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Metabolomics as a Driver in Advancing Precision Medicine in Sepsis

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Abstract

Objective—The objective of this review is to explain the science of metabolomics—a science of systems biology that measures and studies endogenous small molecules (metabolites) that are present in a single biological sample—and its application to the diagnosis and treatment of sepsis. In addition, we discuss how discovery through metabolomics can contribute to the development of precision medicine targets for this complex disease state and the potential avenues for those new discoveries to be applied in the clinical environment.

Methods—A nonsystematic literature review was performed focusing on metabolomics, pharmacometabolomics, and sepsis. Human (adult and pediatric) and animal studies were included.

Main Results—Metabolomics has been investigated in the diagnosis, prognosis, and risk stratification of sepsis, as well as for the identification of drug target opportunities. Metabolomics elucidates a new level of detail, when compared with other systems biology sciences, with regard to the metabolites that are most relevant in the pathophysiology of sepsis, as well as highlighting specific biochemical pathways at work in sepsis. Metabolomics also highlights biochemical differences between sepsis survivors and nonsurvivors at a level of detail greater than that

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demonstrated by genomics, transcriptomics, or proteomics, potentially leading to actionable targets for new therapies. The application of pharmacometabolomics and its integration with other systems pharmacology to sepsis therapeutics could be particularly helpful in differentiating drug responders and nonresponders and furthering knowledge of mechanisms of drug action and response.

Conclusion—The accumulated literature on metabolomics suggest that it is a viable tool for continued discovery around the pathophysiology, diagnosis and prognosis, and treatment of sepsis in both adults and children, and provides a greater level of biochemical detail and insight than other systems biology approaches. However, the clinical application of metabolomics in sepsis has not yet been fully realized. For this to be achieved, prospective validation studies are needed to translate metabolites from the discovery phase into the clinical utility phase.

Keywords

pediatrics; systems biology; systems pharmacology; pharmacometabolomics; pharmacotherapy; critical care

Introduction

Sepsis is one of the leading causes of mortality in the world.[1] Health care costs are significant, as septic patients often require care in the intensive care unit (ICU). Sepsis makes a significant contribution to the overall expense of intensive care medicine, which collectively represents close to 1% of the United States' gross domestic product.[2] Furthermore, the incidence of sepsis is on the rise, which is consistent with an aging population and with an increasing number of immunosuppressed individuals. The most vulnerable populations for sepsis include the elderly and neonates, with a wide variation in incidence between these groups. Sepsis is diagnosed in 0.56 per 1000 children per year in the United States, with a 10.3% mortality rate[3] but accounts for 40% of deaths in children younger than 5 years old worldwide.[4] In adults, the incidence of sepsis is 18.6 per 1000 hospitalizations, with a mortality rate of up to 50% in fulminant septic shock.[5]

Etiology and Definition of Sepsis

The etiology of sepsis varies by age and can include bacterial, viral, and fungal pathogens. In children, the most common pathogens are *Staphylococcus* species, followed by fungal infections, the latter of which are more common in children with cancer.[6] Viral pathogens are common in younger children but are frequently accompanied by bacterial co-infection.[3] In adults, about half of the infections are caused by gram-positive bacteria, about 40% from gram-negative bacteria, and less than 6% from anaerobes and fungi.[7]

Although the pathogens that cause sepsis have not significantly changed, the definition of sepsis was recently updated after more than 20 years. This was prompted by a call to reassess the definition due to an emergence of new knowledge in the field.[8-11] However, these revised Third International Consensus (Sepsis-3) definitions only apply to adults (Table 1),[11] as in children, hypotension presents much later and may indicate nonreversible

cardiac failure.[12] In addition, the classification of severe sepsis was dropped in the most recent adult definitions but remains in the pediatric definition.

The diagnosis, risk stratification, and treatment of sepsis in both children and adults is challenging due to its inherent heterogeneity and the absence of a gold standard for diagnosis. This has traditionally led to poor clinical outcomes and has contributed to a plethora of failed pharmacotherapy clinical trials.[13, 14] Diagnostic and prognostic tools are sparse and are largely based on clinical signs and symptoms. These parameters can vary in children, depending on age.[15] For example, tachycardia in an 8-month-old infant is defined as > 180 beats per minute whereas it is > 140 beats per minute in a 3-year-old. In addition, although the Sequential Organ Failure Assessment (SOFA) score is predictive of outcome in adult patients treated in the intensive care unit, it is not sufficient for pediatric patients with sepsis. The Pediatric Logistic Organ Dysfunction Score is the only pediatric scoring system that has been validated in clinical trials.[16] Collectively, use of scoring systems are cumbersome and typically are not routinely employed as part of patient care. As such, the Sepsis-3 task force recommended that a SOFA score ≥ 2 be used as a criterion for sepsis because it can be calculated from clinical data that are more readily available.[9] Nevertheless, these clinical tools have a low level of accuracy, which creates a particular challenge for achieving precision medicine in sepsis and has hindered the identification of pharmacotherapies targeted at specific subpopulations with this disease. For instance, patients with sepsis can have immune responses ranging from an overactive inflammatory cascade to a highly immunosuppressed phenotype.[17] These phenotypes may not initially be easily differentiated at the bedside or correlate with observable clinical parameters. These points are illustrated in the accompanying case report of pediatric sepsis.[18]

Accurate and early identification of sepsis could influence clinical decision making and direct more precise therapeutic intervention. Therefore, there is an enormous need for new approaches to more accurately phenotype sepsis. The systems biology and pharmacology sciences (Figures 1A and 1B), including metabolomics and pharmacometabolomics, have great potential to aid in defining specific sepsis phenotypes and find much needed predictive and prognostic biomarkers that can lead to more personalized management and therapeutics.[19] In this article, we present an overview of the current knowledge of sepsis and the role that metabolomics and pharmacometabolomics could play in advancing a precision medicine initiative for sepsis. In addition, we depict the utility of metabolomics for the timely identification of pediatric sepsis in the accompanying case report.[18]

Overview of Metabolomics

Metabolomics is a science of systems biology (Figure 1A) that measures and studies endogenous small molecules that are present in a single biological sample.[20, 21] The metabolome refers to the complete set of metabolites in any given biofluid, cell, tissue, or organism.[22] These small molecules, or metabolites, are typically less than 1500 daltons in size and are produced from metabolic processes (e.g., the tricarboxylic acid cycle) and complex biological interactions within an organism as well as interactions between the host and microbes. Unlike genomics, in which a genetic mutation may have little or no impact on the function of a protein or proteomics, which may not identify a functional change in a

protein, clinical metabolomics detects the direct result of a biochemical response to internal and external factors. In addition, virtually any type of biological sample can be assayed, including blood, urine, and tissue.

There are multiple steps that should be followed to conduct a metabolomics study (Figure 2). The generation of reliable metabolomics starts with sample collection (step 1). Use of a standard operating procedure is essential and is particularly important for multicenter studies, as is expeditious and consistent sample handling and storage (e.g., −80°C).[23] For specific analytical platforms and, most often, with the exception of urine, macromolecules need to be removed from the sample (Figure 2, step 2) before assay. There are a number of options for this including sample ultrafiltration or methanol precipitation. The choice depends on the type of sample (e.g., tissue samples require more processing) and the objective of the metabolomics study. For example, if serum samples are filtered, the lipid metabolites will be retained and forfeited in the filter, whereas use of a methanol-chloroform extraction will yield both an aqueous and nonaqueous portion of the sample; this permits dual-platform assays for aqueous and lipid metabolites from a single sample.[24]

Metabolites can be measured by a number of different analytical platforms, but the two most common are one-dimensional (1D) proton (^{1}H) nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) (Figure 2, step 3). The advantages of NMR include that it is routinely quantitative and is nondestructive to the sample. It is especially useful for the detection of medium to high abundant metabolites, polar compounds, and metabolites with a molecular weight less than ~100 daltons, which liquid chromatography (LC)-MS may miss. However, even though nearly every compound has a distinct NMR spectrum, it can be challenging to identify and quantify metabolites that have overlapping spectral peaks that can occur in complex mixtures.[25] Use of commercial spectral processing and analysis software such as Chenomx (www.chenomx.com; NMR Suite 8.0; Chenomx Inc., Edmonton, Alberta, Canada) can be used to optimize metabolite identification and quantification from raw NMR spectra.

MS platforms such as gas chromatography (GC)-MS and LC-MS are more sensitive than NMR and are therefore better suited for the measurement of low-abundant metabolites (< 5 μM) and for the detection of lipid and volatile compounds. Presently, LC-MS is commonly used for what is referred to as “untargeted” metabolomics because it effectively detects a broad range of different types of metabolites. Analytical and spectral processing technology is rapidly evolving such that aqueous and lipid compounds can be simultaneously detected, but LC-MS is highly variable, which makes cross-center studies challenging. In addition, there is no universal LC-MS metabolite library that can be applied to different instruments. This makes careful metabolite identification and quantification particularly important. For more detail, the reader is referred to recent reviews of analytical metabolomics.[26, 27] ^{1}H-NMR spectral acquisition is relatively straightforward, but there are specifications that need to be followed (e.g., the pulse sequence).[28] This is critical for quantitative NMR metabolomics for which a reliable internal standard peak is necessary. If done correctly and the same across clinical centers, the data are highly reproducible. Spectral analysis for LC-MS is more automated than it is for NMR. Most manufacturers of LC-MS instrumentation

have special software that finds and names peaks. Confirmation of metabolite identification and quantification is then done using known standards (Figure 2, step 4).

Following the generation of a metabolomics data set, the statistical analysis is the next step (Figure 2, step 5). This can be quite complicated and typically begins with unsupervised and supervised learning methods[29] such as principal component analysis (PCA) and partial least squares–discriminant analysis (PLS-DA), respectively. Unsupervised methods aid in describing the data based on data-driven group classification and trends that exist within the data. In addition to PCA, other common unsupervised methods include K-means clustering and hierarchical clustering. Supervised learning methods are used to determine the predictive power of the identified metabolites and the outcome (e.g., sepsis vs no sepsis, or severe sepsis vs sepsis). These methods include PLS-DA and support vector machine and are often employed for discovering biomarker candidates.[29] These analyses are often performed using qualitative metabolomics data and are referred to as chemometric analyses. However, nonparametric and parametric statistics can be employed for metabolomics data analysis, particularly for the analysis of quantified data to identify differentiating metabolites. This is preceded by data transformation (e.g., log normalization and range scaling) to achieve a normal distribution. Following the generation of p values, a scheme for the correction for multiple comparisons, such as the calculation of false discovery rate[30, 31], should be employed. Metabolomics data analysis can be accomplished using a number of publically available platforms including MetaboAnalyst (http://www.metaboanalyst.ca/).[32] Importantly, a single study is not sufficient to conduct a robust validation analysis, so it is important that findings are validated using samples generated from separate, independent studies.[33] This level of rigor is critical to test reproducibility and the testing of predictive models that is needed to build biomarker credentials. Following statistical analysis, pathway mapping using tools like Metscape (http://metscape.ncibi.org/), which is a plugin for Cytoscape (http://cytoscape.org/), can be employed to visualize metabolomics data and their associated metabolic pathways.[34]

Although the overarching principles of the above approach remain constant and are independent of the disease process being studied, there are specific considerations when studying the metabolomics of sepsis. The location of the patient within the hospital and at the time of sample collection are important factors to consider. Unlike some other disease states that have been studied using metabolomics (diabetes mellitus, cardiovascular disease), time of sample collection is key in the study of sepsis because the rapid progression of the illness can impact key metabolic pathways. While point-in-time estimates certainly offer information, sequential sample collection from the same subject is an ideal way to study these changes. In addition, a newly identified patient with sepsis who has just arrived in the emergency department may have a markedly different metabolomic profile compared with a patient receiving multiple interventions for several days in the ICU.

Pharmacometabolomics is the application of metabolomics to the prediction of drug response (Figure 1B).[35] The previously discussed principles of metabolomics with regard to study design, work flow, and assays all apply to pharmacometabolomics. Although the strict definition of pharmacometabolomics limits it to prediction, it is important to note that the extent of found associations between pretreatment levels of metabolites and the divergence

of drug-induced metabolic changes and phenotypes needs to be prospectively tested in order to accurately and reliably forecast drug response. In addition to the prediction of drug response, pharmacometabolomics could be a particularly powerful tool for furthering knowledge of the mechanistic underpinnings of diverse drug responses, mechanisms of drug action, and adverse drug reactions either alone or integrated with other systems pharmacology sciences.[36]

Metabolic Diagnosis of Sepsis

The inflammatory response, evidenced by vital sign aberrations, can result from sterile inflammation (surgery, trauma) or as sequelae of infection (sepsis) (Table 1). Therefore, identifying patients who have underlying infection and potential for progression through the sepsis continuum can be clinically difficult for both adults and children. The absence of a validated diagnostic test and the low incidence of detectable bacteria in the blood make sepsis diagnosis particularly challenging. Metabolomics has the potential to provide information to guide this key clinical decision. Metabolite profiles have consistently demonstrated the ability to discriminate sterile inflammation from sepsis in both human and animal studies.[37-39] Among adults admitted to an ICU following traumatic injury, metabolite profiles on admission successfully identified those who proceeded to develop sepsis from those who did not.[40] Similar differences are seen in children, where metabolite profiles clearly differentiate sepsis from sterile inflammation and survivors from nonsurvivors.[41] Among neonates, metabolite profiles discriminate healthy controls from those with sepsis and display distinct patterns between early- and late-onset sepsis.[42] In early sepsis in both adults and children, metabolites involved in energy metabolism show consistent directional changes. Ketone bodies are increased, and levels of citrate, pentose phosphate pathway compounds, ribitol, and ribonic acid are decreased.[41, 42] Furthermore, several studies have identified glucose, lactate, acetate, and citrate as metabolites that differentiate sepsis and systemic inflammatory response syndrome (SIRS) (Tables 1 and 2).[39, 41-44] These findings are presented in a case of a pediatric sepsis where ^{1}H-NMR urine metabolomics detected a large shift in energy metabolites, including increased levels of ketone bodies, which theoretically could have aided in the early diagnosis of sepsis in this patient.[18]

Metabolomics for Prognosis and Monitoring Sepsis

Ultimately, the short-term outcomes of interest for clinicians are mortality and end-organ damage. These endpoints can occur regardless of the specific pathogen and are likely due to a complex interplay between pathogen, patient, and environment. It is beneficial for clinicians to be able to identify at-risk patients early. Patients with evolving sepsis display metabolite profiles that are consistent regardless of pathogen,[37, 39] and distinct metabolite profiles identify specific end-organ damage resulting from sepsis.[39, 45] More recently, the long-term detrimental consequences of sepsis have become apparent in survivors.[46] Specifically, patients display impaired cognition and functionality.[47] Certainly, prognostic and predictive biomarkers of long-term outcome of sepsis survival would be of great value, particularly if targets for drug therapy could be identified. These findings may lie, in part, in the new insights into sepsis pathophysiology that metabolomics offers, which may in turn provide new areas for targeted therapy.

Metabolomics has identified differences in energy metabolism between healthy subjects and patients with sepsis, and between survivors and nonsurvivors of sepsis. Metabolites differentiating these groups are primarily amino acids and their derivatives, and demonstrate the role of energy metabolism in the pathophysiology of sepsis (Table 2). Specific metabolites are identified when comparing adults with both induced endotoxemia and community-acquired sepsis with healthy controls; the direction of change in metabolites is similar as well.[41] When survivors and nonsurvivors were compared, alterations were seen in free fatty acid metabolism, and there was a suggestion of a profound defect in mitochondrial fatty acid beta-oxidation in nonsurvivors; differences in glycolysis, gluconeogenesis, and citric acid cycle were also noted.[48-50] In addition, kynurenine, a by-product of tryptophan metabolism, which also differentiates SIRS (Table 1) from sepsis, is elevated in nonsurvivors compared to survivors (Table 2).[37, 48] The principle of a sepsis-induced energy disruption is evidenced in the accompanying case.[18] We have also demonstrated this in adult septic shock patients in which L-carnitine supplementation appears to be less effective in patients with elevated pretreatment levels of ketone bodies, implying that patients who present with evidence of a disruption in energy metabolism are less likely to survive sepsis.[51]

Ultimately, the strength and “added value” of metabolomics in sepsis for both children and adults may be when it is combined with clinical data and other measurements to improve prediction of outcomes and demonstrate enhanced performance when compared with currently available diagnostic tests including procalcitonin, C-reactive protein, and lactate levels.[43, 52, 53] In an example of this strategy, profiling children early after presentation in the emergency department using metabolomics and inflammatory protein mediators differentiated children who did and did not require ICU care. This application of metabolomics may aid in triage decisions and risk stratification in sepsis, particularly in clinical environments without pediatric expertise.[54]

Precision Medicine and Drug Targets for Sepsis

One of the more intriguing aspects of metabolomics is the potential to provide early, clinically relevant information on an individual's eligibility and response to treatment.[14, 35, 36, 55] With the current focus on resistant pathogens and antibiotic stewardship, pharmacometabolomics could permit customization and targeting of therapy early, thereby decreasing the prevalence of multidrug-resistant organisms. In the case of most infections, results of diagnostic tests for pathogen detection are not available for days to weeks after treatment is initiated. Importantly, specific information to guide antimicrobial therapy choices is not available until the organism has been cultured and sensitivities obtained. Often, particularly in children, the specific pathogen is not identified or cultures are negative. In the case of fungal infections, definitive results may not be available for weeks. In a study of neonates with fungal infections, the amino acid, serine, was elevated compared to healthy controls, and levels gradually declined in response to antifungal therapy, providing treatment-specific feedback prior to the time when culture results would be finalized.[56] In animal studies, expressed metabolites differed in mice receiving effective versus ineffective antibiotic treatment against the bacterium *Staphylococcus aureus* within 2 hours after initiation of therapy.[57] The same authors were able to demonstrate similar changes in response to therapy in vivo, in both *S. aureus* and *Escherichia coli* infections.[57]

As new therapies are developed for sepsis, metabolomics may be used as a tool to elucidate the impacted biochemical pathways, monitor for adverse events, and predict and track treatment responsiveness (pharmacometabolomics)[35, 36] as well as facilitate a precision medicine approach for sepsis.[58] Furthermore, integration of pharmacometabolomics with other systems pharmacology sciences (Figure 1B), such as pharmacokinetics and pharmacogenomics, could lead to further refining of drug response phenotypes.[36] As identification of viable drug targets is critical for drug discovery, metabolomics could be particularly useful in this regard. For example, in an animal study, erythropoietin was found to reduce end-organ damage in sepsis, and distinct metabolic profiles were found between the treated and untreated groups.[59] In addition, based on the metabolites identified, the specific pathways affected by erythropoietin were identified. This insight may lead to further investigation of the experimental therapy as well provide new hypothesis-generating data for the development of other therapies. In a phase I trial of supplemental L-carnitine for the treatment of adults with septic shock, pretreatment metabolite profiles differed between those patients who did and did not respond to the therapy.[51] In addition, the metabolites that differentiated L-carnitine responders and nonresponders highlighted affected biochemical pathways that could aid in identifying drug target opportunities for L-carnitine–nonresponsive patients. Importantly, there were no evident clinical differences between responders and nonresponders prior to treatment. This finding provides a direction for further study in that the metabolomics profile may assist in the early identification of patients most likely to respond to specific therapies who are not readily clinically differentiated using conventional means. Identifying the metabolic phenotypes of responders versus nonresponders to specific therapies could again provide insight into the differences in sepsis physiology and guide more focused precision therapies, thereby providing an approach to overcome what is considered to be one of the major impediments responsible for the repeatedly negative clinical trials, namely a largely heterogeneous patient population. For example, many metabolomics studies have identified mitochondrial beta-oxidation as a key pathway upon which interventions may be targeted (Table 2).[42, 43, 60] Knowledge of these metabolic differences could also be highly valuable for the design of clinical trials in which patients would be enrolled and stratified based on their pretreatment metabolic profiles. Use of metabolomics as an inclusion criterion could result in reduced study patient heterogeneity and improve the likelihood of clinical trial success.[55]

Future Directions

Metabolomics has shown significant potential as a diagnostic tool to differentiate patients with sepsis and sterile inflammation (e.g., SIRS) and for predicting mortality. Metabolomics may also be useful in predicting illness severity in sepsis in both adults and children, with the latter exemplified in our case report,[18] differentiating pathogens to guide appropriate antimicrobial therapy, and identifying the optimal timing for assessing response to therapy. In contrast to the other systems biology approaches, metabolomics comes closest to accounting for the direct interplay between individual, environment, and pathogen. It is precisely these characteristics that lend to its utility in the evaluation of sepsis given its heterogeneity as a disease. As the field evolves, pharmacometabolomics—the application of metabolomics to drug response prediction and phenotyping—will likely emerge as an

increasingly important member of systems pharmacology (Figure 2). Integration of these sciences could be particularly informative of drug response phenotypes and prediction of adverse drug reactions.

One challenge in the clinical application of metabolomics to both adult and pediatric patients is having timely and accessible results. Presently, the metabolomics work flow, which is lengthy, is not conducive to generating real-time data to be used for clinical decision making. Importantly, as key differentiating metabolites of drug response, diagnosis, and prognosis are identified, confirmation of metabolite identification and quantification will be essential. In addition, the findings of metabolomics studies will require validation in prospective studies in order to achieve robust biomarker credentialing.[33] They will also be needed for the development of accurate point-of-care tests that are optimal for the care of critically ill patients. New technologies are being developed, including programmable nano-bio-chips [61] and metabolomics-on-a-chip technology. As metabolomics is applied in future investigations of therapeutic interventions, metabolic phenotyping may be a useful tool in clinical trials for benchmarking or risk stratification of subjects.

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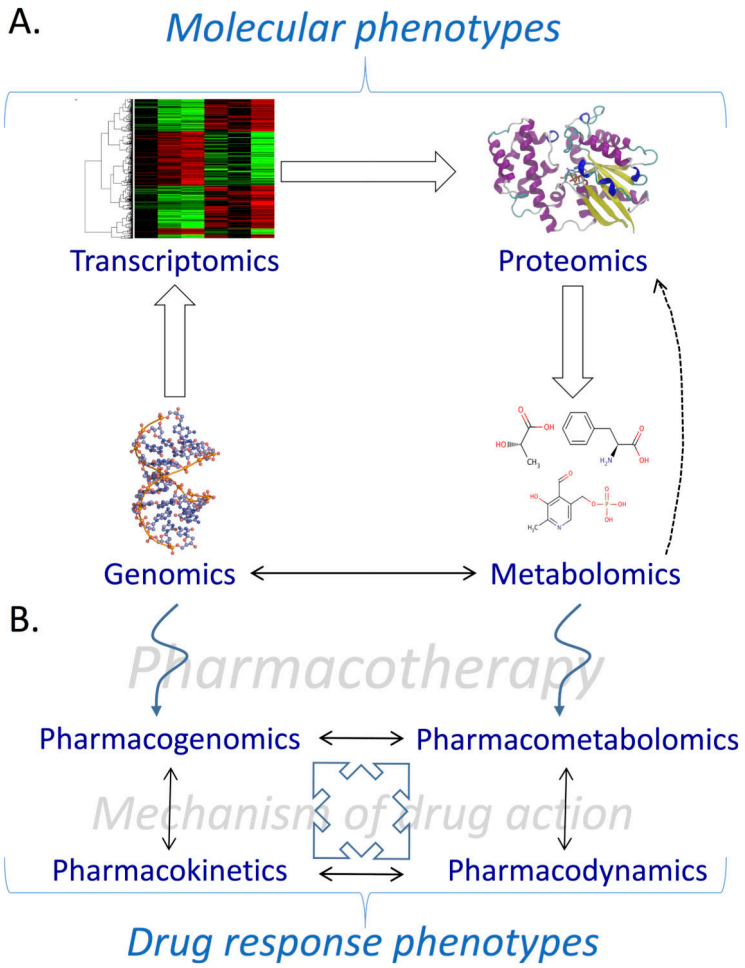

Figure 1.

Overview of the interactions between systems biology and pharmacology sciences. (A) Systems biology consists of genomics, transcriptomics, proteomics, and metabolomics. Although the transition of these sciences is often viewed as linear, it is likely that there are bidirectional interactions among them. For example, metabolites serve as signaling molecules for gene and protein regulation. (B) Systems pharmacology includes pharmacogenomics, pharmacometabolomics, pharmacokinetics, and pharmacodynamics. These sciences interact in such a way that they can inform each other so that more detail about mechanisms of drug action and drug response phenotypes can be learned.

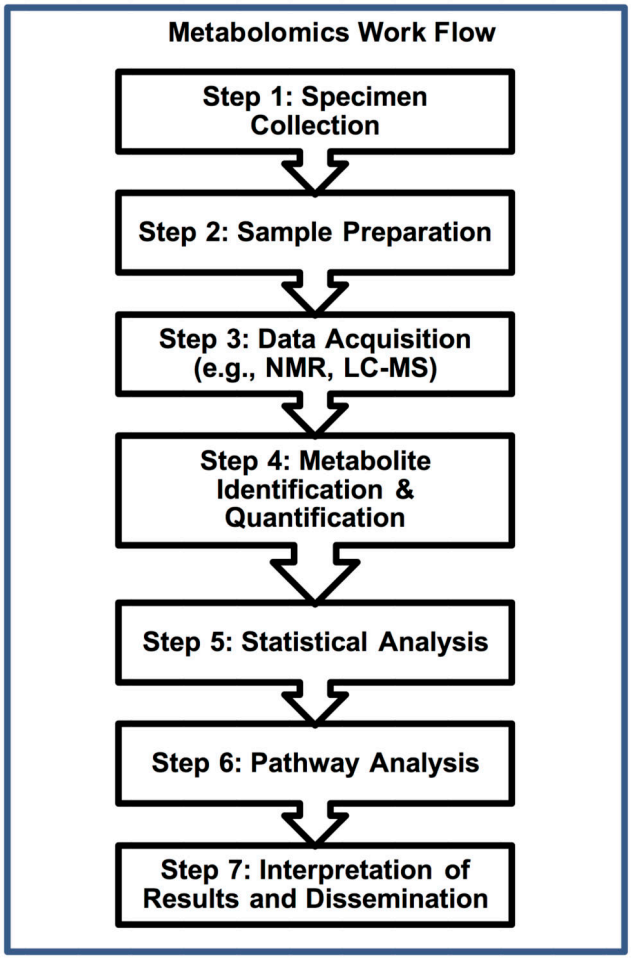

Figure 2.

Representative steps in a typical metabolomics work flow. If prospective sampling is planned, samples should be collected (step 1) using a standard operating procedure to ensure consistent procedures and sample processing. Sample preparation (step 2) will vary depending on the sample type. The most common analytical platforms for metabolomics data acquisition include nuclear magnetic resonance (NMR) and liquid (or gas) chromatography followed by mass spectrometry (LC-MS). A number of different publically and commercially available platforms exist for spectral processing and metabolite identification and quantification (step 4). These include Chenomx (chenomx.com), XCMS (https://xcmsonline.scripps.edu/) and MS-Dial (http://prime.psc.riken.jp/Metabolomics_Software/MS-DIAL/index.html). Statistical analysis (step 5) can be performed using quantified or nonquantified data using a number of different approaches (see text). Pathway analysis or the mapping of metabolites to metabolic networks can be achieved using a number of different tools including Metscape (http://metscape.ncibi.org/) or MetaboAnalyst (http://www.metaboanalyst.ca/).

Table 1

Consensus Definitions of Sepsis

Sepsis-Related Term	Adult[11]	Pediatric[15]
Systemic inflammatory response syndrome (SIRS)	Two or more of the following: • Temperature >38°C or <36°C • Heart rate > 90 beats per minute • Respiratory rate > 20 breaths per minute or $PaC0_2$ < 32 mm Hg • White blood cell count > 12,000 cells/mm^3 or < 4000 cells/mm^3 or >10% immature bands	Two or more of the following: • Core temperature >38.5°C or <36°C • Tachycardia, defined as a mean heart rate >2 SD above the normal rate for age * • Mean respiratory rate > 2 SD above normal rate for age * • White blood cell count elevated or depressed for age or >10% immature bands *
Sepsis	Life-threatening organ dysfunction due to a dysregulated host response to infection	SIRS that results from or occurs in the presence of suspected or proven infection
Severe Sepsis	Definition no longer used	Sepsis plus one of the following: • Cardiovascular organ dysfunction • Acute respiratory distress syndrome • Two or more other organ dysfunctions#
Septic Shock	A subset of sepsis that includes persistent hypotension that requires vasopressors to maintain a MAP ≥ 65 mm Hg and a serum lactate level of > 2 mmol/L despite adequate fluid resuscitation	Sepsis and, despite administration of isotonic intravenous fluid bolus >40 mL/kg in 1 hour, one of the following: • Hypotension (< 5th percentile for age or SBP < 2 SD below normal for age) * • Need for a vasoactive drug to maintain BP in the normal range * Or two of the following: • Unexplained metabolic acidosis; base deficit > 5.0 mEq/L • Increased arterial lactate >2 × upper limit of normal • Urine output < 0.5 mL/kg/hour • Prolonged capillary refill time of > 5.0 seconds • Core to peripheral temperature gap > 3°C

$PaC0_2$ = partial pressure of arterial carbon dioxide; MAP = mean arterial pressure; SBP = systolic blood pressure.

*Age-specific vital signs and laboratory variables are provided in reference 15.

#Organ dysfunction criteria are provided in reference 15.

Table 2
Metabolic Pathways and Associated Metabolites That Are Altered in Sepsis

Metabolic pathway	Sepsis response	Representative metabolites and direction of change
Amino acid metabolism	Increased catabolism of body tissues for energy production	↓ kynurenine, a by-product of tryptophan metabolism found in sepsis survivors, suggests efficient transition to noncatabolic pathways[37] ↓ decreased amino acids correlate with bacteremic sepsis[40, 53, 62] ↑ amino acids in response to effective treatment[57]
Mitochondrial energy metabolism	Increased to meet energy requirement/demand	↑ 3-hydroxybutyrate and acetoacetate (ketone bodies) increase in nonsurvivors suggests compensatory response to decreased ATP[51] ↑ lysophosphatidylcholines[37] ↑ acylcarnitines (transport long-chain fatty acids across mitrochondrial membrane) in nonsurvivors suggests defect in free fatty acid metabolism[42, 44, 48] ↑ linoleic acid in response to effective treatment[57]
Tricarboxylic acid cycle	Utilized substrates for aerobic catabolism	↑ amounts of substrate (citrate, malate, pyruvate, acetate, lactate) in nonsurvivors due to inability to metabolize[42, 48]
Pentose phosphate pathway	Utilized as alternate pathway for glucose metabolism	↓ THBA, ribitol, and ribonic acid suggest compensatory response to decreased ATP[42]

ATP = adenosine triphosphate; THBA = 2,3,4-trihydroxybutyric acid.