humans carry out their activities in close contact with the inhabiting microflora from the first day after birth to the last, i.e., throughout life. In ontogenesis, there created a system of human-microbiota, thanks to close and mutually beneficial metabolic integration [5]. The presence of integration of host-microbial metabolism is confirmed by the fact that

- the number of microbial cells inhabiting the human community is huge, according to some authors exceeds [6] or at least comparable [7] with the number of cells in the human body;
- high speed reproduction of microorganisms and cell renewal of the microbial community indicates active metabolic process in bacteria;
- qualitative and quantitative composition of biological community indicates the existence of mechanisms of regulation within the microbial community, the so-called “quorum sensing;”
- number of mechanisms of immunoreactivity aimed at maintaining a symbiotic relationship that indicates the existence of mutual regulation through a system of common metabolites and/or signaling molecules.

The results of our research allow us to assert that the number of products of microbial metabolism namely phenolic acids entering the bloodstream of critically ill patients in large amounts can block the respiratory chain of mitochondria, disrupting the functions of organs and tissues that are directly involved in the genesis of multiple organ failure (MOF).

These relationships change radically in patients with severe illnesses, reaching a maximum deviation from the norm in critical conditions, until the development of irreversible disorders

of homeostasis and death of the host organisms. Massive tissue damage of any origin is accompanied by hypoxia, shortage of energetically plastic materials, which reflects violations of the metabolic processes of microorganism and influences the metabolism of its microbiota. When the host organism is under extreme conditions (massive hemorrhage, hypoxia, hypothermia, hyperthermia, starvation, hypovolemia, poisoning, irradiation, etc.), that means the rapid changes in the environment of microorganisms inhabiting gut of host.

Sepsis is characterized by sudden depletion and simplification of the microbiota as follows:

- first of all, destroyed biofilms of microbial symbionts, namely indigenous anaerobes (*Bifidobacteria*, *Lactobacilli*, etc.) [8];
- facultative anaerobes (enterobacteria, staphylococci, etc.) form biofilms in the upper digestive tract (bacterial expansion in the small intestine) [9, 10];
- clinically it is manifested by the failure of intestinal barrier and bacterial translocation [11, 12];
- the blood circulating bacteria from gut “in search of” more favorable conditions, which are recorded as “bacteremia” [13];
- resistant to the ongoing antibiotic treatment, bacteria remains in the micro thrombus, exudate, and damaged epithelium/endothelium, forming local foci of infection [14].

3. Why aromatic microbial metabolites (AMM) are the most important in sepsis?

To study the metabolomics profile of biological fluids different technologies including the most gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (with two mass analyzers in one mass spec instrument) (LC-MS/MS), ¹H (H)- (proton nuclear magnetic resonance) spectroscopy (NMR), etc. are used. Global metabolic profiling—spanning hundreds of small molecules—holds the potential to reveal not only host and microbial metabolites, novel biomarkers, but also provides insight into disease pathogenesis.

In our experiments, we used GC-MS analysis of blood serum, as in sepsis in the blood enters the microbial exometabolites from the intestine and from foci of infection. Blood microbial metabolites spread throughout the body to the cells of all organs (**Figure 1**). Comparing qualitative and quantitative composition of low molecular metabolites in the blood of different groups of patients, we found that most of qualitative and quantitative significant differences between the healthy and the sick are observed among aromatic metabolites. In the blood of healthy and sick people, we have identified and measured tens of aromatic metabolites such as phenol, benzole, and their derivatives (p-cresol, benzyl alcohol, benzoic acid, 2,4-dihydroxybenzoic acid, 3,4-dihydroxybenzoic acid, and p-hydroxybenzoic acid), carboxylic acids (lactic, malic, fumaric, succinic, 2-ketoglutaric, 2-hydroxyglutaric, 2-hydroxybutyric, etc.), phenyl carboxylic acids (phenylacetic, p-hydroxyphenylacetic, 2-hydroxyphenylacetic, phenylpropionic, p-hydroxyphenyl propionic, cinnamic, p-hydroxyphenyl cinnamic, phenyllactic,

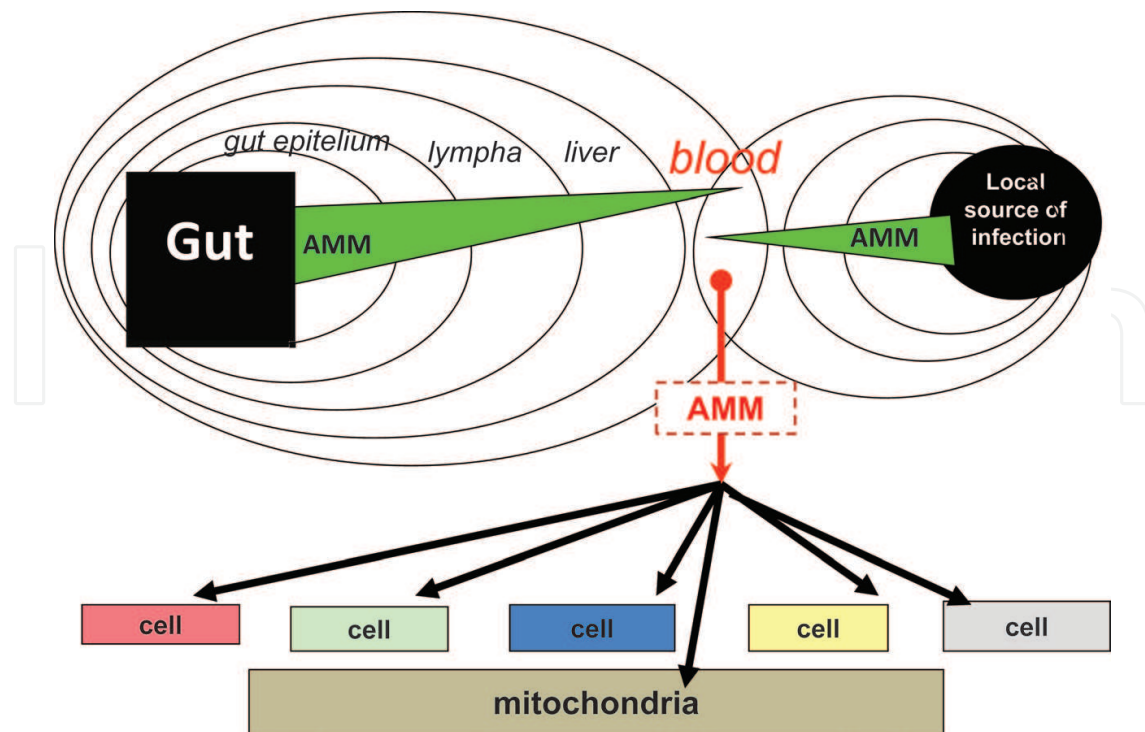


Figure 1. Sources and targets of serum aromatic microbial metabolites (AMM) in sepsis.

p-hydroxyphenyllactic, phenylpyruvic, p-hydroxyphenylpyruvic, and o-hydroxyphenylacetic), indolic acids (1-indoleacetic acid and 3-indoleacetic acid), etc.

It proved the microbial origin of phenyl carboxylic acids in the human body [15]. The examination and comparison of different groups of people discovered that some aromatic metabolites in the blood of patients with sepsis strongly differed from all other groups of patients. We named these compounds as “sepsis-associated” aromatic microbial metabolites (AMM). On chemical structure, they can be attributed to phenolic or phenyl carboxylic acids (**Figure 2**).

Jenner et al. [16] analyzed the profile of aromatic compounds (about 50) in the gut of healthy people. The results showed that they are quantitatively dominated by such metabolites

$$\text{PhAA} > \text{p-HPhAA} > 3, 4 \text{ di-HCinA} > \text{PhPA} > \text{BA} \quad (1)$$

Our research has shown that most of these AMMs are also present in the serum of healthy people [17], but in a different ratio:

$$\text{p-HPhLA} > \text{BA} > \text{p-HPhAA} > \text{PhAA} > \text{PhLA} > \text{PhPA} \quad (2)$$

Differences in the proportion of AMM in the blood compared to the intestine can be explained by the fact that most hydrophilic (p-HPhAA, p-HPhLA, and PhLA) metabolites are excreted by the kidneys, while lipophilic metabolites (BA, PhAA, and PhPA) are absorbed by cells of tissue barriers (intestinal wall, lymphoid tissue, liver, vascular endothelium, etc.).

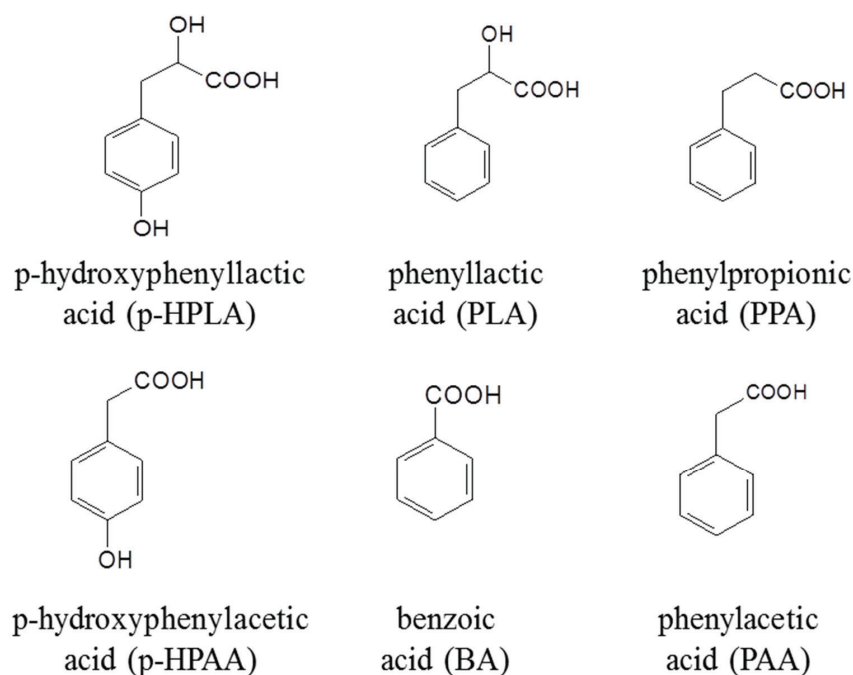


Figure 2. “Sepsis-associated” aromatic microbial metabolites (AMM).

It is important to note that all patients with a diagnosis of sepsis discovered the highest levels in the serum of PhLA, p-HPhLA, p-HPhAA, and always-no PhPA [18].

The comparisons of the levels of microbial aromatic metabolites (AMM) in the blood of septic patients versus a normal level of metabolites in the blood and in bowel (fecal water) of healthy are presented in **Table 1**.

Figure 3 presents visual comparison data on serum concentrations of six AMM (GC-MS, ng/ml) in groups of healthy and patients with chronic heart failure, familial mediterranean fever (FMF), pneumonia, and sepsis. It is important to note that the concentration of lipophilic AMM is so small that the image is almost not visible. At the same time, the concentration of the three “sepsis-associated acids” (PhLA, p-HPhLA, and p-HPhAA) in patients with sepsis greatly exceeds the levels of AMM when comparing groups.

The studies had different questions as follows:

1. Does not such a high level of AMM in violation of their elimination from the body, for example, in renal or hepatic failure (excluding sepsis)?
2. Increase of sepsis-associated AMM in patients with severe trauma without sepsis?
3. What about patients with phenylketonuria?

After the examination of six different groups of patients, such doubts were dispelled and again shows that sepsis is characterized by increase of all three “sepsis-associated” AMM—PhLA, p-HPhLA, and p-HPhAA (**Figure 4**).

AMM	AMM in the blood of septic patients, μM , median (IR 25–75%)	AMM in the blood of healthy, μM , median (IR 25–75%)	AMM in the bowel (faecal water) of healthy, data from [7], μM
<i>Lipophilic AMM</i>			
PhPA	<i>nd</i> *	0,2 (0,1–0,4)	200-600
PhAA	0,4 (0,1–0,7)	0,4 (0,3–0,6)	400-1100
BA	0,9 (0,8–2,0)	0,7 (0,6–0,8)	23-25
<i>Hydrophilic AMM</i>			
di-HCinA	<i>nd</i>	<i>nd</i>	50-200
PhLA	2,7 (1,4–5,1)	0,3 (0,2–0,4)	<i>nd</i>
p-HPhLA	7,6 (2,9–15,5)	1,1 (0,9–2,0)	<i>nd</i>
p-HPhAA	14,1 (7,8–35,9)	0,5 (0,4–0,6)	60-400

* *nd*, not detected.

Table 1. The levels of microbial aromatic metabolites (AMM) in the blood of septic patients versus a normal level of metabolites in the blood and in bowel (faecal water) of healthy.

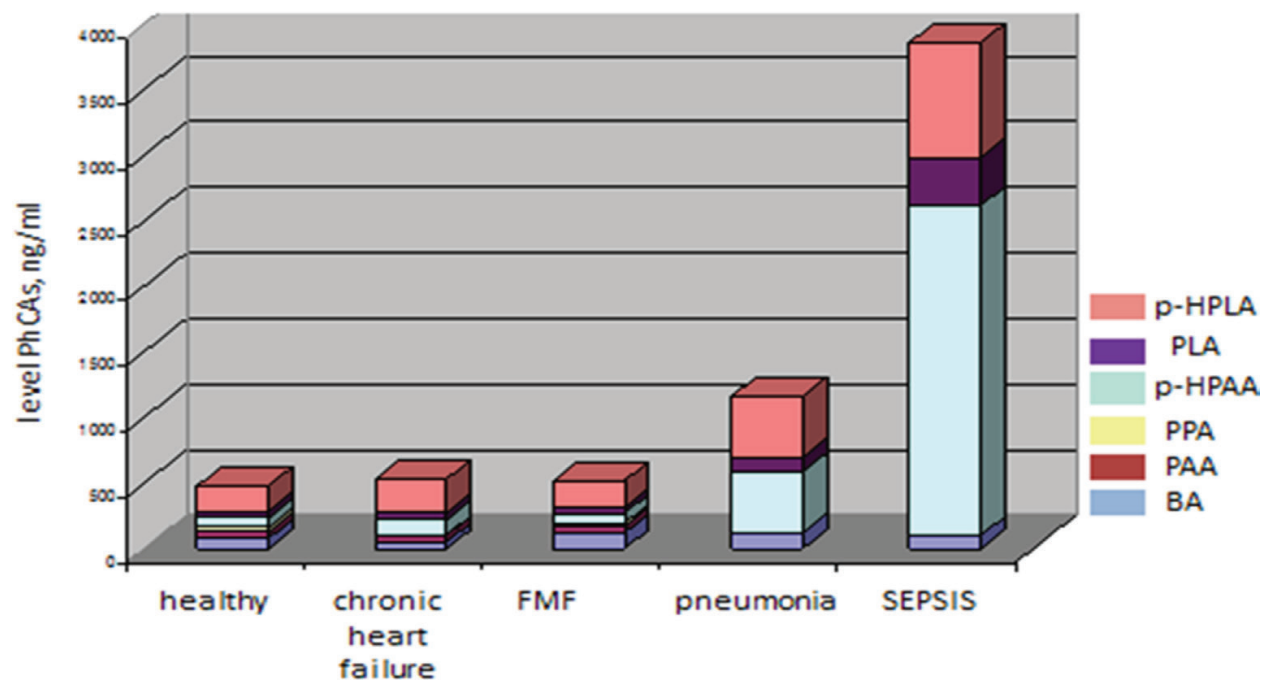


Figure 3. High serum concentrations of p-HPhAA, p-HPhLA, and PhLA (GC/MS, ng/ml) in septic patients versus healthy and chronic heart failure, familial mediterranean fever (FMF), and pneumonia.

The first report on the potential diagnostic value of the level of AMM in sepsis has been reported to us on the International Sepsis Forum (ISF-2007, Paris) and then was published [19]. There was a presentation on the results of GC-MS analysis, which revealed the highest

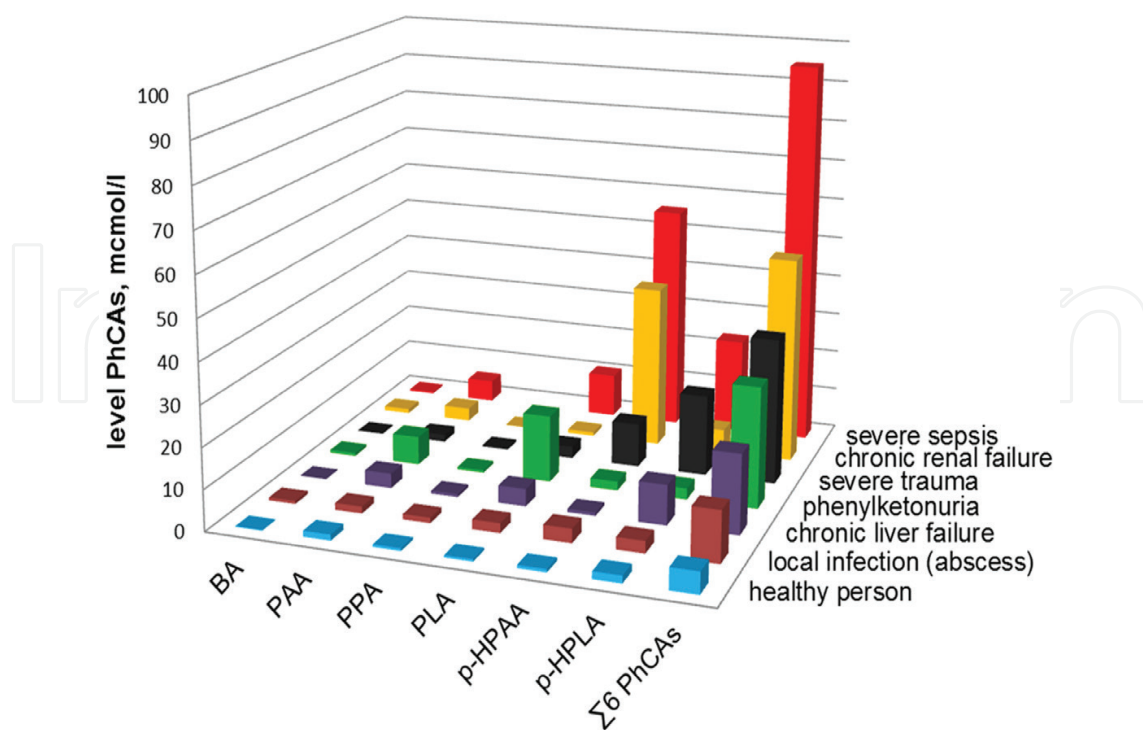


Figure 4. The levels of six phenyl carboxylic acids in blood of six groups of patients (GC-MS, μM).

levels of p-HPhAA, p-HPhLA, and PhLA in 44 septic patients. Then, the levels of AMM in patients were examined in the dynamics. It is shown that aromatic metabolites accurately reflect the efficiency of ICU treatment and the prognostic significance of the some aromatic metabolites is higher than that of lactate [20].

The highest levels of “sepsis-associated” AMM discovered in patients with a fatal outcome in end-stage sepsis, on the last day of life (<24 h before death). **Table 2** shows a significant

Parameter	Time to death (retrospective analysis)		The multiplicity of changes, Me	p=
	48-25 h	24-0 h		
MODS_2	5 (3–7)	9 (6–10)	1,8	<0.001
Lactate, μM	3,6 (2,4–5,1)	4,2 (3,0–10,6)	1,2	0.030
PhLA, μM , median (IR 25-75%)	4,27 (2,14–9,33)	7,97 (5,07–17,90)	1,9	<0.001
p-HPhAA, μM , median (IR 25-75%)	10,45 (1,99–25,81)	20,14 (7,27–65,49)	1,9	<0.001
p-HPhLA, μM , median (IR 25-75%)	9,14 (5,73–25,51)	22,06 (13,64–67,04)	2,4	<0.001
$\Sigma 3$ AMM, μM , median (IR 25-75%)	33,47 (12,40–63,98)	78,02 (38,92–156,26)	2,3	<0.001

Table 2. Some clinical and laboratory data of the monitoring terminal ICU patients in the last 2 days of life (n = 34).

increase in the level of AMM (p-HPhAA, p-HPhLA, and PhLA) in the last 2 days of life in terminal ICU patients (n=34) and significantly the highest concentration for the moment of death.

Later, the relationship was also found between p-HPhLA and 28-day mortality in a large study from Roger et al., after analysis of metabolic profiling of plasma samples from 90 ICU subjects [21].

These facts point to the possible own biological activity of AMM and their direct participation in the mechanisms of pathogenesis and thanatogenesis of sepsis.

4. The role of microbial metabolites in the pathogenesis of sepsis

4.1. Bacterial load and neutrophils

Studies have confirmed that increased bacterial load is accompanied by an increase in the total blood concentration of AMM [22] that contributes to the transition from local infection to sepsis. This can be observed with late diagnosis, inadequate antibiotic therapy, or delayed surgical rehabilitation of the infection focus. So, when comparing patients with different severity of bacterial infection, it is shown that in the group of patients with sepsis, serum concentrations of hydrophilic AMM were significantly higher versus the group of local infections of skin and soft tissues [23].

It is shown that the AMM level reflects the severity of the infectious process and is directly correlated with the number of clinical signs of inflammation, indicators of severity APACHEII and SOFA scores [24].

Neutrophils are the first line of defense against bacteria. They quickly migrate to the site of infection or inflammation, neutralize foreign particles, phagocytose, and destroy bacteria. Polymorphonuclear leukocytes also transmit signals to other cells of the nonspecific immune system about the threat of invasion of foreign agents. Neutrophil dysfunction is one of the key mechanisms of severe infection and sepsis development.

Thus, the microbial load in sepsis associated with microbial metabolites. To test the hypothesis about the influence of AMM on the phagocytic activity of neutrophils, experiments were conducted *in vitro*. It was shown that *in vitro* some sepsis-associated AMM in clinically relevant concentrations are able to inhibit phagocytic activity of neutrophils. In experiments with neutrophils observed the disruption caused by the influence of AMM *in vitro*. They were associated with suppressed production of ROS and similar to those found in patients with sepsis; therefore, among the reasons for mitochondrial dysfunction in sepsis can play an important role in the imbalance of microbial metabolites [25].

4.2. Organ dysfunction

Retrospective analysis of the causes of deaths by group of authors (Vincent, Nelson, and Williams), using a database of 28 countries, which contains all information about 4459 patients with severe sepsis [26]. It has been shown that for the period, by the 28th day from the time

of diagnosis of sepsis died 1201 patients and mortality was 27%. The analysis showed that all the deceased patients showed an increase in the dynamics of the severity of organ disorders, which were objectively evaluated by the growing points on the SOFA scale. The main causes of death were multiple organ failure (MOF) associated with sepsis (43%) and refractory septic shock (22.6%). At the same time, the results of histological findings in postmortem studies of organs and tissues from patients, who died from sepsis, are not consistent with the degree of the organ dysfunction. Cells of heart, kidney, liver, and lungs in the death of sepsis were subjected to minimal changes. In patients with clinical signs of myocardial depression had no data on the damage of cardiomyocytes. Kidney (nephrons and tubules) were intact. That is, the organs looked normal while they are clinically noted MOF with fatal outcome [27]. Thus, it is quite reasonable today in the center of attention—the causes and mechanisms of MOF in patients with sepsis [28].

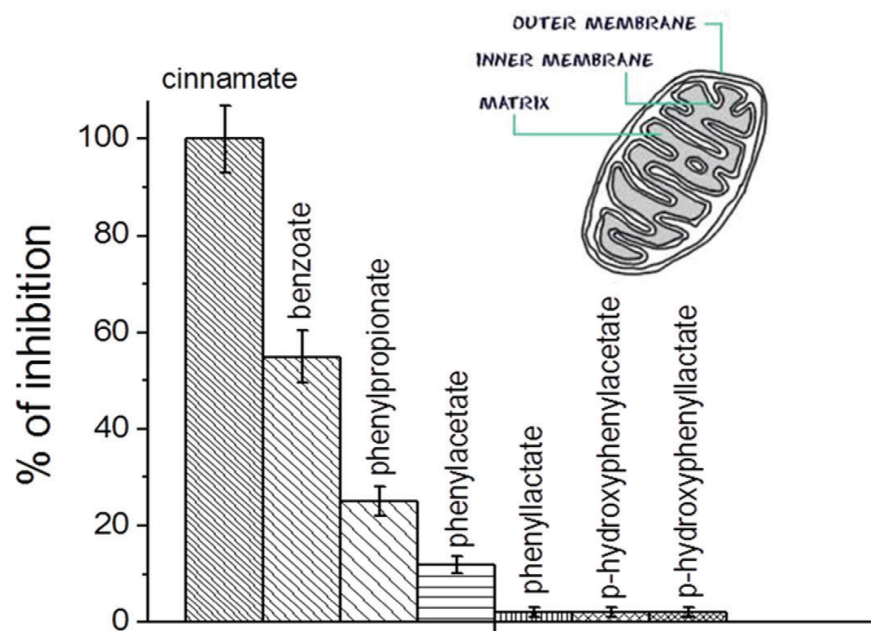
Fortunately, more than half of patients with sepsis survive. Organ dysfunction in sepsis is sometimes called “hibernation-like state” from the point of view of its reversibility [29]. In other words, MOF means something disrupts normal functioning of the cells, in sepsis it is not so much hypoxia, but how much a reduction in energy availability. Unique to sepsis are the coexisting findings: metabolic acidosis means increased oxygen demand, at the same time recorded a decrease of oxygen consumption. The greatest attention is paid to study mitochondrial mechanisms of sepsis-induced organ failure.

It is known that the mitochondria in human cells are similar to bacteria not only in size. Mitochondria have their own DNA, which is similar to bacterial DNA and very different from the DNA of human cells. Mitochondria and bacteria have also some similar pathways for energy production. A number of other biological characteristics also point to a prokaryotic origin of mitochondria in ontogenesis [30].

4.3. Mitochondrial dysfunction

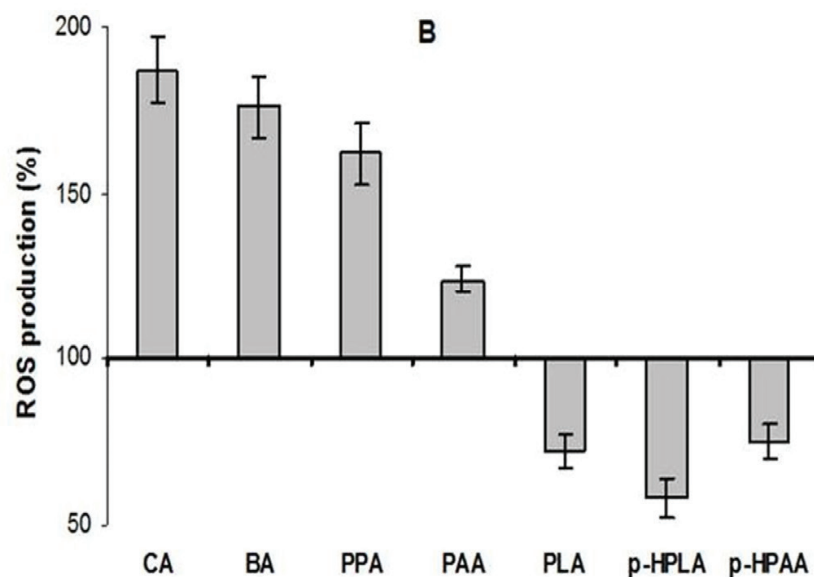
According to the authors, mitochondria reduce the supply of energy to the cell to cause metabolic shutdown. This adaptive mechanism is similar to hibernation, it prevents massive cell death and thereby, gives a chance of complete recovery of organ function after recovery in survivors [31].

The concept of mitochondrial dysfunction and bioenergetics failure during sepsis is not new [32], but it remains unclear why in sepsis does this unique mechanism and what role bacteria play. Our experimental approaches have revealed the influence of AMM on mitochondrial function that have novel and interesting findings [33]. It turned out that some aromatic microbial metabolites are able to inhibit the NAD-dependent mitochondrial respiration. It is shown that the degree of inhibition depends on the chemical structure of metabolites [34]. In the experiment, *in vitro* the degree of inhibition of respiration of mitochondria by cinnamic acid was taken as 100% (**Figure 5**). We can conclude that inhibition of mitochondrial respiration can only occupy lipophilic AMM and its derivatives (benzoic and cinnamic acids are metabolites of PhPA).



The inhibition of NAD-dependent respiration of mitochondria by cinnamic acid was taken to be 100%. The concentration of phenolic acids was 100 μ M. Data are expressed as the means \pm SEM of five independent determinations

Figure 5. Effect of phenolic acids of microbial origin on NAD-dependent respiration of mitochondria.



* cinnamic (CA), benzoic (BA), phenylpropionic (PPA), phenylacetic (PAA), p-hydroxyphenylpropionic (p-HPPA), phenyllactic (PLA), p-hydroxyphenyllactic (p-HPLA)
 Data are expressed as means \pm SEM of five independent determinations at a concentration of 100 μ M

Figure 6. Comparison of the effect of phenolic acids* on menadione-activated ROS production by mitochondria.

When studying the effect of AMM on the production of ROS by mitochondria, activated by menadione, the differences were in the opposite direction: lipophilic AMM (cinnamic CA, benzoic BA, phenylpropionic PPA, and phenylacetic PAA) activated, and hydrophilic AMM (p-hydroxyphenyl propionic p-HPPA), phenyllactic PLA, and p-hydroxyphenyllactic p-HPLA opposite suppressed (**Figure 6**). It is known that a very large number of mitochondrial reactive oxygen species (mROS) directly damage proteins, lipids, and nucleic acids. At the same time, a complete lack of ROS production is also very harmful, so the lower levels of mROS are necessary for normal cell homeostasis. Low levels mROS perform the functions signaling molecules and need to adapt to the stress [35].

It is also shown the *in vitro* ability of microbial metabolites to influence the amount of metabolites of the tricarboxylic acid cycle in the blood of septic patients affects the activity of mitochondrial enzymes [36]. The clinical confirmation and significance of these facts to be assessed in the near future.

4.4. Hemodynamic instability and shock

Some authors view excessive bacterial load as the main cause of uncontrollable progression of hypotension in critically ill patients [37]. It has been shown that the earlier the onset of antimicrobial therapy (immediately on admission of a critical patient with signs of arterial hypotension) may have a significant impact on survival. So, hemodynamic instability and septic shock are undoubtedly connected with bacteria, but how? Attempts to explain the leading effect only of LPS (structural component of cell walls of gram-negative rods or endotoxin) is not satisfied, because they do not allow you to monitor and manage this process.

Our clinical monitoring of patients with high risk of septic shock indicates the potential involvement of AMM in the development of this life-threatening condition, accompanied by high mortality [38, 39]. In our view, an important role is played by disruption of the normal participation of microflora in the metabolism of aromatic amino acids, especially tyrosine and products of its metabolism. First, it is shown that an excess of toxic endogenous and microbial metabolic products of tyrosine and dopamine cannot be fully disposed of anaerobic microbiota [40]. However, these aromatic metabolites on the principle of feedback can inhibit the normal metabolic pathway of synthesis of hormones. Recently, we have obtained indirect evidence of “microbial” mechanism in the development of septic shock through the inhibition of tyrosine hydroxylase. In this way, the products of microbial metabolism may interfere with the normal synthesis of catecholamines from dopamine and to result in arterial hypotension. Search connection between endogenous hormones and bacterial exometabolites potentially related to hypotension in patients with infection will continue [41].

5. Conclusion

Sepsis integration of metabolism of humans and microbiota is disturbed and takes pathological character directed against the owner. Previously, it has been shown that phenyl carboxylic acids could have microbial origin in human blood of healthy people. Microbiota has to survive under adverse conditions, the indigenous anaerobic microflora are not able

to fulfill its “helpful” metabolic functions [14]. Studies have shown that in septic patients, most violations occur in the metabolism of aromatic compounds. We documented imbalance of aromatic acids metabolites in septic patients: absence of serum PPA and high level of PLA, p-HPLA, and p-HPAA are associated with severity and mortality. Experimentally demonstrated the influence of some aromatic microbial metabolites (AMMs) on mitochondrial function, participate in the development of organ dysfunctions in sepsis. *In-vitro* some AMMs in clinically significant concentrations are able to inhibit phagocytic activity of neutrophils. Metabolism of tyrosine is severely disrupted in sepsis. Some microbial exometabolites may suppress the activity of enzymes such as tyrosine-hydroxylase. There is an excess of products of microbial biodegradation of endogenous metabolites of aromatic amino acids in the blood, including the reason of failure function of indigenous anaerobes. The obtained results indicate new promising target in the diagnosis, prevention, and treatment of sepsis.

We believe that the development of laboratory technologies, the use of other more sophisticated methods aimed at detection and measurement of metabolites of microbial origin in the human body, is the real path to success in solving the problem of sepsis in the near future.

6. Methods

We used gas chromatography-mass spectrometry (GC-MS) method to quantify metabolites in human serum from patients with pneumonia or abdomen sepsis, patients with local infection of the skin and soft tissues, healthy (as control). In the process, we adapted the sample preparation and measurement method for working in clinical laboratory conditions on GC-FID [42]. Clinical and laboratory data, APACHE II and SOFA scores in patients were matched. Methods for *in vitro* studies of the biological activity of microbial metabolites on the neutrophils and mitochondria are described in detail in articles listed in references. In microbiological experiments, we measured also the concentration of the metabolites by GH-MS in the nutrient medium after the cultivation of various species of aerobic or anaerobic bacteria. Data were compared by Mann-Whitney U-test; *p*-values less than 0.05 were considered significant.

Acknowledgements

I am grateful to my senior teachers, also to colleagues and associates in experimental and clinical work. This work was supported by the Russian Science Foundation Grant no. 15-15-00110.

Author details

Beloborodova Natalia Vladimirovna

Address all correspondence to: nvbeloborodova@yandex.ru

Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology, Negovsky Research Institute of General Reanimatology, Moscow, Russian Federation

References

- [1] Tannock GW. Understanding the Gut Microbiota. John Wiley & Sons, Inc. Hoboken, New Jersey, - 184 p. ISBN 1118801342, 9781118801345
- [2] Shenderov BA, Midtvedt T. Epigenomic programing: A future way to health? *Microbial Ecology in Health & Disease* 2014;**25**: 24145 - <http://dx.doi.org/10.3402/mehd.v25.24145>
- [3] Beloborodova NV, Osipov GA. Homeostasis of small molecules originating from microbes and its role in microbial relations with the host. *Vestnik Rossiiskoi Akademii Meditsinskikh Nauk*. 1999;**54**(7):25-31. PMID: 10467881 <https://www.ncbi.nlm.nih.gov/pubmed/10467881>
- [4] Wikoff WR, Anfora AT, Liu J, et al. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proceedings of the National Academy of Sciences*. 2009 March 10;**106**(10):3698-3703. DOI: 10.1073/pnas.0812874106
- [5] Beloborodova NV, Osipov GA. Small molecules originating from microbes (SMOM) and their role in microbes–host relationship. *Microbial Ecology in Health and Disease*. 2000;**12**(1):12-21 <http://www.microbecolhealthdis.net/index.php/mehd/article/view/8039>
- [6] Bianconi E, Piovesan A, Facchin F, Beraudi A, Casadei R, Frabetti F, et al. An estimation of the number of cells in the human body. *Annals of Human Biology*. 2013;**40**:463-471. DOI: 10.3109/03014460.2013.807878. pmid:23829164
- [7] Sender R, Fuchs S, Milo R. Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell*. 2016;**164**:337-340. DOI: 10.1016/j.cell.2016.01.013. pmid:26824647
- [8] Shimizu K, Ogura H, Goto M, Asahara T, Nomoto K, Morotomi M, Yoshiya K, Matsushima A, Sumi Y, Kuwagata Y, Tanaka H, Shimazu T, Sugimoto H. Altered gut flora and environment in patients with severe SIRS. *Journal of Trauma*. 2006;**60**(1):126-133. PMID: 16456446 DOI: 10.1097/01.ta.0000197374.99755.fe
- [9] Kirsch M1. Bacterial overgrowth. *American Journal of Gastroenterology*. 1990 March; **85**(3):231-237. PMID: 2178395
- [10] Beloborodova NV, Vostrikova TY, Antibiot Khimioter. Characteristics of fauces microflora in children treated in intensive care units. Article in Russian. 1998;**43**(8):16-22. PMID: 9777101
- [11] Leeuwen PA, Boermeester MA, Houdijk AP, Ferwerda CC, Cuesta MA, Meyer S, Wesdorp RI. Clinical significance of translocation. *Gut*. 1994 January;**35**(Supp 1):S28-S34. PMCID: PMC1378143
- [12] Berg RD. Bacterial translocation from the gastrointestinal tract. *Advances in Experimental Medicine and Biology*. 1999;**473**:11-30. PMID: 10659341

- [13] Deitch EA, Winterton J, Li M, Berg R. The gut as a portal of entry for bacteremia; role of protein malnutrition. *Annals of Surgery*. 1987 June;205(6):681-692
- [14] Beloborodova NV. Integration of metabolism in man and his microbiome in critical conditions. *General Reanimatology*. 2012;8(4):42-54
- [15] Beloborodova NV, Khodakova AS, Bairamov IT, Olenin AY. Microbial origin of phenylcarboxylic acids in the human body. *Biochemistry (Moscow)*. 2009;74(12):1657-1663. PMID: 19961416 DOI: 10.1134/S0006297909120086 <http://link.springer.com/article/10.1134/S0006297909120086>
- [16] Jenner AM, Rafter J, Halliwell B. Human faecal water content of phenolics: The extent of colonic exposure to aromatic compounds. *Free Radical Biology & Medicine*. 2005;38(6):763-772. DOI: 10.1016/j.freeradbiomed.2004.11.020
- [17] Beloborodova NV, Moroz VV, Osipov AA, Bedova AY, Olenin AY, Getsina ML, Karpova OV, Olenina EG. Normal level of sepsis-associated phenylcarboxylic acids in human serum. *Biochemistry (Moscow)*. 2015;80(3) 374-378. DOI: 10.1134/S0006297915030128, PMID: 25761691
- [18] Beloborodova NV, Olenin AY, Khodakova AS, Chernevskaya EA, Khabib ON. Low-molecular phenol metabolites in blood serum: Origin and clinical significance. *Anesteziologiya i Reanimatologiya*. 2012;5:65-72
- [19] Khodakova AS, Beloborodova NV. Microbial metabolites in the blood of patients with sepsis. *Critical Care*. 2007;11(4):P5. DOI: 10.1186/cc5984
- [20] Beloborodova N, Osipov A, Pautova A, Bedova A. Aromatic metabolites clearance reflects the efficiency of ICU treatment more accurately than lactate clearance. *Critical Care*. 2016;20(Suppl 3):P19. DOI: 10.1186/s13054-016-1518-8
- [21] Rogers AJ, McGeachie M, Baron RM, et al. Metabolomic derangements are associated with mortality in critically ill adult patients. 2014; PLoS ONE 9(1): e87538. DOI: 10.1371/journal.pone.0087538
- [22] Sarshor YN, Beloborodova NV, Bedova AY, Osipov AA, Chernevskaya EA, Getsina ML. New criteria of bacterial load in critically ill patients. *Shock*. 2013;40(Suppl 1):31
- [23] Beloborodova NV, Moroz VV, Bedova AY, Osipov AA, Sarshor YN, Chernevskaya EA. Participation of aromatic microbial metabolites in the development of severe infection and sepsis. *Anesteziologiya i Reanimatologiya*. 2016;61(3):202-208. DOI: 10.18821/0201-7563-2016-3-202-208
- [24] Moroz VV, Beloborodova NV, Osipov AA, Vlasenko AV, Bedova AY, Pautova AK. Phenylcarboxylic acids in the assessment of the severity of patient condition and the efficiency of intensive treatment in critical care medicine. *Obshchaya Reanimatologiya*. 2016;12(4):37-48. <http://www.reanimatology.com/rmt/article/view/1538> DOI: 10.15360/1813-9779-2016-4-37-48.

- [25] Beloborodova N, Bairamov I, Olenin A, Shubina V, Teplova V, Fedotcheva N. Effect of phenolic acids of microbial origin on production of reactive oxygen species in mitochondria and neutrophils. *Journal of Biomedical Science*. 2012;**19**:89. DOI: 10.1186/1423-0127-19-89
- [26] Vincent JL, Nelson DR, Williams MD. Is worsening multiple organ failure the cause of death in patients with severe sepsis? *Critical Care Medicine*. 2011;**39**(5):1050-1055. DOI: 10.1097/CCM.0b013e31820eda29
- [27] Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. *The New England Journal of Medicine*. 2003 January 9;**348**(2):138-150. DOI: 10.1056/NEJMr021333
- [28] Abraham E, Singer M. Mechanisms of sepsis-induced organ dysfunction. *Critical Care Medicine*. 2007 October;**35**(10):2408-2416
- [29] Singer M. Cellular dysfunction in sepsis. *Clinics in Chest Medicine*. 2008 December;**29**(4):655-660. PMID:18954700. DOI: 10.1016/j.ccm.2008.06.003
- [30] Friedman JR, Nunnari J. Mitochondrial form and function. *Nature*. 2014;**505**:335-343
- [31] Exline MC, Crouser ED. Mitochondrial mechanisms of sepsis-induced organ failure. *Frontiers in Bioscience*. 2008 May 1;**13**:5030-5041. Review. PMID: 18508567 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3913468>
- [32] Singer M. The role of mitochondrial dysfunction in sepsis-induced multi-organ failure. *Virulence*. 2014 January 1;**5**(1):66-72. PMID: PMC3916385 DOI: 10.4161/viru.26907
- [33] Beloborodova NV, Teplova VV, Fedotcheva NI. Monograph: The Role of Microbial Metabolites in Mitochondrial Dysfunction in Sepsis. Germany: LAP Lambert Academic Publishing; 2013. p. 89. ISBN: 978-3-659-43111-1 (translation)
- [34] Fedotcheva NI, Kazakov RE, Kondrashova MN, Beloborodova NV. Toxic effects of microbial phenolic acids on the functions of mitochondria. *Toxicology Letters*. 2008 August 28;**180**(3):182-188. DOI: 10.1016/j.toxlet.2008.06.861
- [35] Sena LA, Chandel NS. Physiological roles of mitochondrial reactive oxygen species molecular. *Cell*. 2012 October 26;**48**(2):158-167. DOI: <http://dx.doi.org/10.1016/j.molcel.2012.09.025>
- [36] Fedotcheva NI, Litvinova EG, Osipov AA, Olenin AY, Moroz VV, Beloborodova NV. Influence of microbial metabolites of phenolic nature on the activity of mitochondrial enzymes. *Biophysics*. 2015;**6**:IF-0.316. PMID:26841505
- [37] Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, Suppes R, Feinstein D, Zanotti S, Taiberg L, Gurka D, Kumar A, Cheang M. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Critical Care Medicine*. 2006;**34**:1589-1596. DOI: 10.1097/01.CCM.0000217961.75225.E9
- [38] Beloborodova NV, Sarshor YN, Bedova AY, Pautova AK, Chernevskaya EA. Are phenyl-lactic and p-hydroxyphenyllactic acids involved in the development of arterial hypotension? *Infection*. 2015;**43**(Suppl 1):S31. DOI: 10.1007/s15010-015-0827-1

- [39] Sarshor Y, Beloborodova N, Moroz V, Osipov A, Bedova A, Chernevskaya E, Getsina M. Value of microbial metabolites in blood serum as criteria for bacterial load in the pathogenesis of hemodynamic disorders in critically ill patients. *Critical Care*. 2014;18(Suppl 1):P208. DOI:10.1186/cc13398 <http://ccforum.com/content/18/S1/P208>
- [40] Beloborodova N, Moroz V, Osipov A, Bedova A, Sarshor Y, Vlasenko A, Olenin A. Tyrosine metabolism disorder and the potential capability of anaerobic microbiota to decrease the value of aromatic metabolites in critically ill patients. *Critical Care*. 2014;18(Suppl 2):P60. DOI: 10.1186/cc14063 <http://ccforum.com/content/18/S2/P60>
- [41] Beloborodova NV, Chernevskaya EA, Bedova AY, Sarshor YN, Sergeev AA. Connection between aromatic microbial metabolites, hormones and biomarkers in critically ill patients with infection. *Shock*. 2016;46(Suppl 2):P1-9
- [42] Moroz V, Beloborodova NV, Bedova AY, Revel'skii AI, Getsina ML, Osipov AA, Sarshor YN, Buchinskaya AA, Olenin AY. Development of methods of the gas chromatographic determination of phenylcarboxylic acids in blood serum and their adaptation to clinical laboratory conditions. *Journal of Analytical Chemistry*. 2015 April;70(4):495-501. DOI: 10.1134/S1061934815040103

