Metabolomics: Applications and Promise in Mycobacterial Disease

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Abstract

Until recently, the study of mycobacterial diseases was trapped in culture-based technology that is more than a century old. The use of nucleic acid amplification is changing this, and powerful new technologies are on the horizon. Metabolomics, which is the study of sets of metabolites of both the bacteria and host, is being used to clarify mechanisms of disease, and can identify changes leading to better diagnosis, treatment, and prognostication of mycobacterial diseases. Metabolomic profiles are arrays of biochemical products of genes in their environment. These complex patterns are biomarkers that can allow a more complete understanding of cell function, dysfunction, and perturbation than genomics or proteomics. Metabolomics could herald sweeping advances in personalized medicine and clinical trial design, but the challenges in metabolomics are also great. Measured metabolite concentrations vary with the timing within a condition, the intrinsic biology, the instruments, and the sample preparation. Metabolism profoundly changes with age, sex, variations in gut microbial flora, and lifestyle. Validation of biomarkers is complicated by measurement accuracy, selectivity, linearity, reproducibility, robustness, and limits of detection. The statistical challenges include analysis, interpretation, and description of the vast amount of data generated. Despite these drawbacks, metabolomics provides great opportunity and the potential to understand and manage mycobacterial diseases.

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Tuberculosis has been a great public enemy for millennia, and nontuberculous mycobacteria are causing concern in many countries today. The study, diagnosis, and treatment of mycobacterial diseases have been based on culture-related procedures that are more than 100 years old (1). Because these organisms grow slowly, serious complications occur before diagnoses are established. As different species have different growth characteristics, complex culturing procedures require extensive time and highly specialized laboratories (2). As we enter the genomic era, however, these methods are changing. Nucleic acid amplification studies are improving the diagnosis and treatment of mycobacterial disease, with tuberculosis leading the way.

An emerging science of the "omic" era is metabolomics, the study of the set of metabolites produced in response to a disease or perturbation. The metabolites, which may arise from resident or infecting microorganisms or their hosts, have yielded important information about the pathophysiology of many conditions. Metabolites identified by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy, gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectroscopy (LC-MS) have been used to establish diagnosis and prognosis of many infectious and noninfectious diseases (3–12). Metabolomics, as with the other "omics," is made possible only because advanced analytics are now available. In this review, we outline the potential role of metabolomics in mycobacterial disease.

Metabolomics

Metabolites are the low-molecular-weight compounds (<1 kD) resulting from life processes (metabolism). The full set of metabolites reflects the biochemical, physiological, and pathophysiological processes occurring in a life form at a particular time and appears to be unique for the various organisms or tissues sampled (13). Metabolomics is the identification and quantification of these metabolites in an unbiased analytical systems–based approach.

Metabolomics can use targeted and nontargeted approaches to investigate qualitative and quantitative endogenous metabolites. A nontargeted approach is effectively used to identify a number of unknown metabolites known as the *metabolome*, whereas targeted metabolomics is restricted to known metabolites, usually for specific metabolites or within a single class of compounds (14).

The metabolomics approach allows for the analysis of a variety of biological fluids, such as blood, urine, sputum, cerebral spinal fluid, and exhaled breath condensate in humans, or bacterial sources, such as culture media (15).

Metabolites are associated with all biological processes as starting, intermediate, or end products. The profile of these metabolites can provide mechanistic information about the influence of genetics, epigenetics, proteomics, and environmental factors. Metabolites change with time, and metabolomics can track the processes of disease progression and adaptation. The variations in microorganisms also can be plotted. Variation of the metabolome in different tissues may reflect how disease processes affect specific organs. Studying metabolomics with different perturbations (16, 17) can show the effects of the changes on signaling pathways. New biomarkers can be established on the basis of structurally and biochemically annotated metabolites. These biomarkers may be used for the diagnosis and prognosis of many diseases and could be useful in both basic research and clinical trials to monitor the effect of treatment (18). By profiling diseases, biomarkers can be used in drug development and to monitor the response to therapy (19). Metabolomics is a powerful tool with great potential for many areas of research in biology and medicine (19).

The great value of metabolomics is derived from the patterns of many different small molecules, which give a specific profile for different human conditions including infectious, neoplastic, cardiovascular, neurological, metabolic, and inflammatory diseases (19–21).

Metabolomics and Other "Omics"

Metabolomics is complementary to the other "omic" sciences, such as genomics, transcriptomics, and proteomics, but has less limitation because of technical and biological advantages (22). Metabolomics captures the biochemical products of the genes in their environment, and thus can provide fundamental knowledge of the biochemical networks under investigation. In this respect, metabolomics can allow for a more complete understanding of cell functions than genomics, transcriptomics, or proteomics can. This sensitive assessment of cell function at the chemical level has led to a better understanding of the cellular dysfunction caused by several disorders (23). Metabolomics has also clarified the disease development downstream of genetic or environmental changes (24) in contrast to genetics and proteomics, which give insight mainly into the predisposition for a disease.

Metabolomics can show the cellular responses to internal and external stimuli that can help identify early perturbations in cellular metabolism. The perturbations can be caused by the disorders themselves (23), by nature itself (environmental stress), or by humans (answering either research or therapeutic questions).

Analytical Platforms and Multivariate Data Analysis

Several analytical methods are used for metabolomic studies in individual as well as pooled human fluid samples. At present, NMR, GC-MS, LC-MS, and direct infusion tandem mass spectrometry (DI-MS/MS) are the most widely used techniques for the identification of large numbers of metabolites.

Each of these methods carries its own advantages, disadvantages, and predictive power. NMR, GC-MS, and LC-MS are the most common analytical tools used in metabolomics studies, and DI-MS/MS is a suitable technique for rapid diagnosis. Although GC-MS and LC-MS are more sensitive techniques with high separation efficiency, spectral resolution, mass accuracy, and resolution, NMR produces more quantifiable and reproducible data. The nondestructive NMR assay renders it more applicable to intact biomaterials (25). DI-MS/MS has high throughput and sensitivity with relatively good reproducibility (26). Each technique has the ability to detect small disturbances from stimuli, such as those caused by infectious diseases. Because of these differences, a combination of two or three analytical platforms is often needed to identify the various stages of a disease and to differentiate diseases.

Metabolomics aims to comprehensively identify and quantify a large number of metabolites from various classes. To identify the various classes of compounds, such as lipids, carbohydrates, amino acids, organic acids, sugars, sugar phosphates, biogenic amines, nucleotides, vitamins, purines, fatty acids, and steroids (27), one analytical method is not sufficient in a nontargeted approach (28, 29). Table 1 shows the most common analytical tools used (30) and Table 2 shows the strengths and weakness of these tools.

Assigning the vast array of compounds to various disease states is also a statistical challenge. The statistical methods rely on multivariate and pattern recognition approaches, which may be data driven and model driven. The products of the analyses are called "biomarker signatures" or "fingerprints." The large data sets are analyzed by multivariate techniques, such as principal component analysis and orthogonal partial least squares discriminant analysis. Both methods are based on projection methods with the underlying assumption that the system is affected by a limited number of variables (31). Principal component analysis is essential for the visualization and subsequent removal of outliers that may prejudice further analysis. It is unsupervised, meaning no information is given when the data set is entered into a statistical program (32).

Orthogonal partial least squares, another popular technique in metabolic profiling, is frequently applied in

Table 1. Analytical tools most commonly used in metabolomics studies

Technique	Metabolites Seen	Number of Metabolites
NMR	• Amino acids • Polar/nonpolar metabolites • Sugars • Volatile liquids • Large metabolites	50–200
GC-MS	• Volatile/thermally stable metabolites • Nonpolar metabolites • Amino acids • Medium to high lipophilicity • Nucleosides and nucleotides • Carbohydrates • Esters	100–500
LC-MS	• Amino acids • Fatty acids • Polar metabolites • Organic acids • Steroids	100–800

Definition of abbreviations: GC-MS = gas chromatography-mass spectrometry; LC-MS = liquid chromatography-mass spectroscopy; NMR = nuclear magnetic resonance.

discriminant analysis in which biological samples are classified in response to variables (32, 33). This is a supervised analysis when two cohorts take part in the analysis. It is generally used for model separation or classification.

Metabolomics and Biomarker Discovery in Mycobacterial Diseases

Metabolomics has been applied to find specific metabolic patterns for the diagnosis and prognosis of various infectious diseases (34). Studies have shown that mycobacterial exosomes could be used as biomarkers. Exosomes are small vesicles, between 40 and 200 nm, that are released from infected cells and contain mycobacterial proteins, lipoarabinomannan, and metabolites. Exosomes are being evaluated for the diagnosis of tuberculosis, and both immunogenic and nonimmunogenic exosomes are useful in vaccine studies (35).

Lipidomics and Mycobacterial Diseases

Lipidomics is the science that seeks to comprehensively identify and quantify lipid metabolites, making it a subfield of metabolomics. *Mycobacterium tuberculosis* has a great capacity to synthesize lipids, and its lipids are involved in many of its biological processes. They contribute to virulence and drug resistance. Despite mycobacterial lipids being extensively studied, lipidomics has uncovered many unknown lipids and changes in lipids in response to stimuli (36).

Fatty acids are the backbone of the mycobacterial cell wall lipids and contribute to many pathologic processes, such as change in immunity, virulence, antibiotic resistance, and invasiveness (37). Mass spectroscopy and NMR have been able to quantify various lipids including mycolic acids (α-alkyl, β-hydroxy long-chain fatty acids) in *M. tuberculosis* (38, 39). Each strain of bacteria has its own lipidome. Portevin and colleagues showed that mycolic acids have different profiles in different *M. tuberculosis* lineages. Mycolic acids have long been a target for antituberculous drugs, but the more recent findings may give additional clues for new medication (40).

Metabolomics and Diagnostic Biomarkers

Metabolomics can be a powerful tool for the diagnosis of mycobacterial infections. du Preez and Loots used metabolomics to identify new biomarkers for tuberculosis (41). They analyzed the sputum of 34 patients with tuberculosis and 61 control subjects by two-dimensional gas chromatography time-of-flight mass spectrometry. Twenty-two metabolites (14 *M. tuberculosis* components and 8 host-related markers) were identified with high discriminative power. Figure 1 illustrates the potential *M. tuberculosis* metabolites for targeted metabolomics study adapted from the study by Schoeman and colleagues (42).

GC-MS–based lipidomics of tuberculous infected sputum samples showed that 2-acetylamino-2-deoxy-β-D-glucopyranose, α-L-mannopyranose, and D-galactose-6-deoxy could distinguish persons with tuberculosis from those without (43, 44). For these studies, ethanol homogenization was the best extraction method to differentiate the sputum of tuberculous patients from that of control subjects (42). This technique was also used to differentiate isoniazid-resistant strains of *M. tuberculosis* from the wild type. Isoniazid-resistant strains carried *katG* mutations, which produce a specific metabolomic pattern with 29 different compounds in the resistant strains. The metabolites included alkanes, alcohols, fatty acids, surfactant protein, and other compounds involved in the stress alternative energy pathway (45).

Similarly, *rpoB* mutations change metabolites. LC-MS–based metabolomics showed differences in the metabolic profile of rifampin-resistant *M. tuberculosis*: 99 molecular features are different in the rifampin-resistant strains. In addition, the studies showed the major role of *rpoB* mutations in the *M. tuberculosis* metabolism (46).

Using LC-MS–based metabolomics on plasma samples, Frediani and colleagues found 61 metabolites that were significantly different between persons with tuberculosis and their asymptomatic household contacts. These metabolites could be classified in various groups probably related to specific diet, environmental chemicals, and household microbes. This study showed eight metabolites that were specifically up-regulated by the mycobacterial infection, such as glutamate, choline derivatives, *M. tuberculosis* cell wall glycolipids, and lipid mediators of inflammation (47).

Nontargeted ultrahigh-pressure liquid chromatography time-of-flight mass spectroscopy (UPLC-TOF-MS) was able to identify a cohort of patients with leprosy by their bacterial indices (a gauge of bacterial burden in leprosy). Metabolomics

Table 2. Strengths and weaknesses of analytical tools used in metabolomics studies

Technique	Strengths	Weaknesses
NMR	• Nondestructive technique • Versatility for analyzing metabolites in biofluids, tissues, or *in vivo* • Reproducibility and repeatability • More relative quantification • Applicable to intact biomaterial	• Low sensitivity (only metabolites with relatively high concentration [micrograms] can be detected) • Overlap in peaks and high chemical degeneracy (different metabolites have resonances in the same spectral region) • Identifies mainly polar compounds • Usually large sample size
GC-MS	• High-resolution capacity • High spectral resolution • Very sensitive • High mass accuracy to detect compounds • Reproducible retention time • Highly developed compound libraries • Small sample size (50 μl) • High separation efficiency • Ideal for thermostable and volatile and nonpolar metabolites	• High molecular weight analytes • Derivatization required • Fragmentation in MS • Requires technical skill • Extensive sample preparation steps • Poor quantification • Possible variation due to sample preparation • Compound degradation (high temperature) • Problem with ionization
LC-MS	• Short separation time • High resolution • Ideal for nonvolatile compounds • Very sensitive (picogram quantities) • Reasonable robustness • Selectivity • High mass accuracy to detect compounds • Simple sample preparation • Detects a wider range of metabolites than GC-MS • Analysis of more polar compounds without derivatization	• Vaporization errors • More instrumental variables than in NMR and LC-MS • High solvent consumption and lower separation power • Lower reproducibility (within and across laboratories) • Ionization of metabolites • Selectivity • Poor quantification • Lower reproducibility for retention time with different system • Destructive to sample • High instrumental cost
DI-MS/MS	• High throughput • Minimal sample preparation • Rapid analysis (1–3 min) • Good reproducibility • Highly sensitive • Simpler data analysis than LC-MS and GC-MS • Considerable structural information	• Matrix effects • Lack of differentiation between isomers • Lack of accuracy of selection of ions • Competitive ionization

Definition of abbreviations: DI-MS/MS = direct infusion tandem mass spectroscopy; GC-MS = gas chromatography-mass spectrometry; LC-MS = liquid chromatography-mass spectroscopy; NMR = nuclear magnetic resonance.

was able to distinguish those with a bacterial index of less than 1 from those with a bacterial index greater than 4. Serum metabolomics also showed increases in metabolites, such as polyunsaturated fatty acids, eicosapentaenoic acid, and docosahexaenoic acid, in patients with high bacterial indices (48).

Feng and colleagues showed that nontargeted serum-based metabolomics using UPLC-MS was able to distinguish tuberculous patients from healthy controls and patients with other lung diseases, such as pneumonia, lung cancer, chronic obstructive disease, and bronchiectasis. They described 12 metabolites that separated tuberculous patients from control subjects. Fatty acids were the main compounds followed by amino acids, again indicating the importance of lipid metabolism in *Mycobacterium* infection. Palmitic acid, phosphatidylcholine, lysophosphatidylcholine, phytanic acid, and behenic acid were decreased in tuberculous patients compared with control subjects (49).

Volatile organic compounds in patient serum and urine may be biomarkers for mycobacterial infections, although the compounds differ from those found in bacterial culture (50). Taking substances from organisms or culture media or using known bacterial proteins associated with disease may not be the best strategy for discovering lipidomic candidate biomarkers because it is the profile of many metabolites that are the fingerprints of the disease or process (51, 52).

Metabolomics may be useful for distinguishing mycobacterial pathogens from nonpathogens. They could potentially distinguish persons with latent tuberculosis from those with active tuberculosis and uninfected individuals. These biomarkers could potentially also identify vaccine-induced protection against tuberculosis, which would be extremely useful for vaccine development (52).

^{1}H-NMR–based metabolomics has been applied to diagnose *M. avium* subspecies *paratuberculosis* infection in ruminants. The metabolomic study was quicker and more sensitive at distinguishing infected from noninfected animals. The discrimination was significant regardless of infectious burden and time after infection (at 3, 6, 9, and 12 mo) (53). Although the potential is present, more work needs to be done to accurately predict the progression from latent to active mycobacterial disease, relapse after

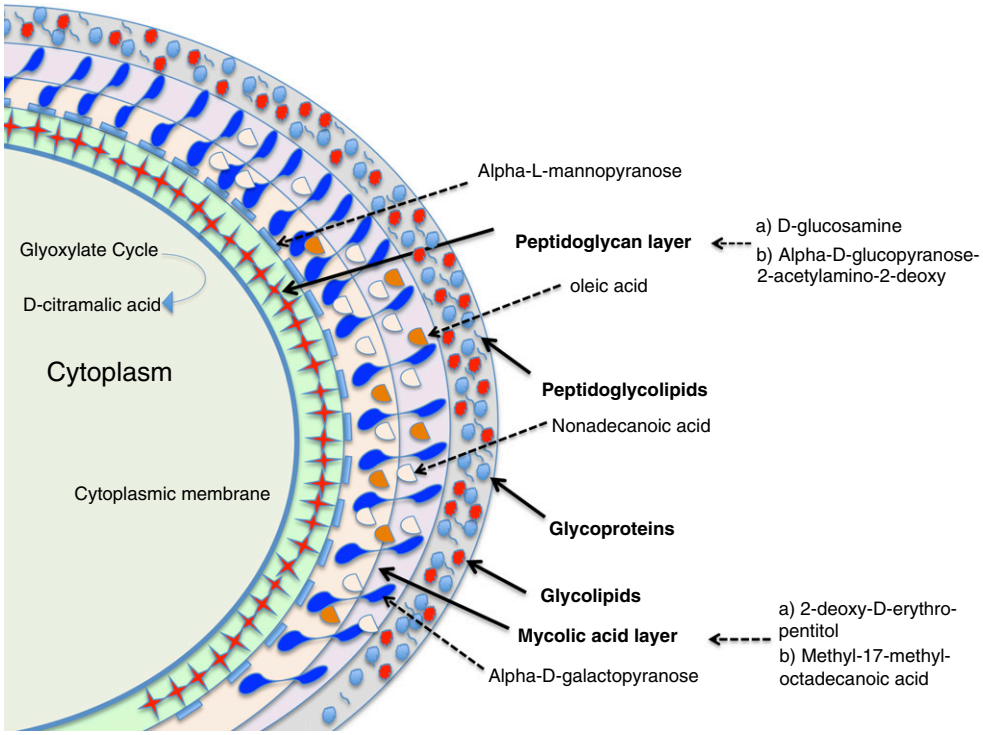

Figure 1. Schematic illustration of the cell envelope of *Mycobacterium tuberculosis* and potential metabolite targets. Metabolites from the peptidoglycan layer (D-glucosamine and 2-acetylamino-2-deoxy-β-D-glucopyranose) can be used for mycobacterial infection diagnosis, and metabolites from the mycolic acid layer (2-deoxy-D-*erythro*-pentitol and methyl-17-methyloctadecanoic acid) can be used to differentiate mycobacterial species.

treatment (54, 55), and completion of treatment (56).

Metabolomics may help increase our understanding of the pathophysiology of nontuberculous mycobacterial diseases. These diseases are rising globally, but are less understood and have fewer treatment options that tuberculosis (57). Biomarkers, once established, may function as important tools in clinical trials (58). Although morbidity and mortality will usually remain the primary end points, biomarkers, once validated with these outcomes, may aid the analysis (compared with the current 130-yr-old gold standard of culture) and study design by allowing fewer participants to be enrolled.

Metabolomics as Predictive Biomarkers

Metabolites can be used as biomarkers of effective treatment. LC-MS–based metabolomics on urine showed specific biosignatures with treatment of tuberculosis in African patients; 45 metabolites changed in the month after treatment completion compared with baseline (59). Biomarkers could also delineate the host immune response against mycobacterial infection. The immune response differs with mycobacterial species and strains, and different inocula.

Metabolomics could clarify the interaction of genetic and environmental factors. For example, metabolomics could show if and how the composition of the diet, the occurrence of life events, and the composition of the microbiome could change the metabolomic patterns under normal conditions and in response to disease processes (60, 61).

Measurement of the number and amounts of metabolites with time provides unprecedented information for basic research of biological and pathophysiological pathways. Using isotopically labeled substrate in a targeted approach is an important area of metabolomics called "fluxomics" (62). Mass spectroscopy–based (LC and GC-MS) fluxomics can track stable isotope–labeled elements, such as ^{2}H, ^{13}C, and ^{15}N, to reproducibly identify their compounds in various biological processes. This is leading to new insights in the metabolic pathways *in vitro* and in experimental models of infection. These methods may show the precise metabolic changes occurring as *M. tuberculosis* enters a dormant state, which is critical for its survival under conditions of stress, and is important for new drug development. ^{1}H-^{13}C-NMR–based metabolomics has been applied to determine the lethal dose of D-cycloserine and to show its effect on the metabolic pathways of peptidoglycan biosynthesis. Cycloserine inhibits several enzymes within the peptidoglycan biosynthesis pathways, particularly D-alanine–D-alanine ligase, a main enzyme of this pathway (63). These methods can be used to study the growth and replication of the organisms.

Metabolomics has shown that when mycobacteria are stressed by nitrogen limitation they produce free glucosylglycerate, by a mechanism specific for nitrogen stress but not oxidative or osmotic stress (64). These metabolic pathways help keep the mycobacteria alive by reducing the growth rate and decreasing the uptake of ammonium (64). A summary of the metabolomic profiles of various mycobacteria, using analytical tools, is shown in Table 3.

Table 3. Summary of metabolomic profiles of various mycobacteria using analytical tools

Analytical Tool	Main Study	Biofluids	Microorganism	Key Metabolites	Ref. No.
FT-ICR-MS	Lipidomics (*Mycobacterium* virulence)	Bacterial culture	*M. tuberculosis*	Phthiocerol dimycocerosate and sulfolipid-1	38
2D HSQC NMR	Lipidomics (lipid profiling and *Mycobacterium* virulence studies on various strains)	Bacterial culture	*M. liflandii*, *M. tuberculosis*, *M. smegmatis*	11 metabolites including triacylglycerol, acylated trehaloses, mycocerosic acid*	39
MRM-based MS	Lipidomics (study on permeability barrier in resistant strain)	Mycobacterial cultures	*M. tuberculosis*	Mycolic acids	40
GC/GC-TOF-MS	Differentiating between TB-positive and TB-negative patients	Sputum	*M. tuberculosis*	22 metabolites including D-gluconic acid lactone, glutaric acid, sebacic acid, ethane, butanal, γ-aminobutyric acid, 3,4-dihydroxybutanoic acid, and normetanephrine*	41
GC-MS	Distinguishing patients with TB from non-TB individuals	Sputum	*M. tuberculosis*	Tuberculosis stearic acid	42
GCxGC-TOF-MS	Metabolic profiling differentiation of drug-resistant *Mycobacterium* strains vs. wild-type *M. tuberculosis*	Mycobacterial cultures	*M. tuberculosis*	23 metabolites (alkanes, alcohols, and fatty acids) including decane, hexadecane, isotridecanol, and octadecanoic acid*	45
GC-EI/MS	Metabolic profile of *M. tuberculosis and* differentiation of positive sputum from negative sputum	Mycobacterial cultures and sputum	*M. tuberculosis*	Mycocerosic acid methyl esters	43
GC/GC-TOF-MS	Metabolomics profiling to distinguish patients with TB from control subjects	Sputum and mycobacterial cultures	*M. tuberculosis*	20 metabolites including nonadecanoic acid, tuberculostearic acid, *myo*-inositol*	44
LC-MS	Role of *rpoB* mutation in rifampin-resistant and -susceptible *M. tuberculosis* strains in metabolomics profiling differentiation	Mycobacterial cultures	*M. tuberculosis*	87 features including diacylglycerol phosphocholine, hexose-*N*-acetylhexosamine-fucose-*N*-acetylhexosamine*	46
LC-MS/MS	Distinction of patients with TB from sputum-negative household contacts	Plasma	*M. tuberculosis*	61 metabolites including trehalose-6-mycolate, phosphatidylinositol, and the D-series resolvins*	47
UPLC-TOF-MS	Identification of patients with leprosy with various bacterial indices	Serum	*M. leprae*	48 features*	48
UPLC-MS	Differentiation of patients with TB from healthy control subjects and non-TB patients	Serum	*M. tuberculosis*	12 metabolites including 3*D*,7*D*,11*D*-phytanic acid, behenic acid, and threoninyl-γ-glutamate*	49
GC-MS	Metabolomics profiling of *M. tuberculosis*	Culture, serum, urine, and EBC	*M. tuberculosis*	Volatile organic compounds	50

(Continued)

Table 3. (*Continued*)

Analytical Tool	Main Study	Biofluids	Microorganism	Key Metabolites	Ref. No.
^{1}H-NMR	Differentiation of ruminants infected with *M. avium* subsp. *paratuberculosis* from noninfected animals	Serum	*M. avium* subsp. *paratuberculosis*	16 metabolites including acetone, asparagine, aspartate, betaine, glycerol, isobutyrate, isoleucine, leucine, mannose, threonine, tyrosine*	53
LC-MS	Metabolic profiling of patients with TB before and after treatment	Urine	*M. tuberculosis*	45 metabolites*	59
^{1}H-^{13}C-NMR	Metabolic profiling for pathway analysis in multi- and extensively drug-resistant strains	Mycobacterial cultures	*M. tuberculosis*	D-Alanine–D-alanine ligase	63
NMR and GC-MS	Pathway analysis in nitrogen stress	Mycobacterial cultures	*M. smegmatis*	Glucosylglycerate	64

Definition of abbreviations: 2D HSQC = two-dimensional heteronuclear single-quantum coherence; EBC = exhaled breath condensate; FT-ICR-MS = Fourier transform ion cyclotron resonance mass spectrometry; GC-EI/MS = gas chromatography-electron impact mass spectrometry; GC/GC-TOF-MS = two-dimensional gas chromatography coupled with time-of-flight mass spectrometry; LC-MS = liquid chromatography-mass spectroscopy; *M.* = *Mycobacterium*; MRM-based MS = multiple reaction monitoring–based mass spectrometry; NMR = nuclear magnetic resonance; TB = tuberculosis; UPLC-TOF-MS = ultrahigh-pressure liquid chromatography-time of flight-mass spectrometry.
*For full list refer to the accompanying reference.

Precision Medicine

Precision medicine seeks to classify diseases into subgroups that in the past have been grouped together because of the lack of good discriminators. The use of metabolomics, in conjunction with genomics, pharmacogenomics, and proteomics, may allow more precise definition and treatment of the mycobacterial infections. Moreover, pharmacometabolomics is emerging as a new science aiming to understand the role of metabolites in antimycobacterial drug development and monitoring (65). Metabolites measured before and after drug administration can be used to evaluate the response to the medicine and could lead to a better understanding of the biological processes involved in effective treatment (19). Metabolomics may be able to identify susceptibility to drug toxicities and drug interactions in patients and healthy individuals (66). Snapshot measurement of hundreds of metabolites, serial analysis, and easy sampling of various body fluids are advantages that metabolomics provides for evaluating drugs in humans.

Metabolomics-based studies on animals, cell lines, and humans can be applied to characterize drug absorption, distribution, metabolism, and disposition. The specific metabolites produced during catabolism of drugs, as well as the metabolic profile changes, can be measured with sensitive analytical tools. A metabolomics approach could be used to study the success or failure of treatments in individuals. This could then determine which treatments will be effective in various individuals, based on their metabolism (67).

Challenges

Although metabolomics has great potential, it also has great challenges and limitations. Many of the studies are exploratory. By the nature of the science, there is variability in metabolite concentration caused by several sources including timing within a condition, biological variation, instrument variation, and sample preparation variation. Instrument and sample preparation variation are important sources of error and can cause both false positives and false negatives, as shown in the separation of nonsurvivors from survivors by Leichtle and colleagues (68).

Reproducibility and specificity are two major obstacles to be overcome before metabolomics can be useful in clinical

Table 4. Potential applications of metabolomics for the diagnosis and management of mycobacterial infections

	Metabolites	Sample	Method (Ref. No.)
Early diagnosis	D-Alanine–D-alanine ligase	Mycobacterial culture	NMR (63)
	Mycocerosic acid methyl esters	Sputum	GC/MS (43)
Latent vs. active TB	3*D*,7*D*,11*D*-Phytanic acid, behenic acid, and threoninyl-γ-glutamate	Serum	UPLC-MS (49)
	Trehalose-6-mycolate, phosphatidylinositol, and the D-series resolvins	Plasma	LC-MS/MS (47)
Distinguishing pathogens from nonpathogenic mycobacteria	Triacylglycerol, acylated trehaloses, mycocerosic acid	Bacterial culture	2D HSQC NMR (39)
	Phthiocerol dimycocerosate and sulfolipid-1	Bacterial culture	FT-ICR-MS (38)
Treatment response	*p*-Aminobenzoic acid, pyridoxal/isopyridoxal, formimino-L-glutamic acid, L-α-aspartyl-L-hydroxyproline, N^1,N^{12}-diethylspermine	Urine	LC-MS (59)

Definition of abbreviations: 2D HSQC = two-dimensional heteronuclear single-quantum coherence; FT-ICR-MS = Fourier transform ion cyclotron resonance mass spectrometry; GC/MS = gas chromatography-mass spectrometry; LC-MS/MS = liquid chromatography-tandem mass spectroscopy; NMR = nuclear magnetic resonance; TB = tuberculosis; UPLC-MS = ultrahigh-pressure liquid chromatography-mass spectrometry.

studies. Impressive results found in studies of a few patients must be repeatable and specific in larger studies (68). Metabolism profoundly changes with age, sex, variations in gut microbial composition, and lifestyle (28, 69).

A great challenge for metabolomics is to describe and accurately classify the many human metabolites and their importance, using different types of instrumental and statistical analysis. Finding and validating biomarkers is a major need (51). The selection of biomarkers of interest by genomics, proteomics, or metabolomics based on performance criteria is difficult and time consuming. The validation of biomarkers is also complicated and must address important parameters such as the range of the measurements, accuracy, selectivity, linearity, reproducibility, robustness, and the limits of detection (70).

The statistical challenges include how to analyze, interpret, and describe the vast amount of data generated. Biomarkers discovered by metabolomics should be validated both internally and externally. Multivariate analyses provide prediction models creating R^2 and Q^2 parameters to assess variability and predictability, respectively. Cross-validation and permutation are methods for internal validation to find false-correlated and overfitted models. Q^2 (goodness of prediction) is obtained by cross-validated and permutation methods in optimized potentials for liquid simulations, a statistical method of partial least squares regression.

Candidate biomarkers need to be externally validated by testing new independent samples, preferably from a new center operating with a different analytical machine and user (71). Tuberculosis biomarker signatures should be validated in different geographically and ethnically diverse populations and take into account coinfection with malaria and HIV, which affect metabolomic biosignatures (52).

Future Perspectives

Although the future of metabolomics for mycobacterial disease is great, the field is in its infancy. It should be integrated with genomics and proteomics to gain a complete picture of biologic and cellular processes. Metabolomics can be applied to the diagnosis, treatment, and prognosis of patients with mycobacterial infections (Table 4). This may be particularly important for infection with the environmental mycobacteria, about which there are more questions and less certainty than concerning infection with *M. tuberculosis*.

Validation studies to confirm biomarkers could be part of large multicenter studies, which may be designed for other goals. The large studies might be able to account for the heterogeneity in genetic backgrounds and environmental factors, which include age, sex, race, comorbidities, types of pathogens, and sources of infection (72). Before metabolomics can be incorporated into routine clinical practice, studies in larger and diverse groups of patients will be needed.

Conclusions

Among "omic" sciences, metabolomics is a promising approach for many different disorders, including mycobacterial disease. Its sensitivity may develop biomarkers better than other "omics," but the field is in its infancy and the great sensitivity and enormous data produced are currently limitations as well as a source of promise. Its ability to render a complete picture of ongoing biological processes is most appealing especially when combined with the other genomic-based methods. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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