Chapter

Diagnostic Evaluation of Tuberculosis: Existing Challenges and Merits of Recent Advances

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Abstract

Tuberculosis remains a major global public health problem despite the modest infectious disease control efforts. Timely and accurate diagnosis is pivotal to the reduction in tuberculosis related morbidity and mortality. In addition, drug resistant form of tuberculosis is a serious threat to the efforts at tuberculosis control and eradication. Hence; there is the need for efficient methods of *Mycobacterium tuberculosis* infection diagnosis and treatment. There are major advances in the laboratory diagnostic methods for detection of *Mycobacterium tuberculosis* which seeks to complement or replace the existing conventional methods in a view to reduction in under-diagnosis and improved infectious disease management. Chest computer tomographies, Cephid GeneXpert, Line probe are some of the *Mycobacterium tuberculosis* diagnostic advances while chest x-ray, sputum microscopy/culture represent some of the conventional methods of evaluation of both *Mycobacterium tuberculosis* infection and its multi-resistant strain. Intriguingly, the conventional tuberculosis diagnostics though time consuming and inefficient, its use still predominates in high disease burden settings. Meanwhile, the slow transition to use of advanced tuberculosis diagnostic methods seems to have an economic undertone. The seemingly lack of cutting edge advanced *Mycobacterium tuberculosis* diagnostics in high disease burden countries is attributable to their suboptimal health financing model and over reliance on the donor organizations thereby retarding progress in tuberculosis eradication.

Keywords: mycobacteria, evaluation, constraints, advances

1. Introduction

Tuberculosis is a chronic granulomatous bacterial infection with a global occurrence [1–3]. This preventable and curable infectious disease has afflicted man since antiquity [4]. Many parts of the world especially the developing nations are still witnessing a rise in the number of new tuberculosis cases with its attendant consequences [3]. This rising prevalence trend of *Mycobacterium tuberculosis* infection is without prejudice to the strides and efforts in infection control [3].

Estimates have it that every minute someone somewhere gets infected with *Mycobacterium tuberculosis* making it a common globally transmitted disease [5, 6]. Epidemiologically, the World Health Organization (WHO) and other multinational health organization have reported tuberculosis as a worldwide pandemic [3, 7].

A recent report by WHO revealed a global tuberculosis incidence of 6.3 million for 2016 reflecting a rise from the preceding year [3]. In the same vein, sub Saharan Africa has a substantial burden of tuberculosis related morbidity and mortality as reported in the 2016 WHO global tuberculosis report [3].

The current epidemiological profile of tuberculosis in Sub-Saharan Africa seems to have an established association of the disease with poverty and social deprivation [3, 5, 8]. Similarly, the burden of tuberculosis remains an obstacle to socioeconomic development with a staggering direct and indirect cost of health financing [3, 9, 10]. The relationship between tuberculosis and social deprivation in addition to the adverse socioeconomic developmental effect of tuberculosis creates a vicious cycle of disease, poverty and poor productivity among the most disadvantaged in the society.

Mycobacterium tuberculosis is the causative organism of tuberculosis which was discovered by Robert Koch [11]. This granuloma forming microorganism is well adapted to live in a man and cause disease under favorable condition(s). In the same token, *Mycobacterium tuberculosis* is ubiquitous and has some unique features in its genetic makeup and cell wall constituents that not only distinct it from other organisms but also confers it with the strength of survival under harsh and stressful conditions [12, 13].

The *Mycobacterium tuberculosis* genome is composed of insertion sequences (IS) and phages which serve as unique features for its identification and speciation [12]. The type of IS that is abundant in *Mycobacterium tuberculosis* is IS6110 [12]. The knowledge of *Mycobacterium tuberculosis* genome is used in assessment of rate of transmission, identification of a regional circulating strain and detection of trans-border spread of the disease.

The main stay of *Mycobacterium tuberculosis* diagnosis has been the sputum microscopy (Ziehl Neelsen) and the solid media culture (Lowestein-Jensen medium) [14]. Although, these conventional methods of *Mycobacterium tuberculosis* identification and isolation are still in use, newer methods of tuberculosis diagnostics which include Ampiclor *M. tuberculosis* PCR test and Cephid GeneXpert is fast gaining relevance and acceptance [15, 16]. The merits of use of molecular and immunological techniques cannot be overemphasized in view of its additional benefit of infectious organism speciation, isolate antimicrobial sensitivity and detection of specific mutations that confer drug resistance (i.e., rpoB and KatG mutation) [15, 16]. The acquisition and deployment of molecular tuberculosis diagnostics in priority high disease burden areas would eventually increase rate of *Mycobacterium tuberculosis* infection identification. This is predicated on the fact that early detection and treatment of smear positive tuberculosis is attributed to improved cure rate [15].

2. Aim and objectives

This chapter is an exploratory study of existing tuberculosis diagnostics aimed at highlighting the conventional and advanced methods of *Mycobacterium tuberculosis* evaluation. The objective is to identify suitable and efficient method(s) of tuberculosis detection by way of profiling their merits and demerits.

3. Methods

This chapter is a descriptive research on the evolutionary trend of tuberculosis diagnosis. Available literature would be used to identify existing methods of *Mycobacterium tuberculosis* detection. A comparative analysis between the merits and

demerits of old conventional and advanced molecular/immunological methods would be carried out to ascertain what best serve the purpose of prompt and efficient detection of *Mycobacterium tuberculosis* infection.

4. Literature review

4.1 Global burden of tuberculosis

Mycobacterium tuberculosis infection remains a significant public health challenge despite the modest achievements in its control at the global, regional and country levels [3]. The alarming global prevalence rate of *Mycobacterium tuberculosis* infection has been put at one-third of the world population [1, 3]. Sub-Saharan Africa, South East Asia, and West Pacific region is home to most of the 22 countries highly burdened by tuberculosis, and this ranking seldom change [3, 17].

World Health Organization reported that 6.3 million cases of tuberculosis were notified in 2016 which might be under-estimation owing to the non-health seeking behavior and poor documentation in some regions [3]. This tuberculosis epidemiological pattern calls for sustained surveillance and prompt treatment to improve the disease related morbidity and mortality [3].

The rising number of drug-resistant form of tuberculosis poses an additional challenge on the inadequate resources allocated to healthcare delivery. In the year 2015, over 100 countries reported extensively drug-resistant tuberculosis [2]. Estimates by the WHO revealed that 490,000 individual developed multidrug-resistant tuberculosis in the year 2016 and only a fraction received treatment [3]. The threat of drug-resistant tuberculosis as affirmed by 2016 global report on tuberculosis may possibly be a consequence of inappropriate anti-tuberculosis medication use [3]. This emerging menace of drug resistant tuberculosis if not properly managed could retard the progress made in the fight against tuberculosis.

The global distribution of tuberculosis infection is in close association with poverty, immunosuppression and social deprivation [3, 5, 8, 17]. Sub Saharan Africa has the world highest tuberculosis incidence of 356 per 100,000 populations per year [2, 3]. Similarly, the social and clinical determinants of tuberculosis which include HIV infection, diabetes mellitus, under-nutrition, migration and smoking have been reported to preferentially afflict the young adult population [3, 5]. Nevertheless, tuberculosis infection generally has no age, gender, racial or regional bias.

Mycobacteria consist of different pathogenic and saprophytic mycobacteria species that are isolated from humans and the general environment [18, 19]. The two broad groups of mycobacteria which are tuberculous and non-tuberculous mycobacteria share some features but are largely distinguishable by molecular analysis and the utilization of some microbiological test which include niacin reduction test, nitrate test, and urease test [18, 19]. Lack of identification of the species of mycobacteria infection from the outset has a clinical, management and prognostic implication. The importance of mycobacteria speciation lie in their differing epidemiology, response to anti-tuberculosis, course of disease and long term consequences [18, 19].

4.2 Pulmonary and extra-pulmonary tuberculosis

Literally, tuberculosis could affect any organ system of the body and clinical presentation depends on the site of involvement. The human host immune system influences the clinical pattern of tuberculosis presentation. Tuberculosis is generally described as pulmonary if it involves the lungs and extra-pulmonary when there is

involvement of other body parts excluding the lungs [8, 20]. This disease has also been described as latent tuberculosis and active/open tuberculosis.

The initial *Mycobacterium tuberculosis* infection could be dormant and symptomless (latent tuberculosis) or it could be symptomatic (progressive primary tuberculosis) manifesting commonly as miliary tuberculosis, primary tuberculous pneumonia, and tuberculous meningitis [21].

Research has shown that 95% of primary *Mycobacterium tuberculosis* infection is contained and represented as Ghon focus on the chest radiograph [21, 22]. Post-primary tuberculosis is the most frequent mode of presentation of pulmonary *Mycobacterium tuberculosis* infection [21, 22]. This form of tuberculosis could result from an endogenous reactivation of the dormant bacilli from the primary infection or a reinfection with *Mycobacterium tuberculosis*.

Clinical manifestations of tuberculosis are body site-specific but may be subclinical thereby posing a diagnostic challenge with frequent missed clinical cases. Commonly, patients with tuberculosis have constitutional symptoms in addition to body site specific symptom(s) [8, 23–25]. The diagnostic challenge of tuberculosis is frequently observed among children and the elderly probably due to their unique immune constitution and the nature of their airway defense mechanism [22, 25, 26].

Clinical manifestation of pulmonary tuberculosis serves as a prototype of *Mycobacterium tuberculosis* infection with specific and non-specific clinical presentations. Cough is the predominant symptom of pulmonary tuberculosis [23–25]. Chronic cough lasting for at least 2 weeks has been used as a flag off symptom for initiating tuberculosis evaluation among individuals at the community level [3]. Difficulty in breathing, chest pain, and chest deformity could be addition complaints by the patients with pulmonary tuberculosis [23–25].

The physical examination equally poses an additional diagnostic challenge as clinical findings could be non-specific and unhelpful in diagnosis. Pulmonary tuberculosis patients usually have respiratory system specific clinical signs of disease or complication that include crepitation, bronchial breath sound, amphoric adventitious sound and pleural rub [23–25]. Noteworthy is the clinical manifestation of extensive lung destruction by way of fibrosis and contraction of lung volume [27].

4.3 Tuberculosis detection methods

4.3.1 Sputum microscopy/culture

The goal of investigation is to make a speedy, definite and accurate diagnosis of *Mycobacterium tuberculosis* infection and to identify complication(s) of the disease. The advances in tuberculosis investigation have provided increased sensitivity and have assisted in improving transit time to diagnosis [28, 29].

Sputum microscopy (Ziehl Neelsen) is a common method for microbiological profiling of *Mycobacterium tuberculosis* infection, especially in resource-limited countries [14, 28]. The fluorescence microscopy for *Mycobacterium tuberculosis* though not readily available is an alternative to sputum microscopy (Ziehl Neelsen) [30]. Both investigations use dye for staining but additionally, the fluorescence microscopy requires the light-emitting diodes (LED) to visualize *Mycobacterium tuberculosis* in sputum sample.

Although, sputum microscopy is cheap and can be rapidly performed, it is operator dependent and its cost-effectiveness is marred by a decreased sensitivity in pauci-bacillary states [15]. This diagnostic limitation has made extra-pulmonary tuberculosis difficult to diagnose. The rate of false-positive results and inter-operator differences in reportage of sputum microscopy has also compelled the need to recognize molecular based investigation (Cephid GeneXpert) as the first

line investigation for tuberculosis [15, 31]. Sputum microscopy is likely to continuously be a cornerstone investigation in the evaluation of tuberculosis patient in the resource constraint nations going by its cost-effectiveness. Hence, the need to improve its operational capacity by way of research and development.

Sputum culture for *Mycobacterium tuberculosis* represents an improvement well and above microscopy, making it a definitive investigation of choice for the isolation and identification of the infective organism [28–30]. Sputum culture has a better sensitivity and specificity when compared with sputum microscopy [15, 31]. In view of this superiority, clinicians are advised to obtain culture confirmation of tuberculosis where feasible.

Mycobacteria solid culture media which is typified by Lowenstein, Stonebrink, or Ogawa medium is the predominant and sometimes the only form of culture used in evaluation of tuberculosis [15, 32]. This mycobacteria culture is cheap, limits contamination but slow taking about 4–8 weeks to detection of culture growth. The merits of mycobacteria solid media culture is beclouded by the long transition to diagnosis which is inimical to tuberculosis control because it delays commencement of treatment. In contrast, liquid media which is represented by Bacteria Mycobacterial Growth Indicator Tube 960 and MB/Bact Alert 10 3D is more sensitive, faster in detection of growth and the gold standard for mycobacteria isolation [15]. The study by Munyati et al., estimated cost of mycobacteria liquid culture (1–3 culture) to be in the range of $53–$163 [32]. By this estimated cost it is considered to be impracticable to recommend mycobacteria liquid media culture in resource limited setting despite its cost-effectiveness in *Mycobacterium tuberculosis* infection detection and isolation. The cost of procurement and maintenance of mycobacteria liquid media culture may have informed the reason for its low availability and utilization.

4.3.2 Radiologic study

Radiographic profiling for tuberculosis is an indispensable investigation in the evaluation of infected patients. This investigative modality had a remarkable evolution from the use of the x-rays to the use of advanced imaging modalities which include the computer tomographic scan thereby upping the radiographic sensitivity and specificity [25, 30].

A normal x-ray does not rule out tuberculosis, and no radiological finding is a sine qua non for tuberculosis. This reality poses a diagnostic challenge for health care providers especially in the evaluation of tuberculosis among the immune-compromised. The x-ray imaging findings are predominantly abnormal in pulmonary tuberculosis among the immune-competent [30, 33]. The upper zone and apical lobe of the lower zone of the lungs are areas of predilection in pulmonary tuberculosis [30]. In pulmonary tuberculosis, the commonest x-ray manifestation is parenchyma involvement which is depicted by linear opacities, cavitations and military shadows [15]. Pleural effusion, pleural thickening, lymphadenopathy, and tuberculoma are the other extra-pulmonary manifestations that could be evident on the chest radiographic examination.

Following chronic inflammation from *Mycobacterium tuberculosis* infection, healing leaves behind nodules and parenchymal scarring. This is the radiologist nightmare and a diagnostic challenge because residual x-ray findings represent a stable disease but does not rule out active tuberculosis. In addition, atypical findings on radiographs of immune-compromised individuals may lead to misdiagnosis of *Mycobacterium tuberculosis* infection.

Chest computer tomography (CT) provides a diagnostic advantage in detecting fine and equivocal lesions among tuberculosis patients when compared with x-ray

films [15]. The findings on chest CT which could represent an active disease or disease complications include features suggestive of consolidation, fibrosis, cavitations and honey combing [15]. Similarly, the chest CT provides a clearer view of extra-pulmonary involvement of *Mycobacterium tuberculosis* infection. For example a "tree in bud" appearance on chest CT sufficiently represents active disease which may not be discernible on x-ray films [15]. Despite, the added advantage of chest CT over chest x-ray, microbiological test for *Mycobacterium tuberculosis* is essential for a definitive diagnosis.

4.3.3 Molecular methods

Tuberculin skin test for the latent tuberculosis is an ancient molecular investigation that still reserves a place in clinical practice [30]. A major drawback of tuberculin skin test is its inability to differentiate the latent tuberculosis from an active disease. This diagnostic drawback has a therapeutic and prognostic implication. Line probe assay and Cephid GeneXpert System are the other advanced molecular investigations that have enabled detection of drug resistant tuberculosis [30]. Cephid GeneXpert System has been recommended as the first choice tuberculosis investigation especially among suspected cases of multidrug resistant disease [34, 35]. This decision may have been predicated on the suitability of Cephid GeneXpert System for use in resource limited environment due to its adequate sensitivity, specificity and the minimal technical knowhow for its operation [34, 35]. However, this guideline should be cautiously recommended for developing nations because they may not cope with its financial implication.

Lipoarabinomannan (LAM) assay in urine has been commercially available for over 20 years but its deployment for use in tuberculosis diagnostics is slow [36]. This molecular investigation is a point-of-care assay that enables detection of LAM which is a mycobacterial cell wall glycolipid antigen [36, 37]. Lipoarabinomannan assay in urine has enabled diagnosis of disseminated tuberculosis especially in pauci-bacillary state and among HIV patients. A meta-analytic study has reported a sub-optimal sensitivity for LAM assay in urine [38]. However, subsequent improvement in LAM assay in urine by way of development of strip test version of this molecular investigation improved its sensitivity, specificity and cost-effectiveness.

4.3.4 Immunological methods

Immunological investigations for active tuberculosis include nucleic acid amplification tests and bacteriophage-based tests. These immunological investigations have allowed detection of nucleic acid sequence of *Mycobacterium tuberculosis* and assisted in genetic finger typing [28–30]. In furtherance, immunological methods of *Mycobacterium tuberculosis* diagnosis provide an opportunity for the infective organism speciation (MicroSeq 500 system, AccuProbe assay) and drug sensitivity test [30]. Worthy of mention in the deployment of immunological investigation for multidrug resistance evaluation are assessment of the rpoB gene mutation for rifampicin resistance, the inhA gene and the katG gene mutation for low and high level isoniazid resistance, respectively [30, 39].

In contrast to smear microscopy/culture, nucleic acid amplification test has a greater positive predictive value (>95%), increase specificity and ability for rapid confirmation of infection [15]. Nevertheless, the smear microscopy/culture still has relevance in modern clinical practice because of its high sensitivity and utility when drug sensitivity test of second line anti-tuberculosis is required [15].

5. Conclusion

In conclusion, there is need to complement and replace where necessary the conventional tuberculosis diagnostics with advanced cutting-edge microbiological, imaging, molecular and immunologic investigations for prompt and adequate diagnosis. The diagnostic and therapeutic advantages of advanced methods of mycobacteria detection call for its speedy deployment to priority areas to enhance accuracy and efficiency of clinical evaluation. The cost of procurement and maintenance of this advanced methods of tuberculosis diagnosis maybe beyond the reach of the health care systems of nations with high burden by tuberculosis. Hence, the reason why donor funding is crucial in provision and maintenance of advanced molecular and immunological tuberculosis diagnostics in high disease burden areas especially in sub-Saharan Africa [40, 41].

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