Review Article

Received: 2024/03/05 Revised: 2024/05/30 Accepted: 2024/06/03

DOI: https://doi.org/10.15441/ceem.24.211

Advances in metabolomics in critically ill patients with Sepsis and Septic Shock

Swarnima Pandey

1 University of Maryland, School of Pharmacy, Department of Pharmaceutical Sciences, Baltimore, MD, USA.

*Correspondence:
Swarnima Pandey
University of Maryland, School of Pharmacy
Department of Pharmaceutical Sciences
20 N. Pine Street,
Baltimore, MD 21201
Phone: (667) 677-0652
mail: pswarnima@rx.umaryland.edu;p.swarnima@gmail.com
Abstract

Sepsis accounts for high cases of morbidity and mortality in hospitalized patients. It has a very complex pathophysiology and swiftly progresses to a severe form of the disease, such as septic shock leading to organ dysfunction, organ failure, and death. Metabolomics has transformed sepsis's clinical and research topography with its application in prognosis, diagnosis, and risk assessment in patients with sepsis and septic shock. Metabolites in blood and urine are detected and analyzed, which helps in understanding the pathogenesis of the disease and aid in better disease management by identifying biomarkers early on. Metabolomics, sepsis and septic shock were the keywords were searched in PubMed and Scopus, from its inception to Dec 2023. This article provides information regarding metabolic profiling performed in sepsis and septic shock. We demonstrated that metabolomics will change the world of sepsis by analyzing and detecting the diagnosis, prognosis, mortality, and treatment response biomarkers. Keywords: metabolomics, sepsis, septic shock, biomarkers, diagnosis, prognosis, monitor

Capsule summary

Sepsis, with its often devastating consequences for patients and their families, remains a major public health concern that poses an increasing financial burden. Early resuscitation together with the elucidation of the biological pathways and pathophysiological mechanisms with the use of “-omics” technologies have started changing the clinical and research landscape in sepsis. Metabolomics (i.e., the study of the metabolome), an “-omics” technology further down in the “-omics” cascade between the genome and the phenome, could be particularly fruitful in sepsis research with the potential to alter clinical practice. In this review, we present recent developments in metabolomics research in sepsis, giving a comprehensive picture of the past application of metabolomics in sepsis and septic shock. In addition it gives a comprehensive reporting of biomarkers by stratifying the biomarkers into categories like treatment responsive and mortality markers along with the various study grouping as that are utilized for metabolomics studies.
Introduction

Sepsis accounts for high mortality in patients admitted to the intensive care unit. Apart from the physical, it is a substantial financial burden due to prolonged hospital stays. A hyperinflammatory response is followed by an immunosuppressive phase during which multiple organ dysfunctions are present, and the patient is susceptible to infection. In 2016, the Sepsis-3 conference defined sepsis as a "life-threatening organ dysfunction caused by a deregulated host response to infection" and septic shock as a "subset of sepsis in which underlying circulatory and cellular/metabolic abnormalities are profound enough to increase mortality substantially."[1]

Early detection of sepsis is the key to preventing its progression to septic shock, which is associated with a mortality rate of 40%-70% 30%-50%[2, 3]. The golden hours for patient survival are the initial hours post-diagnosis, which requires aggressive treatment strategies. It is reported that those children in whom septic shock is recognized early and adequately treated have a much higher survival rate than children who were diagnosed later. [4]. The early diagnosis of sepsis is critical because mortality increases by 7.6% for each hour that appropriate antimicrobial therapy is delayed [5-7]. Thus, developing diagnostic approaches that might accelerate disease recognition is essential to improve patient outcomes and decrease mortality [8, 9].

Diagnostic criteria for sepsis are nonspecific. Hence identifying specific and sensitive biomarker or a panel will aid in reducing the mortality [10, 11].

In 1940 Roger Williams was the first one to introduce the concept of “metabolic fingerprint” like a characteristic trait of every individual: the term “Metabolomics” was then introduced to describe the scientific discipline that deals with the identification of metabolites that characterize the cellular biological processes. Metabolites are the end products of proteomic and genomic processes. They are the ultimate phenotype, making them the ideal biomarkers for diseases or their progression and identifying the drug's efficacy.

Metabolomics has provided various biomarkers to improve the risk stratification of diabetes and its complications and provide novel insights into its diagnosis, prognosis, and therapeutical targets. In 1984,
Professor Jeremy Nicholson demonstrated the possible use of NMR spectroscopy in the diagnosis of diabetes mellitus[12].

Metabolomics provides a holistic view of the complex metabolic pathway. Since subtle changes in genes and protein can bring about substantial changes in metabolite levels, analysis of metabolite would be a compassionate measure to understand the biological status of an individual. The initial alterations metabolic levels may predict disease severity, and changes observed over the longitudinal time course may help characterize therapeutic response, disease progression, or clinical outcome[13, 14]. These differences and modifications can be associated with biological aberrations that provide clues to the pathogenesis of the disease[15-17]. Metabolomics has the potential to provide a unique insight into metabolic changes in living system[18, 19].

This review provides a snapshot of the gist of metabolic outcomes in sepsis and septic shock categorized as and grouped into five issues, including the application of metabolomics for (1) Animal studies diagnosis, (2) Clinical studies, which includes subcategories (a) critically ill patients with SIRS versus healthy controls, (b) critically ill patients with Sepsis versus healthy controls (c) critically ill patients with Sepsis versus noninfected SIRS (d) critically ill patients with Sepsis versus healthy controls vs SIRS (e) critically ill patients with Sepsis versus ICU controls (f) mortality markers (g) treatment response markers.

**Methodology**

The following databases were searched for references: PubMed, Web of Science, Cochrane Library, and Scopus, from its inception to Dec. 2023. The following terms were utilized: ‘Metabolomics’, ‘critically ill’, ‘sepsis’, ‘septic shock’, and metabolic profiling. There were 98 literature searches of which we utilized 68 to write this review. Our major focus was to include studies involving human samples and for the animal studies, we screened the studies that utilized primates and rat as their animal model.

**2.1 Animal studies**

Studies in animal models of sepsis and septic shock, as shown in Table 1. Studies were designed to induce sepsis in rats by the caecal ligation and puncture (CLP) technique and by using lipopolysaccharide-induced (LPS-induced) endotoxemia [20-23]. Studies using CLP-induced sepsis used plasma for NMR analysis and
sham surgery rats as control [21, 23]. The metabolite difference reported between the study groups mentioned above included alanine, acetoacetate, and formate. Apart from that, the studies by Izquierdo-Garcia et al., 2011, reported an increase in phosphoethanolamine in the sepsis group, while Lin reported an increase in lactate and ketone bodies. The study by Lin's group also demonstrated the mortality markers sepsis. The discriminatory metabolites identified in the study were the outcome of anaerobic and fatty acid metabolism aberrations. The rise in formate is due to the increased synthesis of nucleic acids.

The following study includes LC MS-based analysis of plasma [20, 23] and urine [23] in the septic shock-induced rat. Liu et al. showed that there were four groups of subjects with differential combination-induced septic shock (CLP-induced sepsis and sham burns, sham sepsis and burns, sepsis and burns, and shams of both procedures). The Laiakis group had five study groups: one with LPS-induced endotoxemia, three exposed to radiation, and the last control. Though there was a difference in the study design, there were several similarities in the discriminatory metabolites of sepsis. These were pyrimidines, supporting the role of nucleic acids in sepsis.

Furthermore, they also reported a decline in uric acid. The reduction in uric acid is compatible with reducing purine turnover, supporting the increase in nucleic acids study in septic shock. This study illustrated that five metabolites are correlated to dual pathology (sepsis and burns). These studies between experimental models and control illustrated that energy metabolites significantly affect sepsis and septic shock.

Four additional rat model studies compared the experimental animal model of sepsis and control [24-26]. Li and Hou's group performed an LC-MS-based metabolomics study to explore the metabolic changes of lymph and compared them to plasma and lymphatic pro-inflammatory changes (TNF-α, IL 1β, and IL 6). Metabolites in lymph fluid found characteristic of differentiating septic shock were elevated creatinine, phenylalanine, choline, and vitamin B3, while there was a decline in alanine and dimethylarginine. These results support the utilization of lipids, protein, and amino acids as an alternative to glucose as an energy source in septic shock, as reported in the previously mentioned studies.

Two of the studies used a validation cohort to confirm the findings. Steelman et al. used a cohort of horses for their study. Acute laminitis was the subject of interest frequent in horses with sepsis, considered
equivalent to organ dysfunction in horses. The group used serum before and after the induction of acute laminitis. Metabolites discriminating the before and after-induction of acute laminitis included acylcarnitine and alanine, taurine and aromatic amino acids, ketone bodies, acylcarnitine, saturated FAs, and lysophosphatidylcholine.

Metabolic differences between before and after induction samples were correlated to identify the significance of acylcarnitine and saturated FAs in before but not in after induction sample. Amongst the most significant metabolites of discrimination were citrulline and lactate, and they were subjected to validation in the second cohort of horses. This cohort was divided into two parts based on the outcome. They were labeled sick and advanced and compared to healthy control horses. The citrulline concentration was lower in poor outcome cohort than in the healthy group. Thus, this study identified citrulline as a marker for acute laminitis or non-survivors with a sensitivity of 83% and specificity of 62%.

The study by Langley et al. performed a metabolic analysis of bacteremia. They performed a metabolomic and transcriptomic analysis in plasma samples and tissue samples of liver, lung, spleen, and blood on days 1, 2, and 5 of primates inoculated with E.Coli. Upon comparison, it was observed that there was a decline in lysophosphatidylcholines and an increase in kynurenine, bile acids, and TCA intermediates, which could be used as a marker for prognosis and diagnosis. Transcriptomics data analysis revealed that pathways associated with FA metabolism, BCAA catabolism, and inflammation were altered in sepsis. The study concluded that sepsis non-survivors have metabolic and mitochondrial dysfunction, and the lung is responsible for systemic metabolic responses.

The changes in the plasma metabolome of non-survivors identified the positive correlations between TCA cycle, inflammatory response, apoptosis and kynurenine pathway. Additionally, there were negative correlations between acyl-GPCs and lysophosphatidylcholine acyltransferase 2 (LPCAT2)[27]

A regression model was built utilizing lysophosphatidylcholine 1 stearoyl GPC, sulfated bile acid and isovaleryl carnitine. The AUROC curve of the model's ability to differentiate infection from non-infection in this primate cohort. The metabolite model was also shown to be capable of diagnosing sepsis in two
human cohorts: registry of critical illness (RoCI) cohort[28] and Community-Acquired Pneumonia and sepsis Outcome Diagnostics (CAPSOD) cohort[29].

Validation in both cohorts signifies that Fatty acid and amino acid metabolism are correlated with mortality. Other previously reported studies needed more assurance in the exploratory cohort.

Li, Liu et al. aimed to examine the metabolic aberrations associated with the two herbal remedies in a mouse model. The remedies used were called LXHX and QRJD. They used three groups of CLP-induced sepsis and control. A total of 18 metabolites related to energy metabolism, lipid transport, and amino acid were identified.

Another study by Zhang et al. (2019) illustrated that lysine supplementation in septic mice would lead to less inflammation and less hypotension than placebo[30].

The studies mentioned above are consistent with the aberrations in fatty acid and amino acid metabolism to be used as potential biomarkers for diagnosis and mortality.

2.2 Clinical Studies

2.2.1 Critical ill patients with SIRS versus healthy controls

Metabolic profiling in critically ill patients were first performed in trauma patients [31, 32], as shown in Table 2. It includes two study groups: uninfected SIRS and multi-organ failure patients (MOFs); and survivors, and non-survivors of septic shock.

SIRS was correlated with increased BCAA and glucose in the work by Wang et al, while MOFs correlated with increase creatinine, lactate and free fatty acids. The following study by Cohen et al. reported increased lipids and glucose, ketone bodies, and lactate in non-survivors. Above mentioned studies have illustrated the correlation of lactate and lipids with non survivors.

Work by Park included using albumin in treating acute lung injury (ALI).[33]. It was reported that there was an improvement in oxygenation in treatment with albumin compared to placebo. The study was performed by analyzing the metabolomics of the two groups and the metabolic profiles on days 1, 2, 3, and 7, wherein comparisons were made between the two groups and healthy controls. Statistical analysis failed to illustrate any difference in the metabolic profiles of the two groups initially but shows differences from
day 2 onwards. The study reported metabolic differences between the treatment groups, with the elevation of albumin on day 2, LDL and alanine on day 3, and cholesterol on days 2 and 3. The influence of time on the concentration of discriminatory metabolites was assessed. This analysis correlated HDL, alanine, and valine in the albumin group. This provided an insight into the role of these metabolites in the pathogenesis of the diseases. This study also established the significance of serial studies in tracking the metabolic changes related to MOD and clinical outcomes.

The predictive ability of the metabolites was also demonstrated by day 7 of the albumin treatment group. The clustering of the patients with ALI and other underlying disease conditions from both the treatment groups illustrates that the response to ALI has more effect on metabolic profiles than etiology.

2.2.2 Critically ill patients with sepsis versus healthy controls

Review by Pandey et al have presented a integration of clinical data with metabolomics to provide means to understand the patient's condition, stratify patients better, and predict the clinical outcome [34].

Serum and plasma are the two standard biomaterial samples; however, one study used erythrocytes in addition to plasma [35]. One of the earlier works of metabolomics in sepsis was done by Stringer et al. [36]. The study reported a decline in sphingomyelin and elevation in adenosine, glutathione, and phosphatidylserine in ALI compared to healthy control. The pathways obliterated were associated with oxidation, apoptosis and energy utilization. The metabolites related to energy utilization are from the previous animal model studies with the reported levels of pyruvate, ketone bodies, and FA metabolism.

The following study by Bruegel et al. in 2012 was whole blood-based metabolomics using LC-MS-MS [37]. The study included LPS-activated and non-activated entire blood samples which identified amino acids, five arachidonic acids, and two cyclooxygenase metabolites. The LPS-activated blood samples were elevated at a lesser rate in amino acids and two cyclooxygenase metabolites compared to healthy control. A more significant increase in these metabolites between the two groups was associated with favorable clinical outcomes at day 14 and reduced disease severity.
Non-targeted metabolomics was performed by Liang et al [38]. The identified biomarkers as sphingosine, 5-methylcytidine, and 3-dehydrocarnitine. Their study demonstrated that metabolomics can be used for the early diagnosis of septic shock.

A study by Jaurila et al. validated previous findings of septic shock biomarkers [39]. They reported elevated levels of creatinine, 3-hydroxybutyrate, glycoprotein, and glycine and a decline in the concentration of citrate and histidine in septic shock.

Works by Pandey et al. illustrated that diabetes and hypertension[40], gender[41], and progression of the disease has a characteristic biomarker that can be assessed in the serum samples of patients with sepsis and septic shock[42].

A recent study by Li et al. 2023 [43] and Chen et al. identified biomarker with the predictive ability of sepsis and septic shock.

**2.2.3 Critically ill patients with sepsis versus non-infected SIRS**

The previously reported metabolomics studies using NMR and LC-MS-MS are pioneers in the metabolic profiling of sepsis in clinical settings. They are now followed by several other studies to compare the response of sepsis with different controls.

Schmerler et al. (2012) performed the first of these studies using LC MS-based plasma metabolomics[44]. They demonstrated the metabolic difference between sepsis and non-infected SIRS. They reported acylcarnitine and glycerophosphatidylcholines as discriminatory markers of sepsis.

An NMR based plasma metabolic analysis by Blaise, 2013, was performed utilizing sepsis in trauma patients [45]. The study illustrated the elevation of TCA intermediates, BCAA ketone bodies, and allantoin. Allantoin is one of the markers of oxidative stress because, under homeostasis, the end product of purines is uric acid, which is converted by reactive oxygen species (ROS) to allantoin. Thus, oxidative stress is responsible for increasing allantoin and decreasing uric acid, as shown in an experimental model-based study by Liu et al., discussed in section 2.1.

The role of lipids in the differentiation of these groups is in accordance with the two trauma cases mentioned above, which also reported lipids like glycerolipids and fatty acids as significant metabolites of
discriminatory potential and as a mortality marker in sepsis and septic shock. Stringer et al. also demonstrated glutathione as a potential biomarker of ALI-induced sepsis.

The Community-Acquired Pneumonia and sepsis Outcome Diagnostics (CAPSOD) cohort was utilized by Kamisoglu, Calvano, et al., in 2015, to identify the biomarkers of sepsis. This study illustrated that the LPS-induced endotoxemia and sepsis groups had 16 significant metabolites, whereas 18 were found to be significant between non-infected SIRS and LPS-induced endotoxemia. Metabolites common in endotoxemia and sepsis were 2 hydroxybutyrates, mannose, bilirubin and lipids. Acylcarnitine was identified as a mortality marker.

Upon examining survivors, non-survivors and LPS induced endotoxemia group, there were variations in 19 metabolites between LPS induced endotoxemia and survivors that could differentiate between positive and negative clinical outcomes. The results collaborated with the hypothesis that LPS-induced endotoxemia included an induction stage followed by a recovery stage that mirrored responses required for host survival, and it is different from the adaptive response of sepsis associated with mortality. This study also supports the potential of metabolic profiling in distinguishing the infected and non-infected SIRS.

The following milestone study by Langley in 2013 [10]. The study's primary objective was to identify the mortality markers of sepsis. Acyl carnitine was found to be significant in distinguishing sepsis survivors and non-survivors. There were comparisons between sepsis survivors and patients with non-infective SIRS, which reported a decline in citrate, malate, amino acids, and carnitine esters and an increase in six acetaminophen catabolites in sepsis survivors.

Another cohort, Genetic and Inflammatory Markers of Sepsis (GenlMS), which were analyzed using LC-MS-based metabolomics by Seymour et al [46]. Statistical analysis was performed to distinguish sepsis survivors and non-survivors (with 90-day survival) to identify metabolites of oxidative stress, bile acid, nucleic acid, and stress. Amongst all, pseudouridine was highly significant.

In conclusion, all these studies explored and reported variation in patients with sepsis and non-infected SIRS. The significant metabolites included a decrease in BCAA and an increase in non-BCAA, ketone
bodies, and intermediates of TCA. In studies about mortality, non-survivors were characterized by decreased glycerophospholipids and increases in acylcarnitines, nucleic acids, and ketone bodies.

Another study in 2016 compared the metabolic profiles based on the type of infection [47]. The comparison was made between SIRS and sepsis, with patients divided into test and confirmation cohorts. Patients were stratified according to the type of infection: patients with SIRS, community-acquired pneumonia, urinary tract infection, intraabdominal disease, and bloodstream infection. The study reported alterations in acylcarnitine, glycerophospholipids, and sphingolipids in sepsis compared to SIRS. They illustrated that certain specific metabolites could be used for distinguishing the different types of infections. They also analyzed the metabolic profiles of the patients' poor and good clinical outcomes and reported that variations of metabolites depended upon the underlying type of infection.

A recent study by Feng et al. [48] identified the metabolite as biomarkers along with their correlation with clinical variable. Ultimately, succinic acid semialdehyde, uracil, and uridine were shown to be validated with greater potential to be applied in the clinical setup for the diagnosis of multiple traumas complicated with sepsis.

2.2.4 Critically ill patients with sepsis versus SIRS and healthy control

One of the studies employs metabolic analysis of sepsis and septic shock in a pediatric population, wherein serum samples are collected from septic shock, SIRS, and healthy control[4]. The group performed a non-targeted metabolomics approach to identify 2 hydroxybutyrate, lactate, histidine, phenylalanine, and arginine as discriminatory metabolites for septic shock from non-infected SIRS and healthy control.

The third study was performed between patients with sepsis, non-SIRS, and healthy control [49]. Analyzing the group with non-SIRS and sepsis demonstrated a statistical decrease in lactitol dehydrate and S-phenyl-d-cysteine and an increase in S-(3-methylbutanoyl)-dihydrolipoamide-E and N-non-anoyl glycine in sepsis compared to the non-infected SIRS. Metabolites associated with the severity of sepsis and mortality were also examined. The results stated a decline in phospholipid, Ne-dimethyl-lysine, intermediates of phenylalanine metabolism, and cysteine were associated with sepsis severity. In contrast, S-(3-
methylbutanoyl)-dihydrolipoamide-E, glycerophosphocholine, and S-succinyl glutathione were identified as mortality markers in sepsis.

2.2.5 Critically ill patients with sepsis versus ICU controls

Bacteremic sepsis and ICU control were used by Kauppi, Alicia Edin, et al., 2016, using the whole blood from patients [50]. The study identified and validated the metabolites, while reducing the number of metabolites at each stage until final 6 significant metabolites were obtained. Of the six, myristic acid was the most significant metabolite predictive of sepsis, with a sensitivity of 1.00 and specificity of 0.95. Myristic acid was also examined against conventional laboratory and clinical parameters to perform better than various combinations.

Mickiewicz in 2014 demonstrated that 1H NMR could be used as a diagnostic tool for septic shock [51, 52]. They reported the metabolites that can be used as a biomarkers of septic shock and as mortality markers. Most of the identified metabolites were related to energy metabolism. They reported a decline in glutamine, glutamate, BCAA and arginine while an elevation in aromatic amino acids and proline in septic shock patients.

2.2.6 Mortality biomarkers of septic shock

There are several studies reporting the mortality markers of sepsis and septic shock. Non survivors of sepsis was reported to be associated with increased amino acids, ketone bodies and decrease in FA metabolites. [53].

Ferrario et al. designed a study between 28-day and 90-day mortality groups [54]. Spermidine, putrescine, kynurenine, and glucogenic amino acids are elevated and phosphatidylcholines and lysophosphatidylcholines declined in non-survivors of septic shock. Their study illustrated a link between high polyamines to demonstrate a link between pathogens and the host immune system. The study reports that tryptophan catabolism and lipids influence the mortality of septic shock.

Another targeted metabolomics study was performed for lipid profiling and its association with mortality in septic shock [55]. The study demonstrated a significant elevation in prostaglandin F2a and leukotriene B4 in non-survivors. The association of acetylcarnitine with sepsis and its mortality was also studies [56].
They identified that to predict a 28 day mortality risk, non survivors would have elevated plasma acetylcarnitine.

A study by Rogers et al. was designed to identify the metabolites related with 28-day mortality using two different cohort. They identified gamma-glutamyl phenylalanine, gamma-glutamyl tyrosine, 1-arachidonoylGPC(20:4), taurochenodeoxycholate, 3-(4-hydroxyphenyl) lactate, sucrose, kynurenine[57].

Liu et al performed their mortality study in septic shock survivors and non survivors using 0hr and 24 hr serum samples.[59]. The serum collected at the time of admission (0hr) showed the difference in metabolic profiles of survivors and non-survivors. They illustrated an elevation in creatinine, energy metabolites, and amino acid levels and down-regulation in glycoprotein concentration during the evolution from 0hrs to 24 hrs for non-survivors. Significant metabolites reported causing the distinction of septic shock survivors from non-survivors during 24 hrs were alanine, glutamate, lactate, pyruvate, N-acetyl glycoprotein, and citrate. The study demonstrates that monitoring the relevant metabolites could help analyze the patients' early therapeutical response.

21 cohorts with 1287 individuals and 2509 metabolites were utilized by Wang et al to identify amino acids, mitochondrial metabolism, eicosanoids, and lysophospholipids as a biomarker for sepsis in meta analysis.[60].

Another unique study by Garcia Simon's group worked with 0hr and 24hr urine sample to identify arginine, methionine, phenylalanine hippurate, and ethanol as markers of mortality[61]. Phenylalanine and leucine was used for risk stratification in patients with sepsis and septic shock by Huang et al. Another high risk marker, Symmetrical (SDMA) and asymmetrical dimethylarginine (ADMA) were identified in sepsis by Winkler et al.[62][63]

Alice et al.[64] studies revealed a dynamic change in metabolite levels over the study period in severe septic shock patients stratified for mortality. Meanwhile, Evans et al.[65] demonstrated a decreased phenylalanine in septic shock non-survivors at one year.

2.2.7 Treatment Response Biomarkers in Sepsis and Septic
There are two studies reported to monitor the treatment response in sepsis[66, 67]. The first study included patients receiving L-carnitine supplementation [66]. Patients with a good response had a low level of carnitine and acetylcarnitine, where methionine, lysine, phenylalanine, and tyrosine were found to be increased after L-carnitine was given [66]. The second study categorized septic shock patients based upon the sequential organ failure assessment (SOFA) score as responders and non responders [67]. It was observed that myristic acid and oleic acid declined more while creatinine declined comparatively less in responders than the non-responders. Over the time, it was observed that kynurenine was increasing in responders but lower in non-responders.

Another study by Pandey et al. 2023 illustrates the use of metabolomics in monitoring the treatment efficacy during the hospital stay due to sepsis and septic shock. [68]. These findings suggest that metabolomics can be used to monitor treatment response in sepsis effective in patient management.

**Limitations and future prospective of metabolomics in Sepsis and Septic shock**

The current advanced metabolomics technology is just shining the light on precise biomarkers for sepsis. There needs to be more standardization amongst the metabolomic studies, which is one of the significant issues for replicating the results. Moreover, the complexity and heterogeneity of sepsis create a large variety of study populations. Therefore, combining conventional biomarkers and metabolomic profiling is likely a fundamental solution. Moreover, multi-omics should be approached simultaneously since sepsis is also associated with the alterations of protein and gene expression [69, 70]. Metabolomics can be utilized to identify mitochondrial dysfunction or aberrations of the microcirculation in sepsis. Future studies with the metabolomics approach, the aim is to develop bedside laboratory kits, which is significant for clinical practice. Metabolomics to predict outcomes [70] of patients are undergoing research. Identifying mortality markers at an early stage is another remedy to improve patients' outcomes. The integral application of pharmacometabolomics to determine the appropriate drugs or to certify suitable, well-responsive patients is another approach to precision medicine.

Due to the overwhelming metabolomic information in sepsis, artificial intelligence facilitating machine learning experiences may be a utilized to handle this enormous amount of data.
Conclusion

In conclusion, this metabolic profiling of sepsis has established that metabolomics has the potential to be used as a diagnostic and prognostic tool capable of early diagnosis, prognosis, severity determination, and predicting mortality. There are numerous works done in metabolomics-based characterization of biomarkers, but bringing them together under one heading gives us an understanding of what experience of metabolomics has been in sepsis and septic shock, thus helping us better understand what needs to be done in the future for better patient management in critical care.

Declaration

Ethical Approval and Consent to Participate: N/A
Consent for publication: There is no conflict of interest in publication.
Availability of data: N/A
Funding: N/A
Authors’ contributions and materials: Swarnima Pandey collected the articles, compiled and drafted the review
Competing interests: There are no competing interest
Acknowledgments: Swarnima Pandey for the collection of articles and drafting the manuscript.
Conflicts of Interest: Authors have no conflicts of interest to declare.
References


[34] S. Pandey, Sepsis, Management & Advances in Metabolomics, Nanotheranostics 8(3) (2024) 270-284.


[42] S. Pandey, A. Azim, N. Sinha, Longitudinal NMR Based Serum Metabolomics to Track the Potential Serum Biomarkers of Septic Shock, Nanotheranostics 7(2) (2023) 142-151.


<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Platform used</th>
<th>Subjects</th>
<th>Metabolite identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lin et al</td>
<td>2009</td>
<td>^1H NMR</td>
<td>a. CLP -induced sepsis survivors</td>
<td>↑ alanine, formate, lactate, acetoacetate, hydroxybutyrate and acetate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b. CLP induced sepsis non-survivors</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c. Sham controls</td>
<td></td>
</tr>
<tr>
<td>Liu et al</td>
<td>2010</td>
<td>LC-MS/MS</td>
<td>a. CLP induced sepsis</td>
<td>(1) Sepsis and burns single pathology groups:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b. CLP induced sepsis and burns</td>
<td>↓ uric acid and bile acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c. Burns</td>
<td>(2) Sepsis and dual pathology groups:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>d. Sham of CLP induced sepsis and burns</td>
<td>↑ Uracil and nitrotyrosine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(3) Exclusively in dual pathology group:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↑ hypoxanthine; indoxyl sulphate tryptophan; ↑ in glucuronic acid and gluconic acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>and proline↑</td>
</tr>
<tr>
<td>Izquierdo-</td>
<td>2011</td>
<td>^1H NMR</td>
<td>a. CLP induced sepsis</td>
<td>↑ alanine, formate, acetoacetate, creatine and phosphoethanolamine and myoinositol</td>
</tr>
<tr>
<td>Garcia et al.</td>
<td></td>
<td></td>
<td>b. Sham surgical controls</td>
<td></td>
</tr>
<tr>
<td>Laiakis et al</td>
<td>2012</td>
<td>LC-MS/MS</td>
<td>a. LPS endotoxin model</td>
<td>(1) LPS endotoxin group</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b. Radiation with 3Gy of γ rays</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Year</td>
<td>Method</td>
<td>Group</td>
<td>Findings</td>
</tr>
<tr>
<td>-------</td>
<td>------</td>
<td>--------</td>
<td>-------</td>
<td>----------</td>
</tr>
</tbody>
</table>
| Li et al. | 2012 | LC-MS/MS | a. CLP-induced sepsis | Sepsis versus non-infected sham controls:  
↑ palmitoyl-L-carnitine, creatinine, phenylalanine, isonicotinic acid; choline 5-azacytidine and ↓ 1-O-Hexadecyl-2-lyso-glycero-3-phosphorylcholine, alanine, 4-amino-5-hydroxymethyl-2-methylpyrimidine, asymmetric dimethylarginine |
| Li et al | 2013 | ¹H NMR | a. CLP-induced sepsis model | Sepsis versus non-infected surgical sham:  
↑ isobutyrate, 3-hydroxybutyrate, alanine, acetate, lactate and glucose; ↑ TAG and FAs, ↓ proline, taurine, valine, isoleucine, arginine, lysine and ↑ threonine; ↓ choline and trimethylamine N-oxide |
| Steelman et al | 2014 | LC-MS/MS | Cohort 1: | Pre versus post-induction of acute laminitis:  
↑ acylcarnitine and amino acids including alanine, kynurenine, taurine and aromatic amino acids |
Cohort 2:
a. Poor outcome  
b. Good outcome  
c. Healthy controls

(1) Combined good and poor outcome groups versus healthy control:  
↓ citrulline

(2) Citrulline as a predictive marker of the development of acute laminitis or non-survival

(3) Poor outcome and good outcome versus healthy control:  
↑ alanine, valine, glycine, and ↓ serine in the good and poor outcome groups compared to healthy controls.

(4) Poor outcome and good outcome versus healthy controls:  
glycine differed significantly between the two outcome groups.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Method</th>
<th>Study Design</th>
<th>Results</th>
</tr>
</thead>
</table>
| Langley et al. | 2014 | LC-MS/MS       | Inoculation of primates with E. coli                                          | Sepsis non-survivors versus survivors and differentiation of sepsis versus healthy control:  
↓ acyl-GPCs, and↑ kynurenine, bile acids, carnitine, TCA |

**Table 1:** Animal metabolic profiling studies of sepsis. KEY: CLP: caecal ligation and puncture, TCA: tricarboxylic cycle, GPC: glycerophospholipids, TAG: triacylglycerides, FAs: fatty acids, LC-MS/MS: liquid chromatography coupled mass spectrometry, NMR: nuclear magnetic resonance.
<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Platform used</th>
<th>Subjects</th>
<th>Metabolite identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mao et al</td>
<td>2009</td>
<td>$^1$H NMR</td>
<td>a. Severe trauma and SIRS b. Severe trauma and MODs c. Healthy controls</td>
<td>MODS vs SIRS and both trauma vs healthy controls: MODS: ↑ free fatty acids, glycerol, creatinine and lactate; SIRS:↑ amino acids (predominantly BCAAs) and glucose.</td>
</tr>
<tr>
<td>Cohen et al</td>
<td>2010</td>
<td>$^1$H NMR</td>
<td>a. Severe trauma survivors b. Severe trauma and non-survivors c. Healthy controls</td>
<td>Survivor vs non-survivors and both trauma vs healthy control: ↑glucose, glutamate, ketone bodies, lactate, TAGs, mono-unsaturated fatty acids and glycerophospholipids.</td>
</tr>
<tr>
<td>Stringer et al</td>
<td>2011</td>
<td>$^1$H NMR</td>
<td>a. Sepsis related ALI b. Healthy controls</td>
<td>Sepsis related ALI vs healthy control ↑ adenosine, glutathione and phosphatidylserine; ↓ sphingomyelin</td>
</tr>
<tr>
<td>Study</td>
<td>Year</td>
<td>Method</td>
<td>Group</td>
<td>Findings</td>
</tr>
<tr>
<td>---------------------------</td>
<td>------</td>
<td>------------</td>
<td>------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Bruegel et al</td>
<td>2012</td>
<td>LC-MS/MS</td>
<td>a. Sepsis, b. Healthy controls</td>
<td>LPS activated whole blood vs healthy controls: ↓ in AA, PGE2, 11-HETE; TXB2</td>
</tr>
<tr>
<td>Schmerier et al</td>
<td>2012</td>
<td>LC-MS/MS</td>
<td>a. Sepsis, b. Non-infected SIRS</td>
<td>Sepsis vs non-infected SIRS: ↑ acyl-carnitines and glycerophosphatidylcholines: and a diacyl-glycerophosphatidylcholine</td>
</tr>
<tr>
<td>Langley et al</td>
<td>2013</td>
<td>LC-MS/MS</td>
<td>a. Sepsis non-survivors, b. Sepsis survivors, c. Non-infected SIRS</td>
<td>(1) Sepsis non-survivors vs survivors: ↑ 17 amino acid catabolites, 16 carnitine esters, 11 nucleic acid catabolites, citrate, dihydroxyacetone, malate, pyruvate, four free fatty acids; in addition to ↓ seven GPCs and GPE, ↑ lactate, and acyl-carnitines (2) Sepsis survivors vs non-infected SIRS: ↓ citrate and malate, glycerol, glycerol 3-phosphate, phosphate, 21 amino acids and their catabolites, 12 GPCs and GPE esters, and six carnitine esters</td>
</tr>
<tr>
<td>Authors</td>
<td>Year</td>
<td>Methodology</td>
<td>Conditions</td>
<td>Changes</td>
</tr>
<tr>
<td>------------------</td>
<td>------</td>
<td>-------------</td>
<td>--------------------------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Blaise et al</td>
<td>2013</td>
<td>¹H NMR</td>
<td>a. Trauma + sepsis b. Trauma - sepsis</td>
<td>Trauma + sepsis vs trauma – sepsis: ↑ aspartate, citrate, valine,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>hydroxybutyrate and allantoin</td>
</tr>
<tr>
<td>Seymour et al</td>
<td>2014</td>
<td>LC-MS/MS</td>
<td></td>
<td>Sepsis non survivors vs survivors: ↑ taurochenolate sulfate and</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>glycochenolate sulfate; ↑ cortisol, cortisone, and sulfated hormones</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>allantoin, N1-methyladenosine, N2, N2-dimethylguanosine, N6-carbamoyl</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>threonyladenosine and pseudouridine</td>
</tr>
<tr>
<td>Su et al</td>
<td>2014</td>
<td>LC-MS/MS</td>
<td>a. Severe sepsis b. Uncomplicated sepsis c. Non-infected SIRS d. Healthy controls</td>
<td>(1) Sepsis vs non infected SIRS: ↓ lactitol dehydrate and S-phenyl-d-cysteine and ↑ in S-(3-methylbutanoyl)-dihydrolipoamide-E and N-non-anoyl glycine. (2) Severe sepsis vs uncomplicated sepsis: ↓ glyceryl-phosphoryl-ethanolamine, Ne, Ne-dimethyl-lysine, phenylacetamide and d cysteine (3) death within 24 hours:</td>
</tr>
<tr>
<td>Study</td>
<td>Year</td>
<td>Methodology</td>
<td>Groups</td>
<td>Observations</td>
</tr>
<tr>
<td>------------------------------</td>
<td>------</td>
<td>-------------</td>
<td>------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| Kamisoglu et al              | 2015 | LC-MS/MS    | a. Sepsis non-survivors  
  b. Sepsis survivors  
  c. LPS induced endotoxemia in healthy control  
  d. Non infected SIRS | (1) Sepsis and healthy control vs LPS induced endotoxemia vs non infected SIRS:  
  ↑ 2-hydroxybutyrate, mannose, bilirubin and lipids  
 (2) Sepsis survivors vs non-survivors:  
  ↑ acyl-carnitines were the most discriminatory metabolites |
| Mickiewicz et al             | 2013 | ^1H NMR     | a. Septic shock  
  b. SIRS  
  c. Healthy control | Discriminatory metabolite between septic shock, SIRS and healthy control:  
  2 hydroxybutyrate, lactate, histidine, phenylalanine and arginine |
| Mickiewicz et al             | 2014 | ^1H NMR     | a. Septic shock  
  b. ICU controls | Discriminatory metabolite between survivors and non survivors of sepsis |
<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Methodology</th>
<th>Study Type</th>
<th>Discriminatory metabolite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garcia-Simon et al</td>
<td>2015</td>
<td>$^1$H NMR</td>
<td>a. Septic shock</td>
<td><strong>Discriminatory metabolite between survivors and non survivors of sepsis</strong> Arginine, methionine, glutamine, phenylalanine, glucose, ethanol, and hippurate showing differences between non-survivor/survivor</td>
</tr>
<tr>
<td>Liu et al</td>
<td>2016</td>
<td>LC-MS/MS</td>
<td>a. Septic shock</td>
<td><strong>Discriminatory metabolite:</strong> 43 significant metabolites varied in their levels when compared between survivors with non-survivors. Six primary discriminators: Valine, leucine, isoleucine, citrulline, carnitine 2:0, and betanine</td>
</tr>
</tbody>
</table>
| Ferrario et al   | 2016 | LC-MS/MS            | a. Septic shock | **Upregulated in non-survivors:** Polyamines, glucogenic amino acids, and kynurenine  
**Downregulated in non-survivors:** phosphatidylcholines and lysophosphatidylcholines |
<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Methodology</th>
<th>Condition</th>
<th>Discriminatory metabolite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neugebauer et al.</td>
<td>2016</td>
<td>LC-MS/MS</td>
<td>a SIRS, b Sepsis</td>
<td>acylcarnitines, glycerophospholipids and sphingolipids were altered in sepsis compared to systemic inflammatory response syndrome.</td>
</tr>
<tr>
<td>Cambiaghi et al</td>
<td>2018</td>
<td>LC-MS/MS</td>
<td>a. Septic shock</td>
<td>Alteration in the lipidome of non-survivors was found. PCaa C42:6, PCaa C40:6, and lysoPC species</td>
</tr>
<tr>
<td>Dalli et al</td>
<td>2017</td>
<td>LC-MS/MS</td>
<td>a. Septic shock</td>
<td>Elevation in the levels Prostaglandin F2α, leukotriene B4, resolvin E1 resolvin D5, and 17R-protectin D1 were found in non-survivors.</td>
</tr>
<tr>
<td>Chung et al</td>
<td>2019</td>
<td>UHPLC-MS</td>
<td>a. Septic shock</td>
<td>A significantly higher level of plasma acetylcarnitine was found in sepsis non-survivors when compared with survivors.</td>
</tr>
<tr>
<td>Liu et al</td>
<td>2019</td>
<td>1H NMR</td>
<td>a. Septic shock</td>
<td>The concentrations of alanine, glutamate, glutamine, methionine, aromatic amino acids, ketone</td>
</tr>
</tbody>
</table>
bodies, 3-hydroxybutyrate, and acetate were increased in the non-survivors as compared to the survivors. N-acetyl glycoprotein level was found decreased in non-survivors.

| Jaurila et al. 2020 | 1H NMR | a. Sepsis  
| b. Healthy control | **Discriminatory metabolite:**  
| | | Significantly higher serum lactate and citrate concentrations in non-survivors compared with survivors. |

| Reisinger et al. 2021 | 1H NMR | a. Sepsis  
| b. Healthy control | **Discriminatory metabolite:**  
| | | BCAA(valine, leucine, isoleucine, 3-hydroxybutyrate) was significantly lower in sepsis non-survivors. |

| Liang Q et al. 2016 | LC-MS/MS | a. Septic shock  
| b Healthy control | **Discriminatory metabolite:**  
| | | Sphingosine, 5 methylcytidine, 3 dehydrocarntine, 4 acetamido-2-aminobutanoic acid and phenyllactic acid in the Septic shock subjects were significantly different from the controls. |

| Feng et al. 2022 | LC-MS/MS | a Multiple trauma (non SIRS)  
| b Sepsis | **Discriminatory metabolite:**  
<p>| | | Nine potential biomarkers, namely, acrylic acid, 5- |</p>
<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Method</th>
<th>Samples</th>
<th>Discriminatory metabolite:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mecatti et al.</td>
<td>2018</td>
<td>LC-MS/MS</td>
<td>a Septic shock, b Healthy control</td>
<td>Fatty acids and phospholipids detected in plasma and erythrocytes could signal sepsis vs. non-sepsis. Lyso-PCs and SMs were downregulated, whereas the saturated and unsaturated phosphatidylcholines (PCs) were upregulated in the plasma and erythrocytes of septic patients.</td>
</tr>
<tr>
<td>Pandey et al.</td>
<td>2020</td>
<td>¹H NMR</td>
<td>a Septic Shock, b Healthy control, Septic shock with co morbid conditions (diabetes and hypertension), Septic shock with primary diagnosis (respiratory illness and encephalopathy)</td>
<td>The potential biomarkers for septic shock are lactate, 3 hydroxybutyrate, 3 hydroxyisovalerate, proline, 1,2 propanediol, creatine, glycine, phenylalanine, and myoinositol, bile, NAG, and VLDL, which were significantly upregulated in septic shock patients, whereas citrate, carnitine, HDL, LDL,</td>
</tr>
</tbody>
</table>
and lipoprotein with phosphocholine head group were downregulated in septic shock patients.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Methodology</th>
<th>Group Comparison</th>
<th>Discriminatory metabolite:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pandey et al.</td>
<td>2023</td>
<td>$^1$HNMR</td>
<td>a Septic shock pre treatment b Septic shock post treatment</td>
<td>The study showed time-dependent metabolite alteration in ketone bodies, amino acids, choline, and NAG in patients undergoing treatment.</td>
</tr>
<tr>
<td>Cambiaghi A et al.</td>
<td>2017</td>
<td>LC-MS/MS</td>
<td>Treatment response in Septic shock</td>
<td>Lipidome alterations play an important role in individual patients' responses to infection. Furthermore, alanine indicates a possible alteration in the glucose-alanine cycle in the liver, providing a different picture of liver functionality from bilirubin.</td>
</tr>
<tr>
<td>Kauppi et al.</td>
<td>2016</td>
<td>LC-MS/MS</td>
<td>a Sepsis b Healthy control</td>
<td>Six metabolites were identified for bacteremic sepsis.</td>
</tr>
<tr>
<td>Cambiaghi A et al.</td>
<td>2018</td>
<td>LC – MS/MS</td>
<td>a Septic shock</td>
<td>Identified circulating lipids and coagulation cascade in septic shock progression</td>
</tr>
<tr>
<td>Authors</td>
<td>Year</td>
<td>Method</td>
<td>Group</td>
<td>Discriminatory metabolite</td>
</tr>
<tr>
<td>------------------</td>
<td>------</td>
<td>------------</td>
<td>--------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Winkler et al.</td>
<td>2018</td>
<td>LC-MS/MS</td>
<td>Sepsis</td>
<td><strong>Discriminatory metabolite:</strong> SDMS and ADMA associated with sepsis mortality</td>
</tr>
<tr>
<td>Huang et al.</td>
<td>2019</td>
<td>LC-MS/MS</td>
<td>Septic shock</td>
<td><strong>Discriminatory metabolite:</strong> Phenylalanine- and leucine-defined risk classifications provide metabolic information with prognostic value for patients with severe infection.</td>
</tr>
<tr>
<td>Li et al.</td>
<td>2023</td>
<td>LC-MS/MS</td>
<td>a Sepsis</td>
<td><strong>Discriminatory metabolite:</strong> 3-phenyl lactic acid, N-phenylacetylglutamine, phenylethylamine, traumatin, xanthine, methyl jasmonate, indole, L-tryptophan</td>
</tr>
<tr>
<td>Cheng et al.</td>
<td>2022</td>
<td>LC-MS/MS</td>
<td>a Sepsis</td>
<td><strong>Discriminatory metabolite:</strong> Seventy-three differentially expressed metabolites that could predict sepsis were identified.</td>
</tr>
<tr>
<td>Pandey et al.</td>
<td>2022</td>
<td>1H NMR</td>
<td>a Septic shock survivor (M/F)</td>
<td><strong>Discriminatory metabolite:</strong> The energy-related metabolites, ketone bodies, choline, and NAG were found to be primarily responsible for differentiating survivors and non-survivors. The gender-based mortality stratification identified a female-specific association of the anti-inflammatory response, innate</td>
</tr>
</tbody>
</table>
immune response, and β oxidation, and a male-specific association of the pro-inflammatory response to septic shock

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Methodology</th>
<th>Study Description</th>
<th>Drug responsive metabolite:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puskarich MA</td>
<td>2015</td>
<td>LC-MS/MS</td>
<td>Carnitine treatment response in Septic shock</td>
<td>Responsive towards carnitine treatment</td>
</tr>
<tr>
<td>Evans et al.</td>
<td>2019</td>
<td>LC-MS/MS</td>
<td>Carnitine treatment response in Septic shock</td>
<td>Drug responsive metabolite: metabolic signature of L-carnitine-treated non-survivors is associated with a severity of illness (e.g., vascular inflammation) that is not routinely clinically detected.</td>
</tr>
<tr>
<td>Pandey et al.</td>
<td>2023</td>
<td>^HNMR</td>
<td>Treatment response in Septic shock</td>
<td>Drug responsive metabolite: 3 hydroxybutyrate, lactate, and phenylalanine which were lower, whereas glutamate and choline higher in patients showing responsiveness.</td>
</tr>
</tbody>
</table>

Male/Female. Adapted from the Pandey S. Sepsis, Management & Advances in Metabolomics. Nanotheranostics 2024; 8(3):270-284.