Signa Vitae

REVIEW

Recent application of metabolomics in the diagnosis, pathogenesis, treatment, and prognosis of sepsis

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Abstract

Sepsis is a life-threatening organ dysfunction caused by dysregulated host response to infections. It is a leading cause of morbidity and mortality in hospitalized patients. Patients with sepsis often require care in the intensive care unit (ICU) which is costly to the patients and their families. Sepsis has no specific clinical manifestations, and its pathophysiological mechanism is complex. The disease progresses rapidly which makes early diagnosis difficult. Severe forms of the disease, such as septic shock, may lead to organ dysfunction, organ failure, and death. As an emerging "-omics" technology, metabolomics has revolutionized the clinical and research landscape of sepsis. Metabolomics has been applied in the prognosis, diagnosis, and risk stratification in patients with sepsis. This technology provides details on the metabolites and biochemical pathways commonly associated with the pathophysiology of sepsis. At present, it is mostly used to identify metabolites in various diseases. Using this technology, metabolites in body fluids such as blood and urine are detected and analyzed in relation to disease progresssion. The technology therefore helps to understand the pathogenesis of diseases and promote early diagnosis and treatment of the disease. So far, the applicaition of metabolomics in patients with sepsis has not been well defined. This article briefly reviews the application of metabolomics technology in patients with sepsis in recent years, to generate ideas for improving rapid diagnosis and prognosis evaluation of patients with sepsis.

Keywords

Sepsis; Metabolomics; NMR; GC-MS; LC-MS

1. Introduction

Currently, sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection. Organ dysfunction is evaluated using the sequential organ failure assessment (SOFA) score. A SOFA score ≥ 2 indicates organ dysfunction [1]. Sepsis can progress to severe sepsis and septic shock. According to the 2016 guidelines, septic shock is defined as sepsis in which the underlying circulatory and cellular metabolism abnormalities cause death [1]. Sepsis progresses rapidly, and it is a common cause of death in intensive care units. A study published in the Lancet in 2020 showed that about 48.9 million people suffered from sepsis in 2017 worldwide, of which 11 million eventually died. Globally, sepsis contributes to 19.7% of all deaths [2]. In mainland China, the 90-day mortality rate of sepsis patients in the intensive care unit was about 35.5% [3].

Studies have shown that analysis of the human metabolome can reveal the state of sepsis in an individual. Several biomarkers can predict the development of sepsis or response of septic patients to treatment. The discovery and validation of such metabolomic biomarkers enable faster, cheaper and more comprehensive metabolomic analysis for patient with sepsis. As a new "omics" technology, metabolomics is in the same category of systems biology with genomics, transcriptomics, and proteomics. Metabolomics refer to changes in metabolites under normal conditions and in response to changes in the internal environment [4]. This review briefly introduces metabolomics-related technologies and their applications in the diagnosis, pathogenesis, treatment, and prognosis of sepsis.

2. Metabolomics and related technologies

The concept of metabolomics emerged from the term "metabolome", which was first proposed by Oliver in 1998 and revised by Nicholson in 1999 [5, 6]. Over the years, the concept of metabolomics has evolved to include high-throughput identification, quantification, and characterization of endogenous and exogenous metabolites. To date, over 20,000 compounds belonging to various classes of endogenous metabolites (about 8500) and exogenous metabolites (about 11,500) have been identified. Research objects used in metabolomics are small molecules (molecular weight MW

<1500 Da) in organs, tissues, or cells, most of which are intermediate products or the most downstream products of metabolic processes in the body. Metabolites can easily, accurately, and directly reflect changes in the body. Therefore, metabolomics, a new "omics" approach, is being applied in medical practice to promote disease research [7–9]. Two metabolomic approaches are used: targeted or untargeted. The former analyses known metabolites. This approach has high sensitivity and specificity. The latter comprehensively analyses biological samples and compares metabolic profiles associated with different intervention measures. It further reveals differential metabolites based on statistical analysis, which can be used for the discovery of biomarkers [10, 11]. Therefore, untargeted metabolomics does not analyze the specific metabolites.

The analytical techniques used in metabolomics include nuclear magnetic resonance (NMR), gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), Fourier transform infrared spectroscopy, and capillary electrophoresis-mass spectrometry (CE-MS) [12, 13]. Each of these techniques has its own unique characteristics. These techniques can qualitatively and quantitatively analyze body samples to detect small molecular metabolites and reveal the body's metabolic map. Metabolic techniques are critical in discovering the changes and laws of metabolites in the body, as well as new biomarkers, which are essential in the diagnosis, treatment, and prognosis evaluation of disease [14].

Metabolomics has been widely applied in agriculture, forestry, animals and plants, drug research and development, disease research, among other fields. In disease research, metabolomics has mainly been used to investigate tumors, infection, and immune disorders. To comprehensively detect and analyze all metabolites in the body, it is recommended to employ a combination of different analytical platforms [15].

2.1 Nuclear magnetic resonance

Nuclear magnetic resonance (NMR) was first applied to metabolomics in 1974. D Wilson and AL Burlingame used deuterium and carbon-13 traces to study the metabolism of ethanol in rats [16]. Since then, NMR-based metabolomics have been widely applied because it is non-damaging, and can be used for metabolic analysis of living cells. Second, the technology require simple sample preparation and results are reproducible. Third, it has high sensitivity to all metabolites and can quantify all metabolites simultaneously. The NMR technology is often used in non-targeted metabolomics to identify and determine the structure of unknown metabolites using stable isotope labels. The application of NMR technology is, however, limited following that it has low sensitivity and resolution. In addition, the detected metabolites often show multiple overlapping peaks, which is difficult to qualitatively interpret. The minimum concentration of metabolites that can be detected qualitatively and quantitatively is 1–5 μmol/L. The NMR technology can not accurately detect metabolites below the minimum detection concentration [17, 18]. To improve its accuracy, the scanning time and magnetic field strength are often increased.

Currently, the most commonly used NMR technology is 1H NMR, although other technologies such as, 13C NMR, 15N NMR, 13P NMR, and 2D NMR are applied. Therefore, different metabolomics technologies can be employed for different experimental purposes and methods [19–22].

2.2 Gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry (GC-MS) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. The most important feature of this method is its sensitivity to gases and the ability to study volatile substances or substances with lower boiling points. However, this method requires that substances that are not volatile should be derivatized into substances with lower boiling points before analysis. Derivatization treatments are complex, complicated, time-consuming, and expensive. Furthermore, complicated sample derivation processes may lead to the loss of important substances in the sample. Most of the metabolites have high boiling points and are difficult to volatilize. Specifically, when using GC-MS technology, derivatization such as methylation or silanization is required. This limits the application of GC-MS in metabolomics. Nonetheless, GC-MS has been widely used in the study of metabolites [23–27]. This is because it combines the characteristics of high-efficiency separation of chromatograms and structure identification of mass spectrometry. It has high sensitivity and precision and can separate thousands of metabolites of different properties at the same time. And lastly, it can be applied to both targeted and non-targeted metabolomics [28–31].

2.3 Liquid chromatography-mass spectrometry

Liquid chromatography-mass spectrometry (LC-MS) is a technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry (MS). The technique is mainly used to analyze non-volatile substances. Although its sensitivity and resolution are slightly inferior to GC-MS, it is still better than NMR. LC-MS can effectively detect various metabolites and be used in both broad- spectrum targeted and non-targeted manners [23]. The LC-MS technique has the potential to analyze and identify metabolites in samples with a high boiling point even when their content is low. Compared with GC-MS, LC-MS does not require complex derivatization processing. LC-MS is used to compare the metabolic profiles of different treatment groups, which reveals the differential metabolites between groups and helps clarify the mechanisms of disease [32, 33]. At present, LC-MS-based metabolomics is widely used in disease diagnosis, drug development, treatment efficacy evaluation, and outcome prediction [28, 34, 35].

3. Application of metabolomics in sepsis

3.1 Application of metabolomics in the diagnosis of sepsis

According to the guidelines issued in 2016, patients with infections or those suspected of have infections should be scored with SOFA. A SOFA score value ≥2 indicates that sepsis can be diagnosed. However, the SOFA scoring table has complex data that is difficult to apply. For patients with suspected infection, Qsofa (systolic blood pressure <100 mmHg, respiratory rate >22 breaths/min, change of consciousness) is also used. If the score is ≥2 points, sepsis can be diagnosed after further assessment of the organ dysfunction [1]. Progression of sepsis can lead to septic shock, which significantly increases the fatality rate of patients, resulting in poor prognosis. Therefore, early detection and diagnosis of sepsis is necessary for clinical management and treatment.

In sepsis research, interleukin 6 (IL-6), procalcitonin (PCT), C-reactive protein (CRP), and lactic acid (Lac) are the most widely researched biomarkers. However, these biomarkers are not sepsis-specific, and therefore are not ideal for early and accurate diagnosis of sepsis. As an emerging "omics" technology, metabolomics can play a role in the diagnosis of sepsis through combining the current diagnostic standards and commonly used biomarkers [36–38].

To date, several studies have investigated that application of metabolomics in the diagnosis of sepsis. This has been carried out using various models including patients and mice, dogs, horses, fruit flies and other animals. Most studies have focused on finding abnormal metabolites in sepsis to promote early diagnosis of sepsis. For example, Anna M Kauppi used metabolomics method of GC-TOF-MS to analyze blood samples of 65 sepsis patients. The study identified 6 metabolites that could reflect sepsis, of which myristic acid had the highest predictive value. In recent years, there have been more similar studies [39]. Arturas Grauslys used NMR to study 55 infected children (25 bacterial infections, 30 viral infections) and 58 uninfected children undergoing heart surgery. The results showed that metabolomics can distinguish children with infection from children with postoperative inflammation but no infection [40]. James R. Anderson used NMR technology to analyze the synovial tissue of horses. The author concluded that the difference in metabolites can distinguish septic horses from non-septic horses. Moreover, the content of glycine and proline in sepsis horses was significantly increased, which may be a relevant indicator for the diagnosis of sepsis [41]. However, the study findings require further research and verification. Shi-Hui Lin analyzed 31 sepsis patients and 23 healthy controls based on the GC-MS method. The results showed that energy metabolism, amino acid metabolism, and lipid metabolism were lower in patients with sepsis compared with participants in the control group. In addition, energy metabolism was particularly significant [42]. Excessive energy metabolites may be detrimental to the patient's prognosis, and this similar result is by no means accidental. Liu *et al*. [43] used LC-MS metabolomics technology to analyze the arterial blood of 50 cases of sepsis induced by cecal ligation and puncture. The authors found that 13 metabolic regulation substances were mainly involved in the three major metabolisms. D-glucosamine and its phosphorus derivatives gradually declined with the progress of multiple organ failure, which was likely to be a characteristic metabolic marker of sepsis. Yu *et al*. [44] sampled the serum, liver, and lungs of sepsis model mice and sham-operated mice. They employed 1H NMR metabolomics technology for analysis. The results showed that acetate, pyruvate, and lactate were elevated in the serum of septic mice. On the other hand, the levels of alanine, aspartic acid, glutamate, and fumarate decreased in the lungs of the sepsis model mice. Among them, acetate, pyruvate, and lactate showed disturbance in the energy metabolism through the tricarboxylic acid cycle pathway. The authors concluded that changes in alanine and aspartic acid reflected disturbance in amino acid metabolism. Sa Wang divided 57 children with sepsis in the intensive care unit into 27 cases with acute kidney injury and 30 cases without acute kidney injury. Subsequently, they used UPLC-QTOF/MS to analyze the metabolic pathways in their urine samples. The results showed that there was a significant difference in energy, amino acid and lipid metabolism between the acute kidney injury group of sepsis and the group of children with sepsis but without acute kidney injury. They also identified different metabolites and found some possible potential biomarkers. Nevertheless, the potential biomarkers identified at 12 hours and 24 hours were completely different [45]. Metabolomics can also be used to reveal the combinations of differential metabolites which can be used for early diagnosis of sepsis. Presently, there is no biomarker that can be used to diagnose sepsis alone. The use of metabolomics to find a combination of specific biomarkers may help the early diagnosis of sepsis. Moamen Elmassry selected two variables: heat damage and infection with Pseudomonas aeruginosa. The scholar divided them into 4 groups and used GC-MS to analyze the blood of mice. The results revealed that the presence of 26 metabolites. It was further discovered that, of the 26 metabolites, a combination of 5 different metabolites can be used to early diagnose the infection of Pseudomonas aeruginosa in burn patients [46].

The development of sepsis is a complex process that can be triggered by either bacteria, fungi, viruses, or any type of infection, which makes the early diagnosis of the disease difficult [47, 48]. Studies have revealed that the role of metabolomics in sepsis management is not well understood. In addition, potential biomarkers and differential metabolites in such patients or animal models of sepsis have not been profiled. Patients with sepsis are heterogenous in terms of metabolic status and other physiological factors. However, currently, metabolomics is a major milestone in the diagnosis and prognosis of sepsis. The technology has the potential to find differential metabolites, clarify specific biomarkers, and use specific combinations of differential metabolites to enhance early diagnosis of sepsis. Prospect studies should expand the sample size and adhere to strict standards for research.

3.2 Application of metabolomics in the pathogenesis of sepsis

During sepsis, pathogens enter the body and activate immune cells to release various pro-inflammatory and anti-inflammatory factors. In the early stage, the pro-inflammatory reaction produces a strong anti-inflammatory immunosuppressive reaction. Failure of the immune response to suppress the strong inflammatory reaction worsens the patient's condition, leading to a poor prognosis. Currently, there is no clear understanding on the pathogenesis of sepsis. However, inflammatory imbalance, immune disorders, coagulopathy, oxidative stress, endotoxin shift and other mechanisms have been linked to the occurrence of sepsis [49–54]. Recent research has shown that metabolites of metabolic disorders associated with energy, amino acids and lipids are significantly increased in the plasma of patients with sepsis. Incorporating differential metabolites or potential metabolic pathways into metabolomics methods can help to clarify the pathogenesis of sepsis. Lihua Zuo [55] conducted metabolomic analysis on the plasma of septic rats and control rats. On classifying the differential metabolites, they were revealed to involve amino acid metabolism and lipid metabolism only. In a different study, Sarah McGarrity analyzed the serum metabolites of patients with sepsis using the cell endothelial specific metabolism model iEC2812. The results showed that the endothelial metabolism in patients with sepsis was consistent with plasma metabolism. As well, the glucose metabolism in non- surviving patients was significantly up-regulated [56]. Kris M. Mogensen performed metabolomics analysis on blood samples from 85 sepsis patients. The study found that 10 metabolites were associated with malnutrition in patients with sepsis. Of the metabolites, glutathione and purine were involved in cell redox regulation and accelerating tissue adenosine triphosphate (ATP) degradation, respectively [57]. Christopher J. Stewart used LC-MS metabolomics to study blood and stool samples from premature infants with late-onset sepsis and healthy controls. The results showed that the bacterial flora in the blood culture of children with sepsis corresponded to the dominant bacteria of the intestinal flora. The author concluded that the occurrence of late-onset sepsis in premature infants was related to the translocation of intestinal flora [58]. So far, the role of metabolomics in the pathogenesis of sepsis is not well defined and thus, further research is needed to reveal the role of metabolomics in the pathogenesis of sepsis to improve diagnose and treatment of sepsis. Sepsis-related metabolic biomarkers cited in this paper are summarized in Table 1 (Ref. [39, 41–46, 55, 57–64]).

3.3 Application of metabolomics in the treatment and prognosis of sepsis

The most common feature of sepsis is the rapid progression and poor prognosis. The recommended treatment is the "3 h Bundle" plan, although the "1 h Bundle" plan is also used. Early diagnosis, timely and correct intervention are important for improving the prognosis of patients. The application of metabolomics technology in the treatment and prognosis of sepsis has been extensively studied. It has been shown that this technology can decrease mortality rate and improve patient prognosis.

For patients with sepsis, it is important to timely diagnose the infection to initiate early patient treatment and improve prognosis. Several differential metabolite pathways have been applied to evaluate patient prognosis. Few studies have investigated the efficacy of specific drugs used in sepsis treatment, thus more comprehensive research is needed to improve clinical treatment of patient with sepsis. In a study by Henna *et al.* [59], serum samples were collected from 44 sepsis patients and 14 healthy controls. The samples were analyzed using 1H NMR -based metabolomics. A total of 20 non-lipid metabolites were identified. It was found that citrate and lactate were higher in sepsis non-survival group than in the survival group. Previous studies have proved that elevated citrate and its derivatives, acetyl-Coenzyme A and arachidonic acid, can increase levels of nitric oxide and prostaglandins, thereby causing inflammation [65]. Early removal of lactic acid improved the prognosis of patients with sepsis [66]. These results indicate that citrate and lactate reflect poor prognosis of patients with sepsis. In a study involving 33 patients admitted to the intensive care unit, Waqas Khaliq and colleagues divided the patients into four groups: sepsis survival, sepsis death, non-septic survival, and non-septic death groups. Blood samples were collected and analyzed using metabolomics. The results showed that there was a significant difference in lipid metabolism between the sepsis death group and the sepsis surviving group. On the other hand, the metabolism of the non-septic death group and the surviving group had no obvious characteristics [60]. These results imply that patients who die of sepsis may have unique metabolic features. In a related study, Jing Zhu used GC-MS technology to perform metabolomics analysis on 47 sepsis patients and 44 healthy volunteers. In their study, participants were grouped according to the Glasgow Coma Scale (GCS) score. It was found that low GCS scores in patients with sepsis was associated with linear reduction in concentration of 4-HPA (4-hydroxyphenylacetic acid). They postulated that 4-HPA may be an indicator of poor prognosis in patients with sepsis [61].

Metabolomics can also be used to evaluate the prognosis of patients by studying the responsiveness of patients to therapeutic drugs. Charles R. Evans used LC-MS to analyze hematology samples of sepsis patients treated with L-carnitine and placebo-controlled sepsis patients. The results showed that the 1-year mortality rate of patients treated with L-carnitine was significantly smaller than that of participants in the control group (56% versus 75%; *p* = 0.01). Previously, studies have reported that non-surviving patients treated with L-carnitine were related to vasculitis. The metabolites were significantly increased (fibrinopeptide A, lysine, and histamine) [62]. A study by Charles R. Evans showed that metabolomics can be used to evaluate the prognosis of patients with sepsis. Similar to previous studies, the report by Charles *et al.* [62] revealed some vasculitis-related markers can predict drug responsiveness and prognosis of patients. Elsewhere, Li-Wei Liu used LC-MS to analyze the curative effect of biapenem and Xuebijing injection on patients with sepsis. In addition, the author constructed 32 metabolic pathways based on the metabolites found. They found that in sepsis,

TABLE 1. Summary of common metabolomic biomarkers in sepsis.

Metabolite	Sample type	Function	References
Myristic acid	Serum	Discriminate patients with bacterial sepsis and patients with non-bacterial sepsis	[39]
Glycylproline	Synovial fluid	Discriminate sepsis equine SF and healthy controls	[41]
Amino acid, fatty acid, tricarboxylic acid	Serum	Discriminate sepsis patients and healthy controls	[42]
D-glucosamine	Serum	Discriminate sepsis mice and healthy controls	[43]
Acetate, pyruvate, lactate	Serum	Discriminate sepsis mice and healthy controls	[44]
Glycerophospholipid	Urine	Discriminate sepsis children with AKI and sepsis children without AKI	[45]
Trans-4-hydroxypro-line, 5- oxoproline, glycerol-3-galactoside, indole-3-acetate, indole-3-propionate	Serum	Discriminate sepsis caused by *P. aeruginosa* in burn patients and healthy controls	[46]
Amino acid, taurine, phingosine- 1- phosphate	Serum	Reveal underlying therapeutic mechanisms of XBJ on sepsis mice	[55]
Pyroglutamine, hypoxanthine	Serum	Discriminate malnourished and non-malnourished patients in sepsis	[57]
Raffinose, sucrose, acetic acid	Stool	Discriminate preterm neonates with late onset sepsis and healthy controls	[58]
Citrate, 3-hydroxybutyrate, glycine, AGP, histidine	Serum	Discriminate sepsis patients and healthy controls	[59]
Sphingolipid, Lysophoshphatidylcholine, phosphatidylcholine	Serum	Differentiate sepsis survivors from deaths	[60]
4-hydroxyphenylacetic acid	Serum	Discriminate Sepsis-associated encephalopathy (SAE) and healthy controls	[61]
Fibrinopeptide A, allysine, histamine	Serum	Differentiated 1-year survivors of sepsis from nonsurvivors	[62]
Acetate, propionate, butyrate	Cecal contents and serum	Discriminate K. pneumoniae-infected mice and uninfected controls	[63]
Lysophosphatidylcholines, eicosatetraenoic acid, retinol acid, secondary bile acid	Cecal contents	Reduce mortality of sepsis mice by LGG therapy	[64]

Abbreviations: AKI, Acute Kidney Injury; XBJ, Xuebijing; AGP, Acid Glycoprotein; LGG, Lactobacillus rhamnosus GG.

combined application of Biapenem and Xuebijing injection regulated the metabolic pathways more effectively than that of monotherapy, thereby improving the prognosis of sepsis [67]. Ting Wu used GC-MS technology to detect short-chain fatty acids in the serum of mice. Three kinds of single-chain antibodies (acetate, propionate and butyrate) were detected in *Klebsiella pneumoniae*-infected mice at low concentrations. Moreover, mice that were orally supplemented with these three short-chain fatty acids had lower lung bacteria and higher survival rates [63]. The study showed that supplementation of the three fatty acids in patients with sepsis may improve the prognosis of patients and reduce the mortality rate. Chen L applied UPLC-QTOF-MS-based metabolomics to analyze septic mice with cecal ligation and puncture. The analysis showed that *Lactobacillus rhamnosus* GG (LGG therapy) altered bile acid metabolism, lysophosphatidylcholines metabolism, and eicosatetraenoic acid metabolism. In this way, it regulated the intestinal flora to reduce gut microbiota dysbiosis in mice with sepsis [64]. Currently, neither LGG therapy nor short-chain fatty acid supplementation has been applied in the treatment of patients with sepsis. It is expected that using metabolomics technology to evaluate patient prognosis or drug efficacy may help to improve the management of patients with sepsis. Currently, the metabolic features in the biological fluids of septic patients have not been clarified. A literature survey of publications on metabolomics reveals that the metabolic biomarkers reported so far are inconsistent. Recent studies have documented that analysis of death-related metabolic pathways (DRMPs) (Fig. 1) may help to predict the prognosis of sepsis than analysis of blood metabolite biomarkers [68].
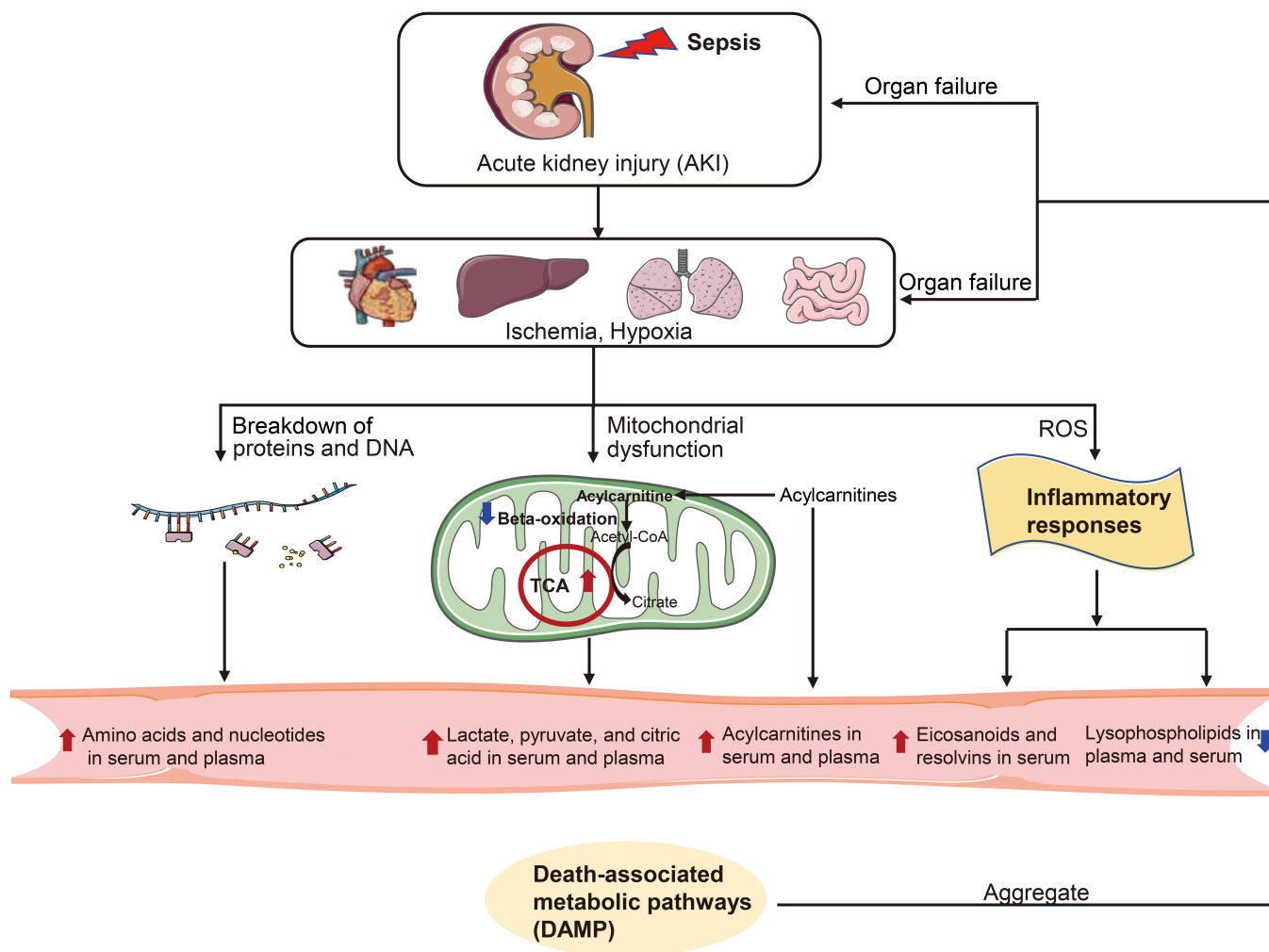
F I G U R E 1. The death-related metabolic pathways (DRMPs) in the blood of sepsis nonsurvivors.

4. Conclusions and perspectives

The application of metabolomics in the diagnosis and prognosis of sepsis is faced with many challenges. This review described the application of metabolomics technology in the diagnosis and treatment of sepsis. Several studies have linked the pathogenesis of sepsis to bacteria, fungus, and viruses and other infections. Metabolomics can use NMR, GC-MS, LC-MS, and other analytical techniques to find metabolic markers or metabolic pathways in patients with sepsis. The most advanced manifestation of any disease is that of changes in metabolites. Metabolomics can reveal changes in metabolites and pathophysiological changes of the body and how the body responds to external intervention. Currently, blood culture is the gold standard method used to identify the cause of infection in patients with sepsis. However, this approach is time-consuming and not effective in all patients. It should be noted that metabolomics analysis techniques are expensive. The aforementioned factors limit the clinical application of metabolomics in sepsis. However, with the continued improvement in the application of metabolomics in sepsis research, it is expected that metabolomics will gain widespread application in the analysis of blood, urine, feces, and other samples. Other omics technologies such as proteomics and transcriptomics can be combined with metabolomics to study the clinical features of sepsis.

AUTHOR CONTRIBUTIONS

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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