Recent advances in the diagnosis and treatment of childhood tuberculosis

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Abstract

Despite over 2.3 million (26% of global burden) cases of tuberculosis (TB) in India the accurate diagnosis of childhood TB remains a major challenge. Children with TB usually have paucibacillary disease and contribute little to disease transmission within the community. Consequently the treatment of children with TB is often not considered a priority by TB control programmes. Adequate and timely assessment of TB infection in childhood could diminish epidemiological burden as underdiagnosed pediatric patients can eventually evolve in to an active state and have the potential to disseminate the etiological agent *Mycobacterium tuberculosis*, notably increasing this worldwide public health problem. In this review we discuss the most important recent advances in the diagnosis of childhood TB: (1) Symptom-based approaches, (2) novel immune-based approaches, including *in vitro* interferon-γ IGRA release assays IGRA tests; and (3) bacteriological and molecular methods that are more rapid and/or less expensive than conventional culture techniques for TB diagnosis and/or drug-resistance testing. Recent advances have improved our ability to diagnose latent infection and active TB in children, nevertheless establishing a diagnosis of either latent infection or active disease in HIV-infected children remains a major challenge.

Key words: Childhood tuberculosis, diagnosis, recent advances, treatment

INTRODUCTION

India had an estimated 2.3 million (26% of global burden) tuberculosis (TB) cases in 2010, and ranked 16th in terms of incidence rate amongst 22 highest TB burden countries. Despite decrease in TB incidence, 8.8 million cases and 1.4 million deaths occurred globally in 2010.[1] The ongoing TB epidemic reflects improper, delayed or missed diagnosis; especially in resource limited countries. Delayed diagnosis of TB not only postpones the required antitubercular treatment ATT, leading to more severe illness and causing irreversible damage to affected organ(s), but also enables un-interrupted transmission of *Mycobacterium tuberculosis* for longer duration.[2]

The natural history of TB in children and pediatric patients follows various phases each representing the progressive nature of the disease.[3,4] There are significant advances in the field of TB diagnostics in last two decades; however the poorly sensitive light microscopy and poorly specific chest radiography still remain primary means for diagnosing TB, in most of the developing countries, including India.[5] The most significant advances in last few years have been BACTEC, (Becton Dickinson, Franklin Lakes, New Jersey, USA). QuantiFERON-TB Gold (Cellestis Ltd., Carnegie, Australia), enzyme-linked immunospot (ELISPOT) (Oxford Immunotec, Oxford, UK) assays and nucleic acid amplification tests such as line probe assay and Gene-Xpert but high cost or sophisticated infrastructure requirements have remained major barriers for their large scale implementation for routine use.[6]

A variety of enzyme-linked immunosorbent assay (ELISA) kits are available commercially for the diagnosis of TB,

however all commercial ELISA tests exhibited highly variable sensitivity (0-100%) and specificity (31-100%).[7] A combination of factors including high costs, limited resources and the poor performance of various diagnostic tests make the diagnosis of TB difficult in developing countries. Additonally the emergence of multidrug-resistant TB (MDR-TB) is a cuase for concern. Extensively drug-resistant TB (defined as MDR-TB strains additionally resistant to a fluoroquinolone and a second-line anti-TB injectable agent such as kanamycin, amikacin, or capreomycin) has been reported in 58 countries.[8] Here we discuss the recent advances in the diagnosis and treatment of childhood TB.

DIAGNOSTIC DIFFICULTIES IN CHILDHOOD TUBERCULOSIS

The diagnosis of TB in children is complicated due to (i) the absence of a practical gold standard diagnostic test (ii) TB can mimic many common childhood diseases, including pneumonia, generalized bacterial and viral infections, malnutrition, and HIV (iii) the inability of preadolescent patients to expectorate sputum (iv) the nonspecific clinical presentation (v) the lower bacillary load which is often smear negative (vi) confirmation by culture of *M. tuberculosis*, the gold standard of diagnosis in adult TB, rarely exceeds 30-40% sensitivity.[9,10]

ADVANCES IN SYMPTOM-BASED DIAGNOSIS

Symptoms of childhood TB are nonspecific and up to 50% of children may be asymptomatic in early stages of the disease. The use of well-defined symptoms with a persistent, nonremitting character considerably improves diagnostic accuracy. The presence of following 3 symptoms at presentation provide good diagnostic accuracy:
1. Persistent nonremitting cough of >2 weeks' duration,
2. Documented failure to thrive during the preceding 3 months and,
3. Fatigue.[11]

In those with an uncertain diagnosis at presentation, clinical follow-up provided additional assistance to differentiate active TB from other common diseases. However, symptom-based diagnosis performs poorly in children with HIV infection and should be used with caution in very young children, due to the rapidity with which disease progression may occur. The most common extra thoracic manifestation of TB in children is cervical lymphadenitis. A simple clinical algorithm, identifying those children with persistent neck masses >2 cm × 2 cm, not responding to a course of oral antibiotics and without a visible local cause, provided good diagnostic accuracy in a TB-endemic area.[12]

RADIOLOGICAL ADVANCES

Evidence of pulmonary TB in chest radiographs varies, but usually radiographs show enlargement of hilar, mediastinal, or subcarinal lymph nodes and lung parenchymal changes. The most common findings are segmental hyperinflation then atelectasis, alveolar consolidation, interstitial densities, pleural effusion, and rarely, a focal mass. Cavitations are rare in young children. High-resolution computed tomography is the most sensitive tool currently available to detect hilar adenopathy and/or early cavitation.[13]

ADVANCES IN IMMUNE-BASED DIAGNOSIS

Serological tests
Enzyme immune assays (EIA) in various formats such as microwell ELISA and immunochromatographic tests have made significant impact in the early and accurate diagnosis of several infectious diseases.[14]

Since first introduction of EIA in 1976 for the diagnosis of TB, several antigens are explored to develop an ideal EIA. First generation EIA tests were based on crude antigens, hence these tests exhibited low specificity. Later, an increased understanding of genomics and proteomics led to the discovery of new *M. tuberculosis* specific purified antigens having highly immunodominant epitopes. These antigens when used singly or in various combinations were reported to provide improved sensitivity and specificity. However on cross validation and field application these tests showed inconsistent results. Inaccurate results were attributed to physiological stage of TB infection, previous bacillus Calmette–Guérin (BCG) vaccination, TB endemicity in the region, exposure to other nontuberculous mycobacteria and varability in host genetics or ethnicity.[15]

Although, no country ever recommended their use, several serological tests for TB diagnosis are marketed and widely used in many parts of the world, especially in developing countries like India with weak regulatory systems.[16] Contradictory reports in support and against the use of these tests are being published by various authors. Of concern the commercial ELISA tests exhibited highly variable sensitivity (0-100%) and specificity (31-100%).[7] As a vast majority of studies conducted were either sponsored by industry, involved test manufacturers, or failed to provide information on industry sponsorship, it is likely that the results of these studies may be highly biased and falsified.[7] However, after the advisory of WHO, the Government of

India has banned these serological tests. Nevertheless, it remains to be seen if the ban will be successfully enforced.

IN VITRO INTERFERON-γ RELEASE ASSAYS

The Mantoux test (tuberculin skin test) is century old and is an inexpensive test for detecting the latent TB infection, however, a negative skin test in an active TB patient may also result from incorrect administration of the test or improper storage of the test reagents, thus compromising the sensitivity of the test. Mantoux test has lower specificity as the test cannot differentiate between infection with *M. tuberculosis*, prior vaccination with *Mycobacterium bovis* BCG or sensitization with environmental mycobacteria.

Highly sensitive and more specific tests for the diagnosis of latent tubercular bacterial infection (LTBI) have been developed recently as a result of advances in genomics and immunology. An alternative to the traditional Mantoux test recently emerged in the form of blood tests that measure interferon-γ (IFN-γ) released by sensitised T-cells after stimulation by *M. tuberculosis* antigens. These T cell assays use antigens that are encoded by the region of difference 1 (RD-1), a genomic region that is present in all *M. tuberculosis* and pathogenic *M. bovis* strains but is absent in all *M. bovis* BCG vaccine strains and most of the environmental mycobacteria of clinical relevance, therefore being more specific to *M. tuberculosis* than tuberculin (purified protein derivative). The two specific antigens used are early secreted protein-6 (ESAT-6) and culture filtrate protein-10 (CFP-10).[17]

Two commercial interferon-γ release assays (IGRAs), whole blood, ELISA-based QuantiFERON-TB Gold (Cellestis Ltd., Carnegie, Australia) and peripheral blood mononuclear cell and enzyme-linked immunospot (ELISPOT) technology-based T-SPOT.TB (Oxford Immunotec, Oxford, UK) tests were subsequently developed and approved by Food and Drug Administration for detecting latent infection. These commercial tests have undergone further improvement since their inception. The newer version of the QuantiFERON-TB Gold assay is called QuantiFERON-TB-Gold-In-Tube (QFT-GIT) (Cellestis Ltd., Carnegie, Australia) that uses ESAT-6 and CFP-10 and TB7.7 (corresponding to Rv2654) peptides as antigens. The newer version of T-SPOT.TB also uses peptides of ESAT-6 and CFP-10 instead of whole ESAT-6 and CFP-10 proteins as antigens (Oxford Immunotec, Oxford, UK).[18] The specificity for TB of QFT-IT was 100% and the specificity of T-SPOT was 98%, both of which were considerably higher than the specificity of TST. Both IGRAs showed high diagnostic value in bacteriologically confirmed childhood TB.[19] Among

Indian poulation both TB-gold QuantiFERON and TST were comparable even in malnourished children.[4] IFN-γ as biomarker in children and positive ELISPOT results predict subsequent development of active TB in recent TB contacts.[20]

ADVANCES IN BACTERIOLOGY-BASED AND MOLECULAR DIAGNOSIS

Although the bacteriological yield in children is said to be low, adolescent children frequently develop sputum smear-positive adult-type disease and sputum microscopy has definite diagnostic value in these older children. In addition, the bacteriological yield in children with TB depends on the specific intrathoracic manifestation of disease. A yield of 77% was reported in children with advanced disease, whereas the yield in those with uncomplicated hilar adenopathy was only 35%, using the MGIT system (Becton Dickinson, Maryland, USA). Automated liquid broth systems such as MGIT and BACTEC offer slightly superior sensitivity and reduced turn-around times compared with conventional Lowenstein–Jensen slants cultures.[21]

In addition to poor bacteriological yield, the collection of bacteriological specimens is often problematic. The collection of a single hypertonic-saline induced sputum specimen reportedly provides the same yield as three gastric aspirate specimens. Moreover sputum induction can be effectively performed and is well tolerated and safe even in infants and this induction is better than gastric lavage for the isolation of *M. tuberculosis* in both HIV-infected and uninfected infants and children.[22] Unfortunately, the safety and feasibility of this technique has not been studied outside the hospital setting. The string test is a novel approach that has recently been evaluated for its ability to retrieve *M. tuberculosis* from sputum smear-negative adults infected with HIV with TB symptoms.[23] The string test was superior in sensitivity compared induced sputum approach and is generally well tolerated by children and infants.[24]

Major limitations of traditional culture methods are slow turn-around times, suboptimal sensitivity, and the excessive cost of using automated liquid broth systems. Bacteriophage-based tests use bacteriophages to infect live *M. tuberculosis* and detect the presence of mycobacteria using either phage amplification or the detection of light. In general, phage assays have a turn-around time of 2-3 days, and require a laboratory infrastructure similar to that required for performing cultures. Phage amplification assays are available as commercial kits; the FASTPlaque-TB (Biotec Laboratories, Ipswich, Suffolk, UK) assay can be used directly on sputum specimens for diagnosis, and a variant, the FASTPlaque-TB Response

kit, is designed to detect rifampicin (RMP) resistance in sputum specimens. No information exists on its utility in the diagnosis of childhood TB. The FASTPlaque-TB Response assay detects RMP resistance, a reliable marker of MDR disease, with a fair degree of accuracy in adults, especially when used on culture isolates.[25] The microscopic observation drug susceptibility assay (MODS) is a novel assay that uses an inverted light microscope and Middlebrook 7H9 broth culture to rapidly detect mycobacterial growth. Early growth of *M. tuberculosis* is visualised as "strings and tangles" of bacterial cells in the broth medium, which may contain antimicrobial drugs for susceptibility testing. MODS also has a shorter time to culture positivity (average of 8 days) compared with Lowenstein–Jensen culture.[26] Although MODS is a promising and inexpensive tool, limited information exists on its utility in children.

Gas sensor array electronic nose (E-Nose)

The potential to detect different *Mycobacterium* species in the headspaces of cultures and sputum samples is another innovative approach that is currently in development. The array uses 14 sensors to profile a "smell" by assessing the change in each sensor's electrical properties when exposed to a specific odour mixture. In an initial study using sputum samples from patients with culture-confirmed TB and those without TB, the E-Nose correctly predicted 89% of culture-positive patients with a specificity of 91%.[27] Further applications of this test, including its potential value in the diagnosis of child TB, are needed.

Polymerase chain reaction

Diagnostic polymerase chain reaction (PCR) is a technique of *in vitro* DNA amplification that uses specific DNA sequences (oligonucleotides) as effective fishhooks for the DNA/cDNA of microorganisms. In theory, this technique can detect a single organism in a lot of specimens such as sputum, gastric aspirate, pleural fluid, cerebrospinal fluid, blood, and urine. Various PCR assays, most using the mycobacterial insertion element IS6110 as the DNA marker for *M. tuberculosis*-complex organisms, have a sensitivity and specificity greater than 90% for detecting pulmonary TB in adults.[28] PCR detection rates for culture-positive specimens were 100% for smear-positive samples and 76.7% for smear-negative samples. The specificity of PCR was 100% in control children. Compared with culture, PCR showed a sensitivity of 90.4%, a positive predictive value of 89%, a specificity of 94%, and a negative predictive value of 95%.[28] This is a rapid, sensitive, specific, and reasonable-cost method for the detection of *M. tuberculosis* in clinical samples. The PCR may be used to:

a. Diagnose TB in difficult samples with negative microscopic examination, negative culture, or with scarce sample;

b. Determine if the organisms in the sample are *M. tuberculosis* or atypical mycobacteria;

c. Identify the presence of genetic variations like a mutations or deletions known to be associated with resistance to some antimycobacterial agents.[29]

GENEXPERT MTB/RIF SYSTEM

It includes the development of integrated DNA extraction and amplification systems. This requires minimal manipulation of sample and operator training. It utilizes real-time PCR (RT-PCR) technology to both diagnose TB and detect RMP resistance. The test amplifies a region of the rpoB gene of *M. tuberculosis*. Mutations of this region give rise to 95% of RMP resistance. In addition, the rpoB core region is flanked by *M. tuberculosis*-specific DNA sequences. Thus, it is possible to test for *M. tuberculosis* and for RMP resistance simultaneously. The simplicity for the user makes this an assay that could feasibly be widely implemented outside centralized laboratories and potentially impacts on TB control.[30] Two Xpert tests doubled the case detection rate compared with smear microscopy (76% vs. 38%), identifying all smear-positive and 61% of smear-negative cases, the specificity was 98.8%. The sensitivies for smear-negative TB were 33.3% and 61.1% when testing one or two samples, respectively. The samplings were induced sputum and they detected three quarters of culture-confirmed TB with very high specificity; the yield of this method was twice that of smear microscopy. Thus suggesting the possibility of replacing the microscopy for this type of methodology which has greater sensitivity especially with a second sample.[31]

ADVANCES IN THE TREATMENT

Treatment of latent Mycobacterium tuberculosis infection

Tracing contacts of infectious pulmonary TB cases (sputum smear-positive) for exposure to tubercle bacilli leading to latent *M. tuberculosis* infection (LTBI) and treatment of latently-infected individuals at high risk of progressing from latent infection to active disease has proven extremely effective in the control of TB in the United States and other low TB-burden countries. The American Thoracic Society (ATS) and Centers for Disease Control and Prevention (CDC) issued guidelines in 2000 for the treatment of LTBI which were also endorsed by the Infectious Diseases Society of America and American Academy of Pediatrics.[32] An update to these guidelines was published in 2005 that also included recommendations for pediatric subjects.[33] The standard regimen for treatment of patients with LTBI in the United States and European countries is 9 months. The efficacy of INH treatment in preventing active TB exceeds 90% among persons who complete treatment.[34]

The ATS and CDC guidelines also included 4 months of RMP alone or 2 months of RMP and pyrazinamide (PZA) as acceptable alternatives for the treatment of LTBI]. The RMP alone is recommended for persons intolerant to isoniazid (INH), close contacts of TB cases in which the isolate of *M. tuberculosis* is resistant to INH or INH resistance is suspected due to the origin of foreign-born persons from countries where INH resistance rates are high]. The advantages with 4 month daily therapy with RMP include lower cost, higher adherence to treatment and fewer adverse reactions including hepatotoxicity.[32] However, treatment with RMP alone is not recommended for HIV-seropositive persons on concomitant antiretroviral therapy as this may lead to the development of acquired rifamycin resistance.[35] The longer half life of rifapentin (RPE), has allowed once weekly dosing of INH + RPE for the treatment of LTBI.[35] A large multi-center study is currently being conducted by the TB Trials Consortium of the CDC to determine the efficacy of once weekly dosing of INH + RPE in preventing active disease among high-risk individuals with LTBI.

Future prospects

Recently, llama (Lama glama) antibodies specific for the 16 kDa heat shock protein of *M. tuberculosis* has been developed[36] and a fast method to diagnose active pulmonary TB by infrared spectroscopy of serum blood sample seems promissory.[37] A method to accelerate *M. tuberculosis* growth in culture applying alternating magnetic fields frequencies of 8 Hz and amplitudes of 80 E for 4-5 days is developed, reducing 7-fold the time in getting results when compared with traditional culture.[38] With another relevant development, it might be possible to discriminate among lung diseases as cancer, asthma, and TB by gas sensor technology, which monitor exhaled volatile organic compounds directly from breath of patients, and this can be applied to diverse equipment similar to above-mentioned E-Nose.[39]

The newer drugs that are in different stages of development may offer better alternatives for the treatment of both, active TB disease as well as LTBI. The new generation fluoroquinolones such as moxifloxacin have excellent (bactericidal) activity against *M. tuberculosis* and may be more effective in the treatment of LTBI than older drugs of the same class.[40] In experimental models, the once weekly regimen of rifapentine + moxifloxacin for 3 months was as effective as daily therapy with INH for 9 months.[41] The PA-824, a nitroimidazo-oxazine, is another promising compound that is active against MDR-TB strains and is also active against nonreplicating persistent bacteria, making it an ideal drug candidate for the treatment of LTBI. The treatment regimen containing PA-824, moxifloxacin, and PZA was highly effective in murine model of TB.[42] A

diarylquinoline (R207910 also known as TMC207) has shown more potent early bactericidal activity than INH during early phase of infection and higher bactericidal activity late in infection than RMP alone and thus may provide another option for the treatment of LTBI.[43] Another promising drug is SQ109 (1,2-ethylenediamine) that is structurally related to ethambutol but is more potent.[44] It is expected that some of these new drugs will provide better options for the treatment of LTBI in the near future.

Another approach that is actively being pursued for controlling development of active disease in persons with LTBI is development of novel vaccines that may prevent TB disease reactivation by efficiently containing the pathogen in a latent state in infected individuals. More than 10 vaccine candidates have entered clinical trials in the past few years.[45] Two of these vaccine candidates are recombinant *M. bovis* BCG constructs designed to improve the antigenicity and/or immunogenicity of the current BCG vaccine.[46] Another seven subunit vaccines are being tested in clinical trials and are being used as booster vaccines designed to reorient the immune response after priming with recombinant BCG vaccines. Three of the subunit vaccines are incorporated in viral carriers while the other four subunit vaccines are being delivered through adjuvant formulations.[47,48]

CONCLUSION

Many promising advances have been made in the development of novel tools to diagnose TB in adults, but none of these tests are currently in position to replace microscopy or culture. Few of these novel approaches have been tested in children, the group in whom the diagnostic dilemma is most pronounced. IGRAs, PCR, E-Nose and FASTPlaque-TB offer improved sensitivity and specificity, which may assist TB eradication efforts in nonendemic countries and the diagnosis of *M. tuberculosis* infection in high-risk individuals. In the presence of necessary infrastructure the GeneXpert would be the best option considering no cross-reactions and higher sensitivity and specificity than culture methods. Nevertheless gold standard for childhood TB diagnosis is an unmet need. Improving the provision of preventive treatment to high-risk children with exposure and/or LTBI and anti-TB treatment to those with active disease will drastically reduce the severe TB-related morbidity and mortality in children in endemic areas. Current TB control efforts in endemic countries are mainly directed towards reduction of transmission by treating sputum smear-positive adults, whereas little emphasis is placed on reducing the vulnerability of communities. At present, the use of adequately validated symptom-based diagnostic approaches

and improved access to chest radiography and anti-TB treatment seem to offer the most immediate benefit to children in TB-endemic countries with limited resources.

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