



TB or not TB? Improving the understanding and diagnosis of tuberculosis through metabolomics

“...metabolomics has resulted in an exponential increase in the number of newly identified tuberculosis biomarkers, which has not only shed light on previously unknown disease mechanisms, but could potentially contribute to all aspects of tuberculosis clinical care...”

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Despite the major discovery in 1882 by Robert Koch that the causative agent for tuberculosis (TB) is the infectious bacteria *Mycobacterium tuberculosis*, and all the genomics, transcriptomics and proteomics data collected on this organism, and the subsequent vaccination, diagnostic and treatment approaches developed since then, TB is still a major global health problem. TB reportedly occurs in approximately a third (1.9 billion) of the world's population either in its active (symptomatic) or latent (asymptomatic) form, which subsequently results in the death of 1.5 million individuals per annum, ranking it the world's foremost cause of death from a single infectious bacterial agent [1].

Smear microscopy is currently the most commonly used method for diagnosing active TB in third-world countries. Despite the fact that it is a low-cost, quick and simple method, it has a sensitivity of only 62%; and it cannot distinguish between various *Mycobacterium* species; nor can it detect drug resistance [2]. The current gold standard for diagnosing TB, however, functions on the basis of bacterial culturing, and typically takes about 2 weeks at best for a result to be obtained [3]. In that period, an individual with active TB could infect 10–15 individuals, depending on his/her social habits. Apart from this serious draw-

back, sophisticated laboratory infrastructure and highly trained staff are required, which are not necessarily available in many third-world communities where the prevalence of TB is at its highest. There are currently other newer TB-diagnostic technologies available which are faster, such as nucleic acid amplification methods [4], phage assays [5] and serological tests [6]. Unfortunately, however, these also fall short with regard to their high cost and limited sensitivities, subsequently missing many TB-positive cases and contributing to the estimated 2.9 million missed TB cases globally each year [1]. Another drawback in current TB-diagnostic methods is that sputum is obtained with difficulty in childhood TB and HIV co-infected cases, subsequently contributing to the aforementioned missed cases. TB-diagnostic methods using urine or blood would be considered less invasive and easier to collect from a patient (when one considered sputum collection in children often requires gastric aspirate) and could potentially pick up patient groups that current diagnostic technologies miss. Considering this, the world is in desperate need for new TB-diagnostic approaches which are fast, inexpensive, sensitive, selective, easy to operate without extensive training or specialized skills and portable (point-of-care devices). They should also be able to identify



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or differentiate between various *Mycobacterium* species, determine their sensitivity or resistance to medication and predict or monitor first-line treatment outcome well before the 6-month treatment period is completed (perhaps even before treatment commences).

“...metabolomics has allowed for better understanding of the adaptations of *Mycobacterium tuberculosis* to the host’s defense and vice versa...”

In an attempt to improve these strategies, some research groups have opted to address the problem by looking at it from a different perspective, using one of the newest omics technologies available, termed metabolomics, in order to identify new TB biomarkers. These new markers can subsequently be used for better understanding of the disease and the host’s response to it, and subsequently the development of improved diagnostic and treatment strategies. Metabolomics is broadly defined as the nonbiased identification and quantification of the metabolites (or small molecular weight chemical compounds) present in a biological system or a sample, using an assortment of highly specialized analytical techniques, in combination with various computational, statistical and mathematical analyses [7]. Since the metabolome is the ultimate downstream product of the genome, transcriptome and proteome, a disturbance in any of these, due to any inherited or acquired perturbation, will alter the host metabolome with regard to the presence or absence of specific metabolites and/or their quantities. Additionally, the direct presence of a pathogen in these samples, or the host’s response to the pathogen, would also modify the metabolome. Subsequently, in the context of TB, a number of metabolomics studies have been carried out to date, searching for new markers using patient collected sputum [8,9]. Because of the fact that these altered metabolite markers or metabolite patterns are not only due to the presence of the foreign body in the host, but also due to a specific host response, patient samples carrying little/none of the infectious organism, such as blood [10,11] or urine [12], can also be used for new biomarker identification. Using serum markers obtained via an LC–MS-based metabolomics approach, Feng *et al.* [10] obtained an area under the receiver operator curve (area under the curve) value of 0.991, and Che *et al.* [11], an area under the curve value of 0.85, when using a GC–MS-based metabolomics approach. The advantages of using these samples over that of sputum are that they are more easily attainable and require less invasive collection procedures, in addition to offering a lower risk of infection during handling and transport. At this point, it is also important to mention how metabolomics has contributed to new bio-

marker identification for the purpose of TB diagnostics, using patient-collected breath, probably one of the least invasive sample collection approaches for diagnosing this disease to date. Using GC–MS-identified volatile organic compound profiles, Phillips *et al.* [13] were able to identify TB with a sensitivity of 84% and a specificity of 64.7%, and Kolk *et al.* [14] with a sensitivity of 62%, a specificity of 84% and an accuracy of 77%. Furthermore, metabolomics has been shown to be a valid approach for identifying markers characterizing/identifying various infectious *Mycobacterium* species with probabilities ranging from 72 to 100%, depending on the species of *Mycobacterium*, using as little as 1×10^3 cells [15], as well as drug-resistant strains [16,17]. Considering this, metabolomics is proving to be a valuable tool for new TB-diagnostic biomarker detection, considering the need for such a method to not only detect TB, but to also differentiate between various infectious *Mycobacterium* species and drug-resistant strains, in a highly sensitive and selective manner. Ideally, these techniques could also predict treatment outcome using patient samples collected via less intrusive methods, with a lower risk of infection to the clinical staff. The costs of the highly specialized analytical techniques required for detecting these markers, and the specialized skills required to operate these apparatus, may be seen as a drawback. The general idea, however, is that these expensive, selective and highly specialized apparatus would only be used in the biomarker identification phase in the context of a research environment, to aid in the identification and development of simpler detection methods specific to the identified diagnostic biomarkers. These could then be applied in the clinical context, for example, using nanotechnology-based detection approaches. This will subsequently allow for the use of these diagnostic biomarkers in the form of a rapid, inexpensive, sensitive and easy-to-use detection device, which does not require extensive training or sophisticated infrastructure, and can subsequently be used for point-of-care/bedside TB diagnostics. Alternatively, this approach can also be used toward high-throughput laboratory-based methods, where hundreds of samples are run simultaneously with minimal preparation, using inexpensive equipment such as a basic spectrophotometer, for instance.

Apart from their diagnostic applications, various newly identified TB biomarkers have additionally contributed to the existing knowledge of the biology of the causative pathogen [16], as well as to various underlying disease mechanisms [9]. These include mechanisms related to *M. tuberculosis* drug resistance [16,17] and virulence [18] including upregulation of various antioxidant pathways, the use of alkanes and fatty acids as alternative energy substrates [16,17,19], a shift in aconitase functionality toward mRNA binding and stability [17] and cell

wall remodeling [17,18]). Anti-TB drug mechanisms [20] and side effects in the host have also been elucidated including anti-TB drug inhibition of the host's electron transport chain resulting in oxidative stress and inducing an abnormal organic acid profile [21]. What is of even greater interest, currently, is that metabolomics has allowed for better understanding of the adaptations of *M. tuberculosis* to the host's defense and *vice versa*, and subsequently the discovery of the presence of a citramalate cycle in *M. tuberculosis*, and the interaction of this cycle with an upregulated glyoxylate cycle during infection; an increased utilization of fatty acids and glutamate by *M. tuberculosis* during infection; an additional mechanism by which the host manufactures hydrogen peroxide in order to eliminate the infecting bacteria; inhibition of the electron transport chain of the host by *M. tuberculosis*, resulting in elevation in various neurotransmitters and organic acids, better explaining the occurrence of various TB-associated symptoms [9]; and clues to improved treatment approaches [12]. Considering this, metabolomics has shed light on never before identified metabolic pathways in both man and the TB-causing bacteria, which in time will undoubtedly contribute to improved treatment strategies, and ultimately assist in curbing the epidemic.

Over the past 8 years, metabolomics has resulted in an exponential increase in the number of newly identified TB biomarkers, which has not only shed light on previously unknown disease mechanisms, but could potentially contribute to all aspects of TB clinical care, especially to that of improved TB diagnostics. Furthermore, metabolomics shows the capacity for identifying markers predicting treatment outcome, and could subsequently accelerate the time-consuming process of new anti-TB medication efficacy testing, and elucidating their mechanisms of action. Considering this, in the future, it would be beneficial to see increased efforts toward validating these newly identified biomarkers for use in a clinical context, especially toward improved diagnostics and anti-TB treatment strategies.

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