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Defining New Standards for the Diagnosis of Tuberculosis by Employing Sputum-Free Diagnostic Techniques

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Abstract

Although Tuberculosis has been a matter of grave concern for decades altogether, the curative for the same is yet to be established. However, years of rigorous research have opened up new avenues for both diagnosis and treatment. To ensure rapid, accurate, and economical testing for tuberculosis, especially in the nascent stages and in special groups, substantial research is required. One of the most promising techniques that ensure the delivery of optimal healthcare services employs sputum-free diagnostic techniques. The development of such techniques would potentially enable the rapid and efficient diagnosis of latent TB infections, in their nascent stages. Additionally, it would facilitate better planning of the available treatment regimen, to minimize adverse effects. This article aims to explore the various sputum-free diagnostic tools for TB, while concurrently stating the advantages and limitations associated with the same. The prime focus of this article will be to underscore the potential of sputum-free techniques and their future prospects in the clinical armamentarium.

Keywords: Tuberculosis; Diagnosis; Sputum-free techniques; *Mycobacterium tuberculosis*

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Introduction

Tuberculosis (TB) is one of the leading causes of death globally, and so is regarded as a grave public health concern. TB is spread through the air by droplets containing *Mycobacterium tuberculosis*, which are released by smear-positive patients with pulmonary tuberculosis while coughing and sneezing and remain suspended in the air. Inhaling these aerosols potentially causes an infection [1]. For a long time, tuberculosis has been associated with poverty, overcrowding, and malnutrition, all of which have a detrimental effect on the ability of sick people to access healthcare. The highest occurrence and fatality associated with TB are found in low-income countries and impoverished neighborhoods inside large cities in rich countries. Furthermore, these environments demonstrate a co-existence of immigration, significant social inequality, HIV infection, and drug or alcohol addiction, all of which are strongly associated with tuberculosis [2,3]. However, the most concerning current health challenge is the increasing frequency of multidrug-resistant TB diagnosed.

M. tuberculosis is known to infect 2,000 million people worldwide, or one out of every three individuals [4]. According to WHO estimates, there were 8.8 million new cases of tuberculosis in 2010 (64% of whom were men) and 1.5 million deaths related to tuberculosis [5]. Most of these cases are estimated to be in Asia (58%) and Africa (27%). The cases in India range from 2.0 to 2.4 million [6], when compared to 0.9 to 1.1 million in China [7], thereby accounting for 38 percent of the total number of cases.

Since 2002, incidence rates have been declining, with an average reduction rate of 1.3% per year, peaking at 2.2% between 2010 and 2011. Likewise, the total number of cases is decreasing, starting in 2006. In 2008, however, there were roughly 440,000 MDR (Multidrug-Resistant) cases of tuberculosis [8]. Furthermore, in some populations, TB can go untreated for a long period, making it an epicenter of infection for the entire community. In order to develop and administer specific therapeutic interventions, that are aimed at addressing the needs of these hard-to-reach groups, the recognition of these groups is imperative [9]. Without a cross-cutting approach to these socioeconomic determinants of the disease, global control and management of this disease will be a distant dream.

Current Landscape for the Diagnosis of Tuberculosis

At present, there is no gold standard test for the diagnosis of Latent Tuberculosis Infection (LTBI). Therefore, its presence is determined by a positive host immune response to the stimulation of the *M. tuberculosis* pathogen, in the absence of the clinical manifestation of TB [10]. On the other hand, LTBI refers to a broad spectrum of conditions, ranging from the elimination of infection to sub-clinical or incipient disease, which is often the first phase of reactivation of TB in which the patient is potentially infectious. The infection is kept controlled in a dormant stage between eradication and subclinical reactivation [10,11]. Generally, the methods employed for the diagnosis of LTBI include the Tuberculin Skin Test (TST), and Interferon-Gamma Release Assays (IGRA). Although these tests do not directly indicate the existence or survival within the host body, they efficiently detect the prior immunosensitization to *Mtb* [12]. However, its utility in diagnosing the nascent stages of LTBI has not yet been demonstrated to be effective. Although there has been substantial research on the development of novel diagnostic kits, the economic and pharmaceutical viability is limited and may require subtle modifications to cater to the needs of the affected cohort.

Sputum-Free Techniques for the Diagnosis of Tuberculosis

The development of feasible and reliable sputum-free diagnosis techniques is necessary to improve the efficiency and quality of primary healthcare facilities. Several other technologies are emerging to offer non-invasive diagnostic alternatives [13].

One such advancement includes the incorporation of Artificial Intelligence (AI) to analyses lung noises and cough patterns to detect TB [14]. TB changes the structure of the lungs, causing changes in chest sounds that can be detected using portable digital signal processing [15]. This technology employs digital recordings, digital stethoscopes, and audio analysis using AI-based cough classification [16]. It not only can screen all facilities entrants quickly and is easily scalable on mobile phones but can also be used to detect other pulmonary disorders by detecting abnormal noises in the absence of tuberculosis [17]. Although it is still in preliminary development stages, it holds great promise for the non-invasive assessment of lung health and TB diagnosis [14,16].

Furthermore, face masks and blow tubes with adsorbent capture filters have been developed to detect the presence of *M. tuberculosis* or pathogenic DNA in aerosols expelled from patients [18]. These techniques can be used on patients to detect TB in its initial stages; sampling aerosol from tidal breathing has given culturable *Mtb* bacilli, implying that cough is not a transmission prerequisite, and hence it will aid in the early detection of the disease [19,20]. This method also allows the detection of subclinical (asymptomatic) TB [21]. Tongue papillae biofilms may also be employed to detect *M. tuberculosis* using TB-LAMP and highly sensitive molecular technologies such as ultra [13].

Biological fluids such as blood, urine, and stool are also finding utility in TB diagnosis [13]. The stool is most commonly employed in pediatric TB detection and extrapulmonary TB in adults [22]. However, urine is a preferred alternative, given its ease of collection, higher accuracy, and utility in detecting extrapulmonary and pulmonary TB [13]. Cellular components of *Mtb*, such as nucleic acids and cells, can pass through the renal barrier and into the urine [23]. Lipoarabinomannan (LAM) is studied as a urine TB biomarker [24]. Additionally, blood biomarkers are also being explored as feasible diagnostic tools for TB. Although there are no validated TB blood tests, antigens, immune cell profiling, host transcriptomics, or cell-free *M. tuberculosis* DNA are viable routes of TB detection [13].

Improvement of TB detection and monitoring techniques could help deliver timely, accurate, and economical healthcare facilities to patients, even in the early stages. Thus, exploration of sputum-free alternatives could overcome the challenges and complexities of this devastating illness.

Advantages of Sputum-Free Diagnostic Methods

To overcome tuberculosis, prompt and efficient diagnosis is imperative, as it helps to improve the specificity and sensitivity of the test. Since the discovery of tuberculosis, microscopic examination and culture of sputum have been employed. Despite its widespread use, it does not identify Tb in patients with immunodeficiency syndrome and young children who are extremely susceptible to the disease [25]. Additionally, there is a growing understanding of the substantial proportion of patients found in prevalence surveys that are asymptomatic or pre-symptomatic (presumed subclinical TB). These emphasize on how the current symptom screening methodologies could very well induce error in the diagnosis of a significantly high number of cases. Furthermore, sputum confirmation testing may not be possible in the nascent stages of the disease, where the patient is infected but does not expectorate sputum [26,27].

Recently, there has been significant research in the development of exclusively sputum-free diagnostics such as the detection of human antibodies targeting *M. tuberculosis* antigens [28], trans renal DNA [29], and detection of biomarkers like gene transcriptional signatures, protein expression signatures and metabolic profiles in urine [30].

As these urine samples include broken-down products from dying cells and microbes, the extraction and amplification of *M. tuberculosis* nucleic acids facilitate the early, specific, and non-invasive identification of tuberculosis. This test is called trans-renal DNA [29].

However, the utility of serum analysis extends beyond the diagnosis of TB, as it also aids in the study of the antibody response to *M. tuberculosis* [30]. Furthermore, it has been found that the amphiphilic molecules of *M. tuberculosis* complex with host lipoproteins such as High-Density Lipoprotein (HDL) in host serum. Thereby, this directs toward a novel approach for the detection of bacterial molecules in the body. Alternatively, the detection of mycobacterial peptides in host exosomes, that help in local and systemic intercellular communication, could facilitate an efficient diagnosis of TB [31]. Thus, the detection of these complexes would help in developing a better understanding of the pathophysiology of TB, and could also serve as a potential biomarker in the early and accurate detection of the same.

A better and deeper understanding of the strengths and shortcomings of the diagnostic tests currently being employed and developed in TB diagnosis could aid in the development of a more logical and rational approach to the aforementioned constraints. For each situation, a judicious mix of the diagnostic approaches mentioned above is justified and may facilitate a better diagnosis for the patients. This would also allow for better stratification of

individuals who could potentially benefit from a particular empiric anti-TB treatment regimen.

Limitations in the Development of Sputum-Free Diagnostic Techniques

The development of precise, rapid, and sputum-free diagnostic methods has been a matter of great interest in TB treatment and prevention. A WHO consortium identified a rapid, non-sputum-based test as one of the highest priority target products for TB diagnostics [32]. Several non-sputum tests are being developed for the diagnosis of tuberculosis. However, they are faced with multiple challenges in their commercial application [13]. A variety of such tests and their potential limitations have been discussed in the following.

Genotypic assays are highly sensitive diagnostic methods propounded by molecular technologies based on the capture of nucleic acid and the detection of *Mycobacterium*. One such test is the Cartridge-Based Nucleic Acid Amplification Test (CBNAAT), used to test both TB and rifampicin resistance [33]. Although it has enhanced sensitivity and specificity, it requires a stable electrical power supply, temperature control, and annual calibration of the instrument. Furthermore, Xpert MTB/RIF assay tests are utilized to diagnose MDR-TB, which requires very expensive infrastructure, often inaccessible to low-middle income families [33]. The recent gene drive test developed to detect resistance to tuberculosis and rifampicin is highly advanced; however, it has not yet met WHO guidelines and shows poor recovery [33].

Furthermore, urine is an attractive alternative sample for TB diagnosis because it is easy to collect and poses a minimal risk of TB transmission. Trans-renal urine cells contain cfDNA, a promising biomarker of tuberculosis, but are challenging to detect due to its short length (100 bp) and low concentration of TB-specific fragments with inconsistent diagnostic sensitivities (0% to 79%) [34].

Furthermore, breath and aerosol tests are also viable routes of *Mtb* detection with high accuracy; however, they are faced with operational challenges, analytical approaches, require volatile organic compounds, and complex hardware [35].

Examination of proposed limitations could vastly improve the accessibility and efficacy of TB diagnosis. Thus, it is vital to explore and overcome these challenges to commercialize the sputum-free diagnosis of TB.

Conclusion and Outlook

Tuberculosis (TB) is an infectious disease caused by *Mtb* and primarily affects the lungs. TB is known to accompany other clinical manifestations such as cough (sometimes blood-tinged), weight loss, night sweats, and fever. Current traditional diagnostic techniques for TB rely heavily on sputum and are associated with compromised sensitivity and poor efficacy in the detection of extra-pulmonary tuberculosis, pediatric tuberculosis, and TB in patients co-infected with HIV. Thus, to address this issue, researchers across the globe are converging on sputum-free alternatives. Additionally, newly emerging technologies such as AI-based cough classification, face masks with adsorbent capture filters, and novel biomarkers in urine and stool are improving the accessibility of non-invasive diagnostic methodologies. Although certain infrastructural and financial obstacles exist in their widespread clinical application, exploration, and examination of non-sputum alternatives. Addressing these issues could potentially change the landscape for the detection and monitoring of tuberculosis, while simultaneously providing patients with timely, accurate, and economical healthcare facilities.

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