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Application of Metabolomics in Laboratory Testing for Bacterial Infections

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Abstract:
Metabolomics is emerging as a powerful tool in the diagnosis of infectious diseases, offering insights into disease mechanisms and enabling the identification of biomarkers for early detection and prognosis. This review highlights the application of metabolomics in diagnosing various infections, including tuberculosis (TB), melioidosis, Clostridium difficile infections (CDI), respiratory infections, and Mycoplasma pneumoniae pneumonia (MPP). Advanced techniques such as UHPLC-QTOFMS, GC-MS, LC-MS, and NMR spectroscopy have been utilized to analyze metabolites in body fluids, including plasma, urine, feces, and sputum, revealing significant alterations in metabolic profiles associated with infection. The identification of specific metabolites, such as acylcarnitines, LysoPE, sphingomyelins, and amino acids, provides valuable diagnostic markers, offering non-invasive alternatives to traditional diagnostic methods. These findings not only improve diagnostic accuracy but also enhance our understanding of infection-induced metabolic changes, paving the way for novel therapeutic strategies and personalized treatment approaches. The potential of metabolomics as a diagnostic tool holds promise for improving infection management, particularly in resource-limited settings.

Keywords: Metabolomics, Laboratory Testing, Bacterial Infections

Introduction

Despite significant advancements in the development of various vaccines and antimicrobial agents, infections remain a leading cause of morbidity and mortality, particularly in low- and middle-income countries. In recent years, the incidence of complications arising from infections has escalated. Timely detection plays a critical role in the diagnosis and treatment of

infections, as well as in minimizing pathogen transmission and preventing long-term complications associated with certain infections (Memar et al., 2019). Conventional laboratory techniques, including serological assays, nucleic acid-based detection methods, microscopy, and microbial cultures, represent the cornerstone of infection diagnosis. Among these, culture-based methods are often regarded as the gold standard for bacterial identification. However, these methods are labor-intensive and time-consuming, and the detection of fastidious or anaerobic pathogens necessitates specialized culture media and incubation conditions. Molecular techniques, such as polymerase chain reaction (PCR), while widely utilized for nucleic acid-based detection, are susceptible to both false positives and false negatives (Kinloch et al., 2020). Hence, there is a pressing need to develop innovative diagnostic strategies capable of reliably detecting specific biomarkers for infection diagnosis.

Metabolomics has emerged as a promising technology in the context of laboratory diagnostics. The metabolome is defined as the complete set of low molecular weight compounds (less than 1500 Daltons) present in a biological sample at a specific physiological state. It encompasses the totality of metabolites within a cell, tissue, organ, or organism at a given moment in time (Bujak et al., 2015). Metabolites are substances that arise from the biochemical and metabolic pathways employed by cells or tissues to sustain vital processes. These compounds are byproducts of both cellular and physiological metabolism. As a rapidly advancing field, metabolomics analyzes a specific subset of metabolites under particular conditions, such as disease states, and offers a promising approach for discovering new biomarkers. Metabolites present in circulating biofluids and tissues can be identified using metabolic patterns, with metabolomics serving as a potent tool for the detection of biomarkers linked to the diagnosis and prognosis of infections. These metabolites provide an accurate representation of an organism's phenotype at any given time, as they reflect the downstream products of the genome, transcriptome, and proteome. In contrast to genomics, transcriptomics, and proteomics, metabolomics provides direct biomarkers of biochemical processes, making it easier to directly correlate with phenotypic outcomes. Investigating the metabolic differences between unaffected and affected biological systems yields crucial insights into disease diagnosis, prognosis, and the underlying pathophysiological mechanisms (Zhang et al., 2015). Given that metabolome patterns are rapidly influenced by even minor stimuli, metabolomics represents an effective methodology for quantifying responses to various factors such as nutritional changes, disease states, host-pathogen interactions, stress, and activated host-defense mechanisms (Puebla-Barragan et al., 2020). The specific methods used for metabolite extraction and analysis vary depending on the metabolites of interest. Nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) are the two primary analytical techniques employed in metabolite measurement. Metabolomics has been instrumental in identifying novel biomarkers, such as agmatine for detecting urinary tract infection (UTI) pathogens, and 5-oxoproline for tuberculosis (TB) diagnosis. More significantly, metabolomics has enhanced our understanding of the molecular mechanisms underpinning infections (J. Li et al., 2022). Furthermore, alterations in specific metabolites can indicate dysregulated pathways that warrant further investigation. This review examines the application of metabolomics in the identification of biomarkers for bacterial infection diagnosis.

Metabolomics: Methods and Analysis

A wide range of matrices, including plasma, serum, cerebrospinal fluid (CSF), pus, saliva, urine, cervicovaginal secretions, and stool, can be utilized for metabolomic studies using accessible tissues or bodily fluids (Bharti et al., 2012). The primary steps in metabolomics analysis are outlined in Figure 2. Mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy are the two most employed analytical techniques for detecting metabolite types and levels. NMR spectroscopy utilizes the unique magnetic properties of specific nuclei

to determine the types and quantities of atoms within a molecule, making it a powerful tool for metabolomics research. One of the key advantages of NMR in metabolomics is its high reproducibility and quantitative capability. NMR facilitates the absolute quantification of metabolites using a single internal reference and can detect unknown or difficult-to-identify metabolites within biological samples. Furthermore, NMR does not require complex specimen preparation or separation, as it can examine intact biofluids and tissues. This makes NMR particularly valuable for tracing metabolic pathways and biological mechanisms using stable isotope labeling. Additionally, NMR can identify metabolites through various nuclei, such as ^1H, ^13C, ^31P, and ^15N, providing complementary information on the sample's composition (Nagana Gowda & Raftery, 2021).

The typical workflow for NMR-based metabolomics involves specimen collection, data acquisition, metabolite detection, and subsequent statistical analysis. This process yields sufficient data to distinguish between healthy individuals and patients by comparing metabolite levels and types. Metabolite profiles are often determined using one-dimensional proton (^1H) NMR, which can detect approximately 60 metabolites in a single spectrum. In addition to metabolite identification, two-dimensional (2D) NMR techniques offer additional structural insights. Proton (^1H) NMR spectroscopy is particularly valued for its high signal intensity, narrow line widths, and broad applicability, making it a commonly used technique in metabolomics studies. The automation, reliability, and speed of one-dimensional ^1H NMR spectra are advantageous in metabolomic research. Modern NMR instruments allow continuous operation and material removal, with spectrum acquisition times reduced to just a few minutes. Using the chemical data from a single ^1H NMR spectrum, 50–100 metabolites can be simultaneously identified and quantified. Several software programs are available to process and interpret ^1H NMR spectra, and there is a large repository of reference spectra for hundreds of known metabolites in public databases. However, challenges such as overlapping peaks and a limited chemical shift range exist in ^1H NMR spectroscopy. To address these challenges, stronger magnets and higher magnetic fields are recommended in 1D NMR studies. Additionally, using larger magnets and selective excitation techniques can improve resolution in 2D ^1H NMR investigations, allowing for the detection of more metabolites by resolving overlapping peaks. However, these experiments require longer analysis times and the expertise of skilled NMR spectroscopists. While ^1H NMR has lower sensitivity compared to MS, its specificity makes it a complementary and indispensable technique in metabolomic research. NMR has gained increasing attention in metabolomics due to ongoing advancements in procedural protocols, data analysis software, and standardization practices.

In metabolomics, MS is an essential analytical tool that enables efficient determination of metabolites in biological samples. MS is capable of analyzing a wide variety of metabolites, offering critical insights into cellular pathways, mechanisms, disease pathobiology, and drug discovery. Its quantitative capabilities allow for accurate, high-resolution mass measurements, thereby enhancing our understanding of biological and cellular processes, and it is particularly useful for evaluating diagnostic biomarkers. To improve its analytical power, MS is often coupled with liquid chromatography (LC) or gas chromatography (GC), which separate metabolites based on their size and polarity before detection. Tandem mass spectrometry (MS/MS), which provides structural information through ion fragmentation, is frequently employed in metabolomics for the identification of metabolites. MS is a highly sensitive technique, able to detect compounds in the femtomolar to attomolar range, making it especially valuable for analyzing complex biological samples (Clendinen et al., 2017).

The preparation of specimens is a critical step in MS-based metabolomics to ensure the generation of accurate, high-quality results. Metabolite purification is carried out using various techniques, such as extraction, precipitation, derivatization, solid-phase extraction, and liquid-liquid extraction. Advances in software, experimental protocols, and instrumentation have led to significant improvements in MS, allowing metabolomics to transition from qualitative to quantitative methods. However, challenges remain in MS-based metabolomics, such as the identification of unknown metabolites, the validation of biomarkers, and the need to maintain interlaboratory reproducibility, all of which warrant further investigation.

Metabolomics Evaluation and Data Analysis

Metabolomics evaluation involves the analysis of large, complex data sets that may encompass hundreds or even thousands of metabolites per specimen. To accurately assess these data sets, expert input from professionals in the fields of statistics, cheminformatics, and bioinformatics is essential. A critical step in metabolomics studies is data normalization, which ensures reliable quantification of the detected features. Normalization helps to minimize systematic biases and background noise, resulting in a modified dataset that better highlights significant differences in metabolite concentrations. In untargeted metabolomics, statistical techniques are routinely employed to assess the diagnostic potential of biomarkers through differential analysis. As each detected metabolite can be regarded as a variable, both univariate and multivariate statistical tests are commonly applied. Principal component analysis (PCA) is typically used for the preliminary analysis of such data, serving as an exploratory tool. PCA aids in the identification of outliers and common sample clusters, which helps to visualize metabolite level differences, such as fold-change disparities between patient and control samples. Statistical significance is generally assessed using techniques like ANOVA and t-tests (Misra, 2020).

Multivariate analysis can be performed through either unsupervised or supervised methods. Supervised data analysis models, such as orthogonal partial least squares discriminant analysis (OPLS-DA) and partial least squares regression discriminant analysis, are used to allocate groups based on prior knowledge. These models are primarily utilized to classify significant factors for creating hypothetical or accurate class assignments. They are also employed to distinguish between established sample groups or to relate unclassified metabolites to known class distributions, such as those found in reference standards. Using common NMR databases, software tools have been developed to facilitate the analysis of NMR data and automate the extraction of quantitative information. One of the challenges in metabolomics is the vast volume of data generated daily from numerous samples, which complicates analysis. To address this, bioinformatics approaches are applied to efficiently process data derived from multiple analytical techniques. Relevant metabolic pathways can be identified using data from various comprehensive databases, such as the Kyoto Encyclopedia of Genes and Genomes (KEGG), the Human Metabolome Database (HMDB), the Madison-Qingdao Metabolomics Consortium Database (MQMCD), and the Biological Magnetic Resonance Data Bank (BMRB), which also provide links to external pathway databases.

Metabolites can be retrieved from these databases using text-based searches or by utilizing software programs like KnowItAll Metabolomics Edition and Chenomx NMR Suite. These tools assist users in identifying metabolites without the need for peak picking by providing access to both proprietary databases and standard spectral libraries. Commercial NMR software programs are often integrated with specific databases. Examples of metabolomics software that incorporate statistical methods (chemometrics) include KnowItAll Metabolomics Edition, MetaboLab, Automics, MetaboAnalyst 2.0, the AMIX toolkit, and dataChord Spectrum Miner. Additionally, for spectral identification and quantification, tools such as Chenomx NMR Suite, KnowItAll Metabolomics, and rNMR are commonly used (Ellinger et al., 2013).

Metabolomics in Sepsis Diagnosis

Sepsis is a leading cause of morbidity and mortality, particularly among critically ill patients. Although mortality rates for sepsis have shown some decline, the condition still threatens the lives of up to 25% of patients globally, with hospital death rates for septic shock approaching 60%. In fact, septic patients exhibit hospital death rates as high as 35.3%. Improving patient care necessitates a more accurate and timely identification of at-risk individuals. Sepsis disrupts the balance between pro-inflammatory and anti-inflammatory responses, leading to a systemic release of host defense mediators, especially cytokines. Both pro-inflammatory and anti-inflammatory pathways are activated, triggering the complement and coagulation cascades in response to pathogen-associated molecules. Sepsis is predominantly caused by Gram-negative and Gram-positive bacteria but can also result from fungal infections. The heterogeneous and non-specific nature of the clinical manifestations of sepsis makes its diagnosis and prognosis challenging. Thus, precise laboratory diagnosis is critical, as it can significantly improve treatment outcomes. Given that the initial hours after diagnosis are considered the "golden hours" for prognosis, it is vital to begin diagnostic-based treatment as promptly as possible.

While microbial culture remains a valuable diagnostic tool, only 30% to 40% of patients with severe sepsis or septic shock yield positive culture results. As a result, the development of effective biomarkers for the diagnosis and prognosis of sepsis is crucial for optimizing treatment strategies. An ideal sepsis biomarker should not only differentiate sepsis from other infections but also distinguish between sepsis caused by various pathogens. Since sepsis and other critical illnesses alter the metabolomic profile, investigating the patterns of metabolites could aid in the development of practical methods for sepsis diagnosis and treatment (Beloborodova et al., 2018). Proton (^1H) NMR spectroscopy has been employed to analyze metabolites in body fluids and tissues, providing valuable insights into the metabolic pathways and etiology of sepsis. Metabolites such as creatine, myoinositol, phosphoethanolamine, taurine, and alanine have been investigated using an animal model of sepsis. Elevated levels of certain metabolites, including acetoacetate and fatty acids, were observed in serum samples from septic rats, while alanine and phosphoethanolamine were found in both lung tissue and serum, and creatine was identified in both bronchoalveolar lavage (BAL) fluid and lung tissue. However, formate levels were reduced in the serum of septic rats. Increased levels of myoinositol were observed in lung tissue specimens as compared to BAL specimens. The sensitivity and specificity of metabolite profiling for sepsis detection, based on analyses of lung, BAL, serum, and combined serum and BAL samples, have been reported as 100% and 92.7%, 92.8% and 85.5%, 100% and 100%, and 100% and 100%, respectively, in septic rats (Izquierdo-García et al., 2011).

Increased pyruvate metabolism and transamination to alanine through the Cori cycle, as observed in sepsis, may contribute to elevated alanine levels in both lung tissue and serum of infected animal models. Creatine, a nitrogenous organic acid, plays a crucial role in energy delivery to muscles and muscle protein turnover. Induced inflammation results in rhabdomyolysis and muscular atrophy. In septic rats, these metabolites have been reported to increase significantly. Elevated levels of phosphoethanolamine in septic animals may indicate phospholipid degradation and cellular injury. Increased fatty acid oxidation in septic rats might be linked to higher acetoacetate levels. Serum formate levels are notably reduced in septic rats, possibly due to greater formate consumption and elevated purine nucleotide levels during sepsis. Myoinositol, which is involved in endotoxin-induced vascular smooth muscle

hypocontractility, is also essential for the signaling pathways regulating vascular smooth muscle contraction.

Twelve metabolites identified in serum have been proposed as potential biomarkers for sepsis, disease severity, and mortality using LC-MS/MS technology. Metabolites such as S-(3-methylbutanoyl)-dihydrolipoamide-E, N-nonanoyl-glycine, lactitol dehydrate, and S-phenyl-D-cysteine have demonstrated diagnostic value in detecting sepsis. Specifically, lactitol dehydrate and S-phenyl-D-cysteine were found to be significantly decreased, whereas S-(3-methylbutanoyl)-dihydrolipoamide-E and N-nonanoyl-glycine levels were significantly increased in the sepsis group. Additionally, decreased levels of D-cysteine, Ne, Ne dimethyllysine, 2-phenylacetamide, and glyceryl-phosphoryl-ethanolamine indicated the severity of sepsis. Furthermore, increased levels of S-(3-methylbutanoyl)-dihydrolipoamide-E, PG (22:2(13Z,16Z)/0:0), glycerophosphocholine, and S-succinyl-glutathione were associated with the mortality group (Su et al., 2014). S-(3-methylbutanoyl)-dihydrolipoamide-E is involved in the synthesis of branched-chain fatty acids and the breakdown of valine, leucine, and isoleucine. Branched-chain amino acids (BCAAs), including valine, leucine, and isoleucine, play vital roles in regulating protein metabolism. Clinical and in vivo studies have shown that BCAA levels decrease in sepsis. Therefore, S-(3-methylbutanoyl)-dihydrolipoamide-E may significantly affect protein metabolism. Critically ill patients often experience severe disturbances in amino acid metabolism. Hydrophilic metabolites, such as lactitol dehydrate, have been reported to effectively identify early stages of sepsis before clinical symptoms manifest. This is further supported by the ROC analysis, which showed lactitol dehydrate as the most sensitive marker, while S-(3-methylbutanoyl)-dihydrolipoamide-E was the most specific. It is hypothesized that during severe sepsis, disruptions in the coagulation cascade and endothelial dysfunction lead to reductions in glyceryl-phosphoryl-ethanolamine. Ne, Ne-dimethyllysine, which plays a role in histone modification, gene expression regulation, and oxidative stress response, may also be a critical marker (Seymour et al., 2013). According to the Human Metabolome Database, 2-phenylacetamide is an intermediary in phenylalanine metabolism, and deficiencies related to amino acid metabolism can be reflected in changes in D-cysteine and 2-phenylacetamide levels. The ROC analysis revealed that 2-phenylacetamide, D-cysteine, and glyceryl-phosphoryl-ethanolamine exhibited higher sensitivities for diagnosing sepsis.

Metabolite patterns related to energy metabolism consistently show directional changes during early sepsis in both adults and children. Notable changes include elevated ketone body levels and reduced citrate levels, along with alterations in molecules involved in the pentose phosphate pathway, ribitol, and ribonic acid (Fanos et al., 2014). Some studies have indicated that metabolites such as glucose, lactate, acetate, and citrate can help distinguish sepsis from systemic inflammatory response syndrome (SIRS) (Kamisoglu et al., 2015).

Differences in free fatty acid metabolism have been observed between sepsis survivors and non-survivors, with significant impairments in mitochondrial fatty acid β-oxidation in non-survivors. Variations in glycolysis, gluconeogenesis, and the citric acid cycle have also been noted. Additionally, kynurenine, a byproduct of tryptophan metabolism, which differentiates SIRS from sepsis, was found to be elevated in non-survivors compared to survivors. Sepsis causes significant disruptions in energy metabolism, and L-carnitine supplementation has shown less effectiveness in patients with elevated pretreatment levels of ketone bodies, suggesting that patients with metabolic disruptions are less likely to survive sepsis (Puskarich et al., 2015). Identifying potential therapeutic targets for non-responders to L-carnitine can be facilitated by metabolite data, which distinguish responders from non-responders. Future research should focus on identifying L-carnitine responders, as these individuals may be more easily identified based on similar metabolomic profiles and responses to specific treatments.

By targeting the metabolic disruptions in sepsis, such as those in mitochondrial β-oxidation, therapies can be developed to address the heterogeneous nature of sepsis physiology.

Metabolomic data derived from quantitative 1H NMR spectroscopy used to study plasma during sepsis revealed significant differences in the levels of sphingomyelin, adenosine, phosphatidylserine (PtdSer), and total glutathione between sepsis patients and healthy controls. The elevated levels in sepsis-induced acute lung injury reflect the complex pathophysiology of the condition and indicate involvement of various processes, such as oxidative stress (glutathione), energy balance (adenosine), apoptosis (PtdSer), and endothelial barrier function (sphingomyelin).

Ultra-performance liquid chromatography mass spectrometry (UPLC-QTOF/MS) has been utilized to explore serum metabolomics in patients with severe sepsis. Several metabolites, including 3-phenyllactic acid, 2-phenylaminoadenosine, 3-hydroxyhippuric acid, ketoleucine, and 3-methoxytyrosine, have shown diagnostic efficacy for early detection of severe sepsis. The sensitivity and specificity of 3-hydroxyhippuric acid in predicting severe sepsis have been reported as 98.2% and 99.3%, respectively. Moreover, serum biomarkers such as 3-hydroxyhippuric acid and 3-phenyllactic acid, with an AUC greater than 0.95, have demonstrated a high predictive value for severe sepsis patients. These biomarkers have shown promising diagnostic efficacy in differentiating severe sepsis patients from controls in terms of accuracy, sensitivity, and specificity (Liang et al., 2016).

Analysis of urine specimens from septic newborns using 1H NMR and GC–MS revealed decreased levels of pseudo-uridine, 2,3,4-trihydroxybutyric acid, 2-ketogluconic acid, 3,4-dihydroxybutanoic acid, and 3,4,5-trihydroxypentanoic acid, compared to control groups. In contrast, metabolites such as acetate, acetone, glycine, lactate, lysine, and glucose were upregulated, while citrate and creatinine were downregulated in the septic group. These findings suggest that sepsis alters metabolic profiles, including variations in lactate, acetate, and glucose levels. Sepsis-induced changes in glucose metabolism are thought to involve a shift from mitochondrial oxidative phosphorylation to other metabolic pathways, such as the pentose phosphate pathway and anaerobic lactate production. LC-MS analysis of peripheral blood specimens in septic patients has identified differential metabolites, including 3-phenyl lactate, N-phenylacetylglutamine, phenylethylamine, traumatin, xanthine, methyl jasmonate, indole, and levotryptophan. 3-phenyl lactate, which is significantly upregulated in sepsis patients, plays a key role in phenylalanine metabolism. Higher levels of acetylglutamine and phenylacetylglutamine have been found in plasma of early diabetic nephropathy patients compared to individuals with normal renal function, suggesting that these metabolites may indicate early renal function failure in diabetic individuals. N-phenylacetylglutamine has been particularly expressed in the sepsis group, where it is primarily involved in phenylalanine metabolism. Phenylethylamine-induced hyperthermia (PIH) activates a series of events that may lead to coagulation cascades, rhabdomyolysis, and mortality. Additionally, the analysis of metabolomic pathways revealed that sepsis patients show elevated phenylethylamine levels through the phenylalanine metabolic route. L-tryptophan and indole exhibited low expression levels in sepsis patients, while traumatic acid, which has antioxidant properties and stimulates collagen biosynthesis, showed reduced expression in sepsis patients (Jabłońska-Trypuć et al., 2016).

Metabolomics in UTI Diagnosis

Urinary tract infections (UTIs) are among the most common infections acquired in both community and healthcare settings, affecting both men and women. Due to anatomical differences in the lower urinary tract, women are more susceptible to UTIs than men. Although

urine culture is considered the gold standard for microbiological detection of UTIs, it is generally unnecessary to perform a urine culture in the diagnosis of uncomplicated UTIs, as diagnosis is typically based on clinical symptoms. Urine culture is a time-consuming and labor-intensive process that may delay the initiation of antibiotic therapy. Consequently, a panel of biomarkers, including metabolomic profiles, may offer valuable indicators for UTI diagnosis. The identification of pathogen-associated metabolites and their levels, using high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS), has been evaluated for UTI detection. Two metabolites, agmatine and N6-methyladenine, have demonstrated the ability to accurately identify infections caused by more than 14 different microbial species, which were isolated from over 90% of UTIs. Agmatine and N6-methyladenine are produced during normal microbial metabolic processes when UTI-causing agents grow in urine. The metabolism of arginine and N6-methyladenine, a modified nucleobase present in prokaryotes, leads to the formation of agmatine. Urinary agmatine levels have been significantly associated with UTIs caused by Enterobacterales, as well as with culture-negative urine specimens, exhibiting a sensitivity and specificity of 94% and 97%, respectively. A diagnostic threshold of 170 nM for agmatine has been reported to indicate infections caused by Enterobacter, Citrobacter, Proteus mirabilis, and Escherichia coli, all of which exhibit significantly higher agmatine levels compared to urine specimens from healthy controls. N6-methyladenine MS levels were also effective in distinguishing culture-negative urine specimens from culture-positive specimens, with sensitivity and specificity of 91% and 89%, respectively (Gregson et al., 2024).

Quantitative metabolomic analysis has uncovered the potential of siderophores such as aerobactin, enterobactin, yersiniabactin, salmochelin, and the metabolite HPTzTn-COOH as reliable biomarkers for distinguishing Uropathogenic E. coli (UPEC) from non-UPEC in UTIs. The purine synthesis pathway has been identified as a specific signature for UPEC isolates from asymptomatic bacteriuria. UPEC strains do not rely on de novo synthesis pathways to meet their purine requirements. Instead, they can fulfill these needs by converting adenosine to xanthosine, and subsequently to guanosine. Metabolites and metabolic pathways associated with biofilm formation in UPEC have been identified using metabolomic techniques. By comparing the metabolism of planktonic and biofilm forms, several studies have identified different metabolic patterns depending on the growth conditions. Analysis of multiple differentially generated metabolites enabled the identification of metabolic pathways activated during biofilm formation, which are linked to glycerolipid and amino acid biosynthesis, as well as glucose metabolism (Lu et al., 2019).

NMR spectroscopy analysis of urine specimens with a colony count exceeding 10^5 CFU/mL revealed that acetic acid and trimethylamine (TMA), a mammalian-microbial cometabolite, are significantly elevated in infected urine. Regardless of the etiological agent, acetic acid levels were found to be indicative of bacterial UTIs. However, TMA was more specific to UTIs caused by E. coli. Overall, the acetic acid per creatinine ratio, when detected at a cutoff of 0.03 mmol/mmol for UTI diagnosis, exhibited sensitivity rates of 96.3%, 100%, and 100% for cases associated with E. coli, Klebsiella spp., and Enterobacter spp., respectively. These findings suggest that acetic acid could be comparable to other commonly used tests in clinical laboratories, such as the leukocyte esterase test, nitrite test, and urinary microscopy for white blood cells. Studies have demonstrated that acetic acid can achieve a 95% accuracy rate as a standalone marker for UTI diagnosis. Furthermore, acetic acid levels in urine can be used to monitor the effectiveness of treatment. In UTI patients, persistently high acetic acid levels may signal antibiotic resistance or poor treatment outcomes. Increased levels of TMA in patients with E. coli-associated UTIs may be attributed to the activity of Trimethylamine N-oxide (TMAO) reductase. This bacterial enzyme converts TMAO to TMA. Typically, food is

the primary source of TMA, which is then converted to TMAO by the enzyme flavin-containing monooxygenase (FMO3). A deficiency in FMO3, known as trimethylaminuria or fish odor syndrome, can result in elevated urinary TMA levels. However, since trimethylaminuria is rare, it is unlikely that the TMA observed in E. coli-associated UTI is related to this syndrome. The bacterial enzyme TMAO reductase, which converts TMAO back to TMA in the bladder, is the product of a combined metabolic process between microorganisms and the host's main metabolome. Thus, TMA can serve as a specific biomarker for bacterial load and activity. The presence of TMA in freshly collected urine reduces the likelihood of contamination during collection, thereby supporting a positive bacterial culture result. TMA may indicate bacterial growth within the host's bladder epithelium (Lam et al., 2015). Two metabolites, 1,3-propanediol (1,3-PD) and 6-hydroxy nicotinic acid (6-OHNA), may be specific to UTIs caused by Pseudomonas and Klebsiella, respectively. These compounds can be detected objectively using 1H NMR spectroscopy to identify Pseudomonas and Klebsiella-associated UTIs. Higher levels of metabolites such as 1-methylnicotinamide, citric acid, creatinine, glycolic acid derivatives, and hippuric acid have been reported in UTI patients compared to control groups using 1H NMR. In contrast, lower levels of acetic acid, acylcarnitine, lactic acid, taurine, and trimethylamine have been detected in UTI patients. Additionally, urine specimens collected 30 days after enrollment showed lower levels of taurine and higher levels of furoylglycine, para-aminohippuric acid, scyllo-inositol, trigonelline, and glycolic acid derivatives compared to baseline levels.

Evaluation of 60 metabolites in plasma via MS using capillary electrophoresis revealed that several metabolites were elevated, such as glutamate, and others were decreased, such as arginine, asparagine, inosine monophosphate, citrulline, glutamine, adenosine diphosphate, and adenosine triphosphate. These metabolites were found to be associated with male lower urinary tract symptoms (LUTS). These variations ranged from negligible to significant. The circulating amino acid profiles might serve as a risk factor for LUTS in men. Some metabolic pathways, particularly those involved in amino acid metabolism, may be implicated in LUTS. The profiles of amino acids in plasma may also be linked to metabolic syndrome. Gas chromatography–MS analysis of the urinary metabolome of females with overactive bladder syndrome (OAB) revealed significantly increased levels of metabolites, including intermediates of the Krebs cycle such as 2-hydroxyglutarate, fumarate, malate, and pyroglutamate, as well as metabolites from glycolysis, such as itaconate, hydroxyisobutyrate, and hydroxybutyrate. These metabolites are related to inflammation (itaconate), ketoacidosis (hydroxyisobutyrate and hydroxybutyrate), and mitochondrial dysfunction (2-hydroxyglutarate, fumarate, malate, and pyroglutamate) (Mossa et al., 2020).

The GC-TOF MS assay was employed to assess the potential of urine and serum metabolites in detecting diabetic nephropathy (DN). Benzoic acid, fumaric acid, erythrose, and L-arabitol were found to be significantly elevated in the DN group compared to the Type 2 diabetes mellitus group. Conversely, glycerol 1-octadecanoate, taurine, L-glutamic acid/pyroglutamic acid, fructose 6-phosphate, and L-glutamine levels were significantly lower in DN patients. Additionally, metabolites such as D-glucose, L-valine, L-histidine, sucrose, gluconic acid, glycine, L-asparagine/L-aspartic acid, L-xylonate-2, and oxalic acid were significantly higher in urine specimens from DN patients compared to those with Type 2 diabetes mellitus without nephropathy. Fumaric acid, L-glutamine, and L-glutamic acid were detected at significantly higher levels in the serum of DN patients than in T2DM patients without nephropathy. Conversely, serum taurine levels were lower, and serum levels of benzoic acid and fumaric acid were higher in DN patients, indicating various abnormalities in organic

acid metabolism in DN patients. Lesser-known organic acids such as taurine, which perform a variety of functions, including osmoregulation, stimulation of prolactin and insulin release, bile acid conjugation, cell viability, and avoidance of oxidant-induced tissue damage, were also implicated in these metabolic changes. To maintain blood glucose levels, L-glutamic acid plays a role in insulin secretion and β-cell regulation. Furthermore, changes in the levels of fructose 6-phosphate and L-glutamic acid/pyroglutamic acid could serve as early indicators of impaired glucose tolerance and diabetic nephropathy (Shao et al., 2020). Further exploration of metabolic alterations will aid in enhancing the understanding of metabolic pathways involved in kidney diseases.

Metabolomics in Meningitis Diagnosis

Meningitis refers to the inflammation of the meninges and subarachnoid space, typically caused by infections. Among the various forms of meningitis, bacterial meningitis is often regarded as the most severe type. The diagnosis of bacterial meningitis is primarily based on the analysis and culture of cerebrospinal fluid (CSF). However, aseptic inflammatory responses can also lead to alterations in the CSF, complicating the diagnostic process. Furthermore, antibiotic prophylaxis administered before CSF analysis can obscure the identification of bacterial meningitis through traditional methods. As a result, reliance on conventional CSF analysis alone for diagnosing meningitis is insufficient. This underscores the need for a rapid, sensitive, and accurate diagnostic approach that can effectively distinguish bacterial meningitis from viral and aseptic cases. The development of biomarkers for central nervous system (CNS) disorders is essential to aid in diagnosis and therapeutic decision-making. Several studies have shown that CSF metabolites can be readily analyzed using nuclear magnetic resonance (NMR) spectroscopy, with these metabolites demonstrating associations with specific clinical conditions. Recent studies have suggested that CSF metabolite patterns can be used to differentiate between bacterial meningitis, tuberculosis (TB) meningitis, and viral meningitis. The application of H-NMR to detect CSF metabolites revealed significantly higher lactate levels compared to glucose, while the citrate signal was completely absent. These metabolites are currently being evaluated for their potential in diagnosing various types of meningitis (Yekani & Memar, 2023). The H-NMR approach has also demonstrated that lactate levels, when semi-quantitatively assessed in CSF specimens, are elevated in bacterial meningitis and cerebral infarction cases, but not in viral meningitis. This suggests that H-NMR spectroscopy of CSF samples could serve as a promising method for the biochemical diagnosis of CNS infections, including bacterial meningitis. Through H-NMR, several metabolites such as glucose, lactate, pyruvate, citrate, glutamine, glutamate, creatine, creatinine, 3-hydroxybutyrate, acetoacetate, acetone, acetate, alanine, valine, isoleucine, and leucine have been identified. Visual inspection of the data revealed clear biochemical alterations, including elevated lactate levels and depleted glucose levels in bacterial meningitis cases. Furthermore, bacterial meningitis has been associated with elevated pyruvate levels and increased concentrations of amino acids, particularly alanine, isoleucine, and leucine.

NMR spectroscopy has also been used to identify differences in metabolite levels between patients with TB meningitis and viral meningitis. TB meningitis patients exhibited higher levels of 14 metabolites compared to those with viral meningitis, while the reverse was true for 11 metabolites, which were found at lower concentrations in TB meningitis cases. These findings suggest that NMR spectroscopy could differentiate between TB and viral meningitis based on specific metabolite patterns (Z. Li et al., 2017). In addition, the use of liquid chromatography-tandem mass spectrometry (LC-MS/MS) and direct injection MS/MS analysis of CSF from patients with enteroviral meningitis revealed reduced levels of 23 metabolites. These included phosphatidylcholine (PC.ae.C36.3), asparagine, and glycine, which collectively served as an accurate combined indicator for enteroviral meningitis.

Furthermore, kynurenine was identified as a potential biomarker for enteroviral meningitis. However, the mechanism by which PC.ae.C36.3 emerged as the most reliable biomarker for enteroviral meningitis, even in the presence of a normal cell count, remains unclear. Interestingly, a combination of PC.ae.C36.3, PC.ae.C36.5, and PC.ae.C38.5 resulted in near-perfect classification of enteroviral meningitis cases. Specific metabolites, such as kynurenine, have shown promising diagnostic potential as indicators of inflammatory status. Additionally, LC-MS analysis of CSF has highlighted several metabolites that differentiate TB meningitis from other types, including viral meningitis, bacterial meningitis, and cryptococcal meningitis. Sixteen metabolites were identified when comparing TB meningitis with viral meningitis, and nine when comparing TB meningitis with cryptococcal meningitis. Except for a lower concentration of 3,4-dihydroxybenzoate in TB meningitis relative to viral meningitis, and lower levels of phosphatidic acid and chenodeoxycholate in TB meningitis relative to bacterial meningitis, most metabolites showed higher concentrations in the CSF of TB meningitis patients compared to those with other forms of meningitis. Despite a few exceptions, the majority of biomarkers were found to be elevated in TB meningitis cases (Dai et al., 2017).

Metabolite profiling of CSF using 1H NMR spectroscopy has revealed significant differences in 20 metabolites between patients with TB meningitis and control groups. These differences include decreased glucose levels and increased lactate levels, indicating a disruption in glucose metabolism. Additionally, elevated levels of proline and creatine metabolism, as well as alterations in the glutamate-glutamine cycle, have been observed. The metabolic changes associated with impaired glucose metabolism, disrupted amino acid metabolism, and oxidative stress have been identified in the CSF of individuals with TB meningitis. These findings contribute to a deeper understanding of the pathogenesis of TB meningitis and have the potential to inform future diagnostic and therapeutic strategies (Dai et al., 2017).

Metabolomics in Tuberculosis Diagnosis

Tuberculosis (TB) represents a major public health issue globally, especially in developing nations, where approximately 80% of TB cases occur. The World Health Organization (WHO) emphasizes the necessity for rapid and precise diagnostic methods for the effective control and treatment of active TB. Traditional diagnostic approaches for TB primarily involve sputum smear microscopy, though this method may fail to detect Mycobacterium tuberculosis during the early stages of infection. Another conventional method, sputum microbial culture, while highly sensitive, requires extended periods to yield results. Molecular techniques, although more advanced, necessitate modern equipment and highly skilled personnel. The tuberculin skin test measures the body's delayed hypersensitivity response to an injected protein derivative, yet it lacks both sufficient sensitivity and specificity. Recent studies have explored metabolomics as a promising avenue for biomarker discovery in TB diagnosis (Collins et al., 2018).

The GC/TOF-MS technique has demonstrated that the levels of 5-oxoproline are consistently lower in TB patients compared to healthy controls, with reported sensitivity and specificity values of 78.3% and 78.2%, respectively. Therefore, 5-oxoproline levels may serve as a valuable biomarker for diagnosing active TB and assessing lung tissue damage associated with the disease. High-resolution LC-MS has identified 61 metabolites in the plasma of newly diagnosed pulmonary TB patients when compared to Household Contacts (HHCs) controls. These metabolites include glutamate, choline derivatives, M. tuberculosis-associated cell wall glycolipids, and resolvins, all of which exhibit potential for upregulation. Specific metabolites

such as M. tuberculosis-derived glycolipids and resolvins may be valuable as biomarkers, providing insights into the pathophysiology and clearance of TB (Frediani et al., 2014).

Further analysis using GCxGC-TOF MS of urinary biomarkers in pulmonary TB patients, compared with both healthy controls and non-TB disease controls, has revealed significantly elevated levels of various metabolites. These include oxalic acid, L-rhamnulose, quinolinic acid, ribitol, indole-3-carboxylic acid, kynurenic acid, and glycerol monostearate. These alterations suggest disruptions in the host's fatty acid and amino acid metabolism, particularly in tryptophan, phenylalanine, and tyrosine pathways. These findings may offer significant potential for advancing TB diagnostic methods and treatment strategies. Additionally, HPLC-MS has been employed to determine metabolites linked to inflammatory mediators in response to TB, revealing biomarkers such as neopterin, diacetyl spermine, sialic acid, and N-acetyl hexosamine in urine. These metabolites have demonstrated high sensitivity and specificity (> 95%) in distinguishing TB patients from healthy controls, as well as patients with non-TB pulmonary diseases. Furthermore, levels of these metabolites decreased after 60 days of anti-TB treatment (Isa et al., 2018).

The apLC-MS and xMSanalyzer platforms detected three metabolites, phosphatidylglycerol, lysophosphatidylinositol, and acylphosphatidylinositol mannoside, in the plasma of both TB patients and the HHC group, linked to M. tuberculosis. These metabolites were significantly upregulated in active TB patients, suggesting their potential utility for distinguishing TB patients from HHCs. GC–MS methods combined with chemometric analysis have identified 23 breath molecules as potential biomarkers for distinguishing active TB patients from non-TB controls, with a sensitivity of 82% and specificity of 92%. These metabolites appear to provide valuable information for differentiating active TB from other conditions, according to preliminary research.

H-NMR spectroscopy has also been utilized to examine differences in the serum metabolomic profiles of healthy controls, asymptomatic HHCs (asymptomatic household contacts), and active TB patients, along with HHCs having latent TB infection (LTBI). Active TB patients exhibited significantly lower levels of amino acids, including alanine, lysine, glutamate, glutamine, citrate, and choline, compared to the HHC group. However, no significant difference was found between the HC-TB and CH-HHC groups in terms of these metabolite levels. LC-MS analysis of lung fluid specimens has shown promising results in detecting over 400 markers with high differential diagnostic capacity, suggesting the potential for a new clinical test involving peripheral lung fluid to identify active TB patients (Chen et al., 2020).

In comparison of iron metabolism between patients with LTBI and those with active TB, the ROCHE COBAS 8000 platform revealed significantly higher serum levels of ferritin and soluble transferrin receptors in active TB patients. Notably, active TB patients showed a decrease in serum iron, transferrin, total iron binding capacity, and unsaturated iron binding capacity compared to the LTBI group. The combination of iron metabolism indicators with the TB-specific antigen/phytohemagglutinin ratio (TBAg/PHA ratio) yielded an accuracy of 88.80% and specificity of 90.10%, making these markers promising candidates for differentiating active TB from LTBI in T-SPOT-positive patients.

Using GC–MS and UPLC-MS alongside transcriptomics analysis, 33, 7, and 49 differential metabolites were identified between TB and HC, LTBI and HC, and TB and LTBI, respectively. OPLS-DA plots clearly differentiated active TB, LTBI, and HC groups. Seven specific metabolites—5-hydroxyindoleacetic acid, isoleucyl-isoleucine, heptadecanoic acid, indole acetaldehyde, 5-ethyl-2,4-dimethyloxazole, 2-hydroxycaproic acid, and an unknown metabolite—were selected as potential biomarkers for TB based on significant differences observed in serum metabolite profiles between TB patients and healthy controls.

Additionally, studies of the gut microbiome and metabolic changes in the feces of untreated active pulmonary TB patients compared to healthy controls using GC–MS and V3-V4 16S rRNA gene sequencing have shown a significant decrease in short-chain fatty acid (SCFA) synthesis, including acetic acid, propionic acid, isobutyric acid, butyric acid, 2-methylbutyric acid, and valeric acid in TB patients. These patients also exhibited reduced cholesterol levels. Fecal metabolomics analysis identified four bacterial genera (Fusobacterium, Fusicatenibacter, Tyzzerella, and Anaerotruncus) that showed combined diagnostic potential in distinguishing TB from healthy controls. Furthermore, a combination of five metabolites showed improved discrimination for TB, suggesting they could serve as both preventive and therapeutic targets as well as diagnostic biomarkers for TB.

Urine metabolomics analysis using UPLC-Q Exactive MS in TB patients, LTBI, and non-TB controls revealed six distinct metabolites: 3-hexenoic acid, glutathione (GSH), glycochenodeoxycholate-3-sulfate, N-[4′-hydroxy-(E)-cinnamoyl] L-aspartic acid, histamine, and deoxyribose 5-phosphate. These metabolites were analyzed in both positive and negative cases, with histamine and glutathione identified as viable biomarkers for distinguishing both TB and LTBI based on their relative quantitative levels. Cysteine, however, did not show promise in differentiating LTBI from non-TB controls.

Analysis using UPLC-MS/MS on human THP-1 macrophages, plasma, and sputum samples revealed that sputum cholestenone levels, but not plasma cholestenone levels, could differentiate TB patients from non-TB individuals exhibiting TB-like symptoms. Cholestenone, a metabolite not typically found in significant amounts in humans, may thus serve as a valuable biomarker of TB infection in clinical specimens.

GC–MS or UPLC-MS/MS methods have also been used to investigate the metabolomes of small molecules in the serum of HCs, LTBI, and active TB patients. These analyses revealed significant anti-inflammatory metabolomic changes in TB, including alterations in 20 metabolites such as histidine, cysteine, threonine, citrulline, tryptophan, glutamine, aspartate, and urea. These metabolites showed significant differences between TB patients and healthy controls, with certain amino acids, including pyroglutamine, phenylalanine, and kynurenine, exhibiting higher levels in the TB group. The analysis also revealed decreased phospholipase activity, increased adenosine metabolism products, and increased activity of indoleamine 2,3-dioxygenase 1 (IDO1) in TB patients, along with markers of fibrotic lesions when comparing latent TB infection to active disease.

LC-MS/MS techniques demonstrated lower blood levels of glutamine, methionine, and asparagine, and higher levels of glutamate, sulfoxy-methionine, and aspartate in the serum of active TB patients compared to those with LTBI and healthy controls. Among active TB patients, no significant variation in the blood levels of these metabolites was observed based on the severity of infection or likelihood of relapse. These metabolites, including glutamate, sulfoxy-methionine, aspartate, glutamine, methionine, and asparagine, may serve as quick and non-invasive markers for the detection of pulmonary TB (Cho et al., 2020).

UPLC-MS techniques have shown significantly increased levels of fatty acid and amino acid metabolites in the plasma of TB patients, further supporting their role as potential biomarkers for TB (Cho et al., 2020).

Metabolomics in Diagnosis of Other Infections

UHPLC-QTOFMS analysis of plasma metabolomic patterns in three groups—melioidosis patients, patients with other bacteremia, and healthy controls—has revealed significant differences in the levels of 12 metabolites in melioidosis patients compared to those in the plasma of patients with other bacteremia and healthy controls. LC-MS/MS analyses

demonstrated that these metabolites belonged to four lipid classes, as determined by elution order, MS/MS fragmentation, and predicted molecular metabolite classes. These classes included acylcarnitines (six metabolites), lysophosphatidylethanolamine (LysoPE) (three metabolites), sphingomyelins (two metabolites), and phosphatidylcholine (PC) (one metabolite). Elevated plasma concentrations of six medium- to long-chain (C13 to C19) acylcarnitines in melioidosis patients may suggest alterations in fatty acid β-oxidation during the infection. Acylcarnitines are synthesized from acyl-CoAs through the transfer of a hydroxyl group to carnitine, enabling their transport from the intermembranous space into the mitochondrial matrix for fatty acid β-oxidation.

The increased levels of three LysoPE (16:0/0:0), LysoPE (0:0/18:0), and LysoPE (18:0/0:0) in the plasma of melioidosis patients may reflect changes in phospholipid metabolism or cellular damage induced by the systemic infection. LysoPE, a component of cell membranes, is derived from the hydrolysis of phosphatidylethanolamine (PE) by phospholipase A2 (PLA2). However, due to the extensive breakdown of cell membranes in rats with induced liver lesions, higher plasma levels of LysoPE have been observed. Hence, the elevated LysoPE levels may be attributed to significant organ damage in systemic melioidosis. Elevated levels of sphingomyelin (d16:1/16:0) and sphingomyelin (d18:2/16:0) could indicate changes in sphingolipid metabolism occurring during melioidosis. Sphingomyelin synthase transfers phosphorylcholine to ceramides, which are subsequently broken down by sphingomyelinase to form sphingomyelin, a structural component of cell membranes. It would be crucial to compare phosphorylcholine (16:0/16:0) levels in plasma from individuals with pneumonia caused by other factors to determine whether it serves as a generic pneumonia biomarker or is unique to melioidosis.

The NMR technique used to assess metabolites in urine samples of children with respiratory infections, including Respiratory Syncytial Virus (RSV), viruses other than RSV, bacterial infections, and controls, analyzed 86 metabolites and revealed no consistent differences between RSV and non-RSV infections. Notably, however, variation was observed when comparing bacterial infections and RSV-infected children. The analysis demonstrated that metabolomic variations in the urine of children infected with RSV differed from those in healthy controls and patients infected by pathogens other than RSV, with the variations being influenced by the infection's severity (Adamko et al., 2016).

GC–MS analysis of fecal metabolites identified shifts in metabolomes associated with both microbes and hosts, which could distinguish patients with Clostridium difficile infections (CDI) from those with non-C. difficile diarrhea and C. difficile colonization. Compared to uncolonized controls, patients with CDI exhibited a unique chemical pattern of Stickland amino acid fermentation. Thirteen indicators, including fatty amides, sphingosine, bile acids, amino acids, carnitine, lysophosphatidylcholine (LPC), sphingomyelin, and esters, were identified through UPLC–MS analysis of CDI metabolomics. When comparing C. difficile-positive samples to C. difficile-negative and healthy controls, higher relative abundances of sphingosine, chenodeoxycholic acid, and LPC were observed in the positive samples. In contrast, diarrhea samples with C. difficile showed decreased relative abundances of fatty amides, tyrosine, linoleyl carnitine, and sphingomyelin. When compared to healthy controls, samples from patients with C. difficile-positive diarrhea showed lower levels of glycochenodeoxycholic acid and phenylalanine. Elevated levels of sphingosine and LPC in fecal samples could suggest malabsorption in C. difficile infections. Damage to the digestive tract caused by LPC could trigger an inflammatory response, and increases in sphingosine and decreases in sphingomyelin in the gastrointestinal tract may disrupt the microbial barrier, promoting the proliferation of C. difficile.

Significant alterations in bile salt profiles have been linked to recurrent CDI, with secondary bile acids being more prevalent in stool. These secondary bile acids inhibit the germination of C. difficile spores, while primary bile salts increase due to antibiotics, which reduce the metabolic capacities of the microbiome. The ratio of stool deoxycholate to glycoursodeoxycholate may be a potential predictor of secondary bile acids, with noticeably higher levels in controls compared to those with recurrent and initial CDI.

The isomeric amino acid allo-isoleucine, previously not linked to C. difficile or CDI, has emerged as a biomarker for branched-chain ketoaciduria, an inherited metabolic disorder causing maple syrup urine disease. While the exact source of allo-isoleucine in feces is not well understood, it serves as an indicator of the condition. A total of 43 metabolites were found to distinguish CDI from uncolonized controls, many of which are associated with bile acid metabolism and Stickland fermentation. Patients without CDI exhibited active bile acid metabolic pathways, with cholenoic acid and monohydroxycholenoic acid being the two noncanonical, unsaturated, dehydroxylated bile acids most significantly associated with CDI. Moreover, 5-aminopentanoic acid was found to significantly correlate with CDI. CDI specimens generally showed lower levels of amino acids compared to controls, suggesting their consumption by C. difficile, a metabolically active bacterium that produces Stickland fermentation products. Of the 43 metabolites, eight out of ten (80%) were significantly associated with CDI specimens compared to controls. LC-MS analysis has also been applied to determine the serum metabolites of Lyme disease. The development of a metabolic biosignature identified 95 molecular characteristics that differentiate early Lyme disease patients from healthy controls. Around 44 molecular features from the biosignature showed a sensitivity of 88% (84%–95%) and specificity of 95% (90%–100%) in distinguishing early Lyme disease patients from healthy controls. Notably, 77% to 95% of individuals with serology-negative Lyme disease were accurately diagnosed by metabolic indicators. These results demonstrate the potential of metabolic profiling for early Lyme disease diagnosis, providing significantly higher diagnostic sensitivity than the serology method, while maintaining excellent specificity. Among the identified metabolites were 11 polyunsaturated fatty acids (PUFAs) or lipids containing PUFAs, along with products of prostaglandin metabolism, fatty acid or cholesterol metabolism, sphingolipids, plasmalogens, metabolites of tryptophan, purine, and heme metabolism, an endogenous alkaloid, and seven peptides.

The plasma metabolites of patients with Mycoplasma pneumoniae pneumonia (MPP) were evaluated through UPLC Q-TOF MS, which revealed that MPP was associated with elevated levels of fatty acyls, sphingolipids, and glycerophospholipids. Three significant metabolites (411.3208, 568.5661, and 459.3493) exhibited high diagnostic value. Metabolite 411.3208 demonstrated 100% sensitivity and specificity in differentiating MPP from both healthy controls and infectious disease controls. In comparison, the sensitivity and specificity of metabolites 459.3493 and 568.5661 for diagnosing MPP varied when compared to healthy controls or infectious disease controls. The metabolism of glycerophospholipids and sphingolipids appeared to be most affected by these altered metabolites, suggesting immunological activation and cellular membrane injury in MPP patients. Markers of extrapulmonary complications were found to correlate with lipid metabolites differentially expressed between severe and moderate MPP cases, indicating their potential association with the severity of MPP disease. These findings could provide new insights into the pathophysiology of MPP in children and assist in biomarker selection.

Conclusion

Metabolomics has proven to be a transformative approach in the diagnosis of infectious diseases, enabling a deeper understanding of the metabolic alterations that occur during infection. By analyzing the metabolic profiles of patients, metabolomics can provide early and accurate detection of various infections, including TB, melioidosis, CDI, and respiratory infections. The ability to identify specific metabolites that reflect disease states offers a significant advantage over traditional diagnostic methods, which often require time-consuming culture or molecular testing. Furthermore, metabolomics-based biomarkers can help differentiate between different types of infections and assess disease severity, improving patient outcomes and guiding therapeutic interventions. While challenges remain, particularly in standardizing methods and integrating metabolomics into clinical practice, the growing body of research underscores its potential as a vital tool in the diagnosis and management of infectious diseases.

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