Diagnosis and Management of Tuberculosis

Chapter 1

Current Status of Pediatric Tuberculosis Diagnostics, Needs, and Challenges

Shailja Jakhar[1]; Kiersten Lenz[1]; Harshini Mukundan[1*]

[1]MSJ567, Physical Chemistry and Applied Spectroscopy, Chemistry Division, Los Alamos National Laboratory, Los Alamos, New Mexico 87545.

*[*]**Correspondence to: Harshini Mukundan,** MSJ567, Physical Chemistry and Applied Spectroscopy, Chemistry Division, Los Alamos National Laboratory, Los Alamos, New Mexico 87545.*

Email: harshini@lanl.gov

1. Overview and Statement of Problem

Tuberculosis (TB) is a chronic bacterial disease caused by *Mycobacterium tuberculosis* (Mtb). About one third of the world's population is infected with Mtb, of which only 5-10% develop clinical symptoms and progress to an active disease state. The rest of the population are carriers of the disease, a condition referred to as latent TB infection (LTBI). The transition from latent carriers to active disease can be influenced by many factors such as HIV co-infection, age, co-morbidities such as malaria and other factors, and poses a problem for reliable diagnosis of TB [1].

TB is a major public health concern in all age groups, but presents a bigger challenge in pediatric populations, primarily owing to the lack of reliable diagnostics. Young children (<3-4 years of age) are most commonly exposed to Mtb infection from adults in the family. Some of the factors that increase risk of exposure are age, physical structure of the child's house, and sleeping practices. The chance of infection differs with age: there is a 20-30% risk in children aged 1-2 years, 5% risk in children aged 3-5 years, 2% risk among children 5-10 years, and 5% risk among children older than 10 years [2]. The burden of TB is much higher in developing countries due to various factors including poverty, malnutrition, HIV, HIV-TB co-infection, and increased drug resistance. TB reporting gaps are the most profound among younger children, as 55% of children estimated to have the disease are not reported to national monitoring and surveillance programs, as compared to a rate of 35% in adults. This reporting disparity amongst children varies with age: 69% of cases are unreported in children younger

than 5 years of age, and 40% of cases are unreported in children 5-14 years [3]. Because of the disease progression and risks to children, more focus needs to be placed on understanding, diagnosing, and treating pediatric TB.

There are three major challenges associated with presentation of TB disease in pediatrics, which complicate diagnostics and therapeutic intervention:

1. Pediatric TB is paucibacillary – i.e. clinical samples and isolates from infected children contain few bacteria, making culture and isolation of the causative agent challenging [4]. For this reason, pediatric TB is considered a minor contributor to the spread of infection in a population. However, young children have a high risk of disease progression following infection, and are more likely to develop severe or disseminated disease. Indeed, in some of the higher burden regions, children account for more than 20% of TB cases [5].

2. Pediatric TB is also often disseminated. As with adults, disseminated TB is much more difficult to treat and control, because of the uncharacteristic presentation and unclear treatment regimens. This same challenge also extends to children presenting with drug-resistant forms of the disease, because the dosage and regimens for treatment in children have not been well established. Dodd *et al*. reported that of the estimated 850,000 children diagnosed with TB in 2014, about 7% were isoniazid resistant, 3% were multidrug resistant (MDR), and 4.7% of MDR cases were extreme drug resistant (XDR). It has been demonstrated that more children are infected with drug resistant TB than are actually diagnosed [6]. Thus, drug resistant TB presents an significant challenge in children, especially those living in the vicinity of adults with similar variants of the pathogen, because it is difficult to diagnose, track, and treat in this population.

3. TB can manifest itself in pulmonary and extra pulmonary forms, of which extra pulmonary TB (EPTB) is more difficult to diagnose, especially in pediatric populations. Pulmonary TB (PTB) commonly presents as a cough that lasts greater than 4 weeks, dyspnea, asthenia, chest pain, hemoptysis, persistent evening fever, night sweats, and weight loss. In children, these symptoms can be easily mistaken for infections associated with other respiratory, and present with similar clinical and radiological findings, making it more difficult to diagnose the disease in this population. EPTB presents an even greater challenge. The disease can be disseminated, or it can present in the genitourinary, gastrointestinal, cerebral, lymphatic, or skeletal systems [7].

Together, these factors complicate our ability to identify and control pediatric TB. The goal of this chapter is to summarize the problems, challenges, promising future avenues, and future needs for combating the problem of pediatric tuberculosis, with specific emphasis on diagnosis of the condition.

2. Challenges

The inability to diagnose TB poses the biggest challenge to management of the disease in children. Early symptoms of TB are very similar to other childhood diseases, including viral and bacterial infections, pneumonia, and other respiratory diseases. As mentioned earlier, TB presents in paucibacillary form in children [8]. Sputum culture, which is the gold standard diagnostic for adults, remains negative in ~70% of pediatric cases because of the low bacterial load in this population [5]. This problem is further confounded by the fact that many children present with disseminated disease, thereby producing no sputum. And even in those with pulmonary disease, young children are unable to expectorate sputum, making it a difficult approach for reliable diagnosis in this population. These factors and the differential pathology of the disease impact diagnosis by traditional culture-based methods, sputum microscopy, and newer approaches such as Gene Xpert based detection. Indeed, the percentage of children with active TB that were missed by confirmatory tests are 40% missed by culture, 50% by gene Xpert, and 77% by microscopy [9]. Therefore, the diagnosis of childhood TB is currently based on history, clinical symptoms, the Tuberculin skin test (TST), and chest radiography, [10] each of which is associated with high rate of failure and unreliability. The consequent alarming percentage of missed diagnoses points to an urgent need for a rapid and sensitive point-of-care diagnostic for tuberculosis in children.

3. WHO Goals for Pediatric TB (2018)

Throughout the world, TB remains the leading cause of pediatric mortality from a single infectious agent. In 2017, 1 million children younger than 15 years of age (10% of total TB cases) developed TB, of which 52% were less than 5 years of age. 80% of TB-related pediatric deaths were among children 5 years old or younger, and 17% of those were co-infected with HIV. 15% (233,000) of the pediatric TB-related deaths were among children that had poor access to diagnosis and treatment. There were about 150-400 cases per 100,000 people in high-burden, low income countries, as compared to only about 10 cases per 100,000 in high income countries. This disparity highlights the need to increase access to proper diagnosis and treatment in low income regions, which would help prevent many of the TB-related deaths in children. Because of these reasons, the World Health Organization (WHO) is on a mission to reduce the absolute number of TB deaths by 90% and incidence rate by 80% by the year 2030 (as compared to 2015) [11].

The WHO report clearly identifies the need for an effective diagnostic in pediatric populations in order to achieve this goal. According to the WHO, successful diagnosis and treatment of TB can prevent millions of deaths each year. In order to develop a successful diagnostic, understanding the reasons for under-diagnosis are critical. Today, under-diagnosis of TB in pediatric and adult populations can be due to various factors such as poor access to

healthcare, lack of symptoms, healthcare providers failing to test for TB, in addition to the poor sensitivity and specificity of the diagnostic itself. These factors are all compounded in pediatric populations owing to the differential presentation of the disease. Further, most of the gaps in detection and treatment were observed in African regions, where HIV-TB coinfection rate is significantly high, and in resource-poor regions of the world (e.g., parts of India and China). [12] To close the gap between detection and treatment, a new WHO initiative called "Find. Treat. All." was established in 2018. The goal of this initiative is to detect and treat 40 million people, including 3.5 million children, from 2018-2022. Such initiatives and the WHO report establish the clear need for new and effective diagnostics for pediatric TB.

4. Current TB Diagnostic Tools

Approaches to diagnose Mtb infection can be broadly divided into two categories:

1. Detection of the human immune response to Mtb infection (e.g.; detection of antibodies and activated T cells); and

2. Direct detection of Mtb and Mtb Signatures (e.g.; microscopy, culture, antigen and nucleic acid detection assays) Several diagnostics have been developed under each of these categories for the diagnosis of TB infection, and many have been adapted or evaluated in pediatric populations. A complete review of all of them is beyond the scope of this chapter. Thus, we will primarily focus on non-nucleic acid-based diagnostics for pediatric TB in this manuscript.

4.1. Assays for the detection of the human immune response to Mtb infection:

1. *Tuberculin skin test (TST)* - One of the earliest diagnostic assays that was developed for the diagnosis of TB infection is the Tuberculin skin test (TST). The technique involves the application of tuberculin/purified protein derivative to the skin, and is also known as the Mantoux test, Mendel- Mantoux test, Heaf test, or Pirquet test. The antigen is injected intradermally and the human immune response to the pathogen-specific antigens is assessed by measuring the diameter of the inflammatory response on the skin. If the diameter of induration is greater than 10 mm within two days after injection, the result is considered positive for TB exposure. Thus, the results are subjective, qualitative, and require two visits to the physician for final diagnosis [13]. TST cannot discriminate exposure from infection, and is currently only prescribed for the diagnosis of LTBI. Another disadvantage of the TST is poor specificity in individuals with prior exposure to non-tuberculous *Mycobacteria* (NTM), or those who have been vaccinated with Bacillus Calmette-Guerin (BCG), both of which can result in false positive outcomes. TST may also have low sensitivity in younger children and those with advanced TB, immunity, or malnutrition [14]. The sensitivity of this test is reported to be 63-75% in immunocompetent TB-suspected individuals, 44-56% in malnourished individuals, and 36-69% in HIV-infected

individuals [9,15]. Depending on the antigen preparation and methods used, there is significant disparity in the efficacy and use of the TST. This also depends on the population in question, and health care infrastructure therein. A Gambian study showed that TST is slightly more sensitive than enzyme linked immune absorbent spot (ELISpot, which measures release of IFN γ, a host immune biomarker, see below for further information) in children exposed to Mtb, and it is not confounded by prior BCG vaccination [16]. On the other hand, a study performed in the UK showed that ELISpot had a higher sensitivity when compared to TST in children exposed to a confirmed TB case in school [17]. Similarly, a study in children with a history of exposure to TB showed that a variant of the ELISpot assay, focused on measuring IFN γ release, named T.SPOT, resulted in a test sensitivity of 50%, and was no better than TST (80%) in culture-confirmed cases. Thus, TST cannot be used to exclude active disease [18]. However, another study performed in Australia showed higher specificity of QGIT compared to TST and a high discordance between both tests [19]. It is noted that both IGRAs (see below) and TST measure the host immune response to the pathogen, and hence, can be influenced by infection with similar organisms, or previous infection with the pathogen in question.

2. *Interferon gamma release assays (IGRAs*)– Originally developed by Oxford Immunotec, UK, Qiagen, USA, the IGRA is based on the release of interferon γ (IFN γ) when T cells of individuals are exposed to Mtb. This release of IFN γ can be measured quantitatively *in vitro*, which makes the assay less subjective as compared to TST. This technique has currently been approved by the WHO for diagnosis of LTBI. Mtb antigens such as the Culture Filtrate Protein 10 (CFP10) and Early Secretory Antigenic Target 6 (ESAT6), proteins encoded by genes within the Region of Difference 1 (RD1) of Mtb genome, elicit interferon γ response in the human host, *via* activation of innate immune receptors. The BCG vaccine strain of Mtb, and some non-mycobacterial species, do not contain these two antigens. Hence, even though they are based on host recognition mechanisms, IGRAs have been shown to have better sensitivity and specificity than the TST, and can differentiate between BCG vaccination and infection, unlike TST in a variety of studies [13]. Some of the commercially available IGRAs include QuantiFERON-TB Gold (QFT-G), QuantiFERON-TB Gold in-tube (QFT-G-IT) and T-SPOT TB (T-SPOT), [13] which vary in the mode of detection (lateral flow assays, ELISA-based, and other). However, there exist some studies which question the superior performance of IGRAs over TST. For instance, Kampmann *et al*. showed that TST had better sensitivity than IGRA (QFG-IT and T-SPOT.TB) in predicting definite TB cases, but showed similar performance of both IGRA tests in LTBI cases. Further, TST and T-SPOT.TB had reduced sensitivity in EPTB compared to PTB, whereas QFG-IT demonstrated similar performance in both [20]. IGRAs have been evaluated for diagnostic efficacy in pediatrics. Connell *et al.* showed similar concordance (93%) between both IGRAs in LTBI children [21]. Bianchi *et al.* showed a good agreement between positive QFG-IT and active disease, and an intermediate agreement between IGRA and TST [14]. A meta-analysis done by Laurenti *et al.* showed

no difference in sensitivity between TST, QFT-IT, and T-SPOT.TB among immunocompetent children, but found that the specificity of QFT-IT and T-SPOT.TB was much greater in this population when compared to TST [22]. Other reviews support the observation that IGRAs demonstrate higher specificity over TST, but suggest that their sensitivity is in fact lower (66% pooled sensitivity from 20 different studies) than TST [23]. ELISpot assays have been shown to demonstrate a sensitivity of 83% in all TB cases, and ~75% in individuals with HIV co-infection and/or malnutrition [15]. Most recently, Lehman *et al.* performed an analysis of the use of IGRA in children, as compared to TST. They found that IGRA testing has greater specificity compared to TST in children > 5 years of age, and recommended it to be the test of choice for diagnosing TB in this age group. However, in younger children (< 5 years of age), both TST and IGRAs were shown to have similar sensitivity. In all of these studies, a negative IGRA does not rule out TB, particularly in children < 1 year of age and those with central-nervous system affliction, suggesting that better and more reliable methods are required for pediatric disease, especially EPTB.

On the whole, these findings suggest that IGRAs are a valuable platform for the diagnosis of TB. In theory, because IGRAs measure host immune recognition of Mtb antigens, they should be effective in PTB as well as EPTB. However, the efficacy of this method in EPTB and pediatric populations has not been well established [24]. IGRAs are more expensive compared to TST, but have the advantages of being free from human errors, and they only require one visit to a clinic [15,25,26].

4.2. Assays for direct detection of Mtb and Mtb Signatures

1. *Sputum smear microscopy* – The acid-fast nature of Mtb provides for a simple staining based microscopic identification in people presenting with PTB [27-30]. This is the primary method to diagnose TB in low- and middle-income countries. Both light and light emitting diode microscopes have been endorsed by the WHO for diagnosis and treatment monitoring of TB using this method. The technique is simple, rapid, and inexpensive, with high specificity in high burden TB areas and the sensitivity is moderate in PTB patients [27-31]. There are several challenges in the use of this technique for the reliable diagnosis of TB. For one, the WHO requires at least two (but preferably three) sputum specimens to be collected from each patient suspected of having PTB. The results depend on the skill of a microscopist, and are impacted by the overall health of the patient and ability to expectorate sputum, complicating results and therapeutic intervention. Since sputum microscopy requires the actual presence of bacteria in the chest expectorate, the method cannot be applied to patients with EPTB [32,34]. Even in cases of PTB, collecting adequate sputum samples from children and immunocompromised individuals presents another challenge [35]. Only 15% of children diagnosed with TB have a positive smear from either sputum or from gastric aspirate [36]. The sensitivity of the method is reported to be between 12-22% in immunocompetent TB-suspected individuals, while the

specificity is 100% [9,37]. One challenge in the application of the method for diagnostics in pediatric populations is the paucibacillary nature of the disease, which complicates the ability to acquire three repeated positive smears from a single patient. Further, the sensitivity of microscopy for induced sputum as compared to culture range from 20-57% in children making it unreliable for diagnosis [38]. A sputum sample is extremely infectious; handling and processing of the sample for microscopic characterization increases the risks associated with this method.

2. *Culture-based methods* - Culture is the current gold standard TB diagnostic in adults, but even this method fails quite often in children because of the differential manifestation of the disease. Mtb can be cultured, albeit requiring a longer time compared to most common bacterial pathogens, and the requirement of laboratory infrastructure and trained personnel complicates the process as well. The two culture-based diagnostic systems approved by the WHO are 1) the liquid culture system with rapid speciation, and culture-based phenotypic drug sensitivity testing (DST) using 1% critical proportion in LJ,7H10,7H11 (culture media) and 2) the mycobacterial growth indicator tube (MGIT) media. Of the two, MGIT provides a significantly faster diagnosis when compared to conventional solid culture, but has the disadvantage of a high cost [11]. A lower cost alternative to these methods is microscopic observation drug susceptibility (MODS), which has also been shown to be more sensitive in pediatric populations [11] when compared to conventional modalities, and allows for the simultaneous assessment of drug resistance. The main disadvantage of culture-based methods is they may take up to 12 weeks for the test results to come back due to the slow reproduction rate of *Mtb*. The ability to culture the pathogen in clinical samples varies with various factors such as age, HIV status, disease progression, and clinical presentation [11]. Whereas culture is well established in adults, there is a scarcity of data in children. The paucibacillary nature of pediatric TB results in reduced sensitivity of culture in children [39]. Only 40% of children diagnosed with TB receive a positive culture test result [40]. The sensitivity ranges from 44-60% in immunocompetent TB-suspected individuals, while specificity is 100% [9,37]. A study in Vietnam showed a sensitivity of 81.3% for MODS and 88.6% for liquid culture [41]. As with smear microscopy, negative culture results cannot be used to rule out TB in children, [42] due to the complications associated with presentation of the disease. However, when positive, culture can be useful to distinguish between non-mycobacterial and mycobacterial disease in HIV-TB coinfection [42]. With the PTB and EPTB presentation of the disease, and the paucibacillary nature of pediatric TB, culture cannot be used to exclude the disease when negative, but is definitely confirmatory when positive. The choice of the sample, and the concentration of the bacteria for growth are critical considerations in the use of culture.

3. *Rapid molecular tests* - Xpert Mtb/RIF assay (Cepheid, USA) is the only rapid molecular test currently recommended by the WHO for pediatric TB today, and can provide results within

2 hours of sample collection. Current policy recommends it to be used as an initial diagnostic test in children suspected of having MDR-TB or HIV-associated TB. The WHO acknowledges the inability to get microbiological confirmation in children, and allows for the use of data from adults to guide this recommendation in children. The test can simultaneously detect TB and resistance to Rifampicin, by detecting a DNA sequence specific to Mtb through polymerase chain reaction. Steingart *et al.* showed a sensitivity of 65.1-75.9% for children [43]. In order to increase accessibility to rapid molecular testing for TB, Cepheid developed the Edge platform, which is a single-module instrument that connects to a tablet, facilitating storage and transfer of data. This allows for the instrument to function in more decentralized settings, at the same level as microscopy, as it includes an auxiliary battery. The WHO meeting report shows that the next-generation Xpert Mtb/RIF Ultra cartridge will offer enhanced sensitivity as compared to current Xpert Mtb/RIF cartridge in detecting *Mtb* in paucibacillary specimens, including smear-negative culture-positive specimens (e.g. those from people living with HIV), extrapulmonary specimens (notably cerebrospinal fluid), and specimens from children [11]. The sensitivity is 49% in immunocompetent TB-suspected individuals, while specificity is 100% [9]. However, the Ultra cartridge has the disadvantage of a short shelf life, which makes it difficult to use in low-resource countries. Yet, Xpert offers the most promise for the application of molecular diagnostic technologies for the diagnosis of pediatric TB, and variant manifestations of the disease.

4. *Detection of Lipoarabinomannan, an Mtb Biomarker*– Lipoarabinomannan (LAM) is one of the most studied Mtb biomarkers [44,45]. LAM is secreted by Mtb, and is a conserved lipoglycan involved in virulence. The biomarker is known to activate Toll-like receptor 2 mediated innate immune pathways during Mtb infection [46]. Investigators have demonstrated the secretion of LAM in urine and its presence in blood [47,48]. Detection of LAM in multiple patient samples, as described below, offers a promising strategy for the diagnosis of TB. *LAM in urine* - Detection of LAM in urine has allowed for the development of point-of-care tests for TB. However, most of these tests present with low sensitivity and hence, are not suitable for use as general screening tests for TB. However, the sensitivity for the diagnosis of TB among individuals coinfected with HIV, especially among patients with low CD4 counts, is significantly elevated and these assays are therefore being widely used in this population. The urine LAM strip-test (Determine®-TB Alere, USA) is currently recommended by the WHO in HIV-positive adults with CD4 counts less than or equal to 100 cells/μL with signs and symptoms of TB. Since 2015, new evidence has emerged that might justify the use of the test in a broader group of people living with HIV [3]. LAM detection in urine has the advantage that it allows for simple, non invasive sample collection, is associated with low cost, less bench time, and does not require highly trained personnel. A WHO update on LAM assays reported a pooled sensitivity of 47% and pooled specificity of 82% among various studies performed in children with HIV [49]. *LAM in blood* - LAM produced by Mtb at the site of

infection quickly enters the blood stream. However, LAM is amphiphilic, as are other bacterial pathogen associated molecular patterns (PAMPs) that activate immune recognition, and is unstable in aqueous blood [50,51]. Because of this biochemistry, LAM seeks to associate with host membranes or carrier complexes for biochemical stability. In blood, host lipoproteins such as high- and low-density lipoprotein (HDL, LDL) sequester LAM, resulting in modulation of the inflammatory response [52,55]. Understanding this host-pathogen biology, Mukundan *et al.* have developed a novel lipoprotein capture assay using a biosensor platform developed at Los Alamos National Laboratory to directly and quantitatively detect LAM in blood [54]. The fact that LAM is expressed, albeit hidden in lipoproteins, in the blood of patients with active TB, and that this expression occurs irrespective of whether the patient has pulmonary or disseminated disease makes it a promising target for diagnosis of pediatric TB [53,56].

5. The Lack of Gold Standard Diagnostic and Implications

One of the major challenges in diagnosing childhood pulmonary TB is the lack of a reliable gold-standard diagnostic, which leads to significant under or over treatment of children with suspected disease [35,57–60]. Culture is considered the gold standard in adults, but has been shown to be imperfect in detecting childhood TB [35,57]. Less than 15% of pediatric cases are sputum smear positive, and culture detects around 30-40%, due to reasons outlined earlier in this chapter. Therefore, childhood TB is diagnosed based on a triad of close contact with a TB patient, positive TST, and abnormal chest radiograph, [35] which results in significant misdiagnosis and under diagnosis, both of which have societal and individual implications.

Evaluation of new diagnostic tools for the detection of childhood TB is difficult due to the absence of accurate comparative matrices and reference assays [9]. One of the key factors that can be considered when evaluating new diagnostics is the duration and proximity of suspected case of pediatric TB to confirmed cases – i.e. transmission cohort studies. Some studies have emphasized the significance of exposure to confirmed TB cases, as positive results increased with increased exposure [16,17].

6. Pediatric TB in Context of HIV

TB is the most common opportunistic infection and leading cause of death in people with HIV, including children. The immunocompromised status of HIV positive individuals may allow for the activation of TB in latent carriers, increase risk and susceptibility in non-carriers, and enhance unconventional disease presentation and possibility of disseminated disease, all of which challenge conventional diagnostic approaches. Children with a low CD4 count have a five-fold risk of contracting TB as compared to those with mild immunosuppression [61]. The risk of infection by drug-resistant TB also increases with HIV coinfection [62]. HIV-TB coinfected patients are 37% more likely to develop resistance to at least one drug, versus 19% of patients with TB only [63]. HIV-TB coinfection has been reported to be over 50% in some

high burden African settings [64]. Globally, 11% of HIV-positive TB patients died during treatment, and the possible reason for poor outcome is late detection of HIV-associated TB, as well as delayed start of treatment. Whereas most of this data is accrued on adult populations, the ramifications apply to children – if only more significantly than to adults. Therefore, the WHO has recommended treatment for latent TB infection in HIV individuals and children under 5 years, who are living in households with individuals/family members with bacteriologically confirmed TB. The 2018 WHO report recommended the use of GeneXpert assays and lateral flow urine LAM assays in HIV clinics to help ensure early diagnosis and reduced mortality [65]. The relationship between TB and HIV coinfection, and the implications of this association on disease manifestation, need to be considered in making treatment decisions.

7. Research Needed on Pediatric TB

The WHO has developed a roadmap to end TB in children and adolescents, with the goal of developing new diagnostic approaches for systematic TB detection in vulnerable children and to develop child-friendly point of care tests with requisite accuracy by the year 2023. According to the WHO 2018 report, a major technological breakthrough is required by 2025 so that TB incidence rate can fall to much lower levels, and the spread of the disease can be curtailed. There is a significant dearth in the investment and development of new diagnostics, which has delayed such breakthroughs and effective control of disease spread. Consequently, there is an urgent need for a new diagnostic that can minimize barriers to healthcare access, ensure quality testing in difficult to diagnose groups, is affordable to use, and has low maintenance costs, especially in pediatric populations. Early diagnostic tests, which are usable at the point of care, and can accurately diagnose PTB and EPTB in children with/without HIV co-infection can provide that much required breakthrough and allow for the realization of the WHO goals for global TB control.

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