RECENT ADVANCES

Recent advances in the diagnosis of childhood tuberculosis

Ben J Marais, Madhukar Pai

Arch Dis Child 2007;**92**:446–452. doi: 10.1136/adc.2006.104976

Children account for a major proportion of the global tuberculosis disease burden, especially in endemic areas. However, the accurate diagnosis of childhood tuberculosis remains a major challenge. This review provides an overview of the most important recent advances in the diagnosis of intrathoracic childhood tuberculosis: (1) symptom-based approaches, including symptom-based screening of exposed children and symptom-based diagnosis of active disease; (2) novel immune-based approaches, including T cell assays and novel antigen-based tests; and (3) bacteriological and molecular methods that are more rapid and/or less expensive than conventional culture techniques for tuberculosis diagnosis and/or drug-resistance testing. Recent advances have improved our ability to diagnose latent infection and active tuberculosis in children, but establishing a diagnosis of either latent infection or active disease in HIV-infected children remains a major challenge, particularly in high-burden settings. Although improved access to diagnosis and treatment is essential, ultimately the burden of childhood tuberculosis is determined by the level of epidemic control achieved in a particular community. Several recent initiatives, in particular the United Nations Millennium Developmental Goals, deal with the problem of poverty and disease in a holistic fashion, but global political commitment is required to support these key initiatives.

Children with tuberculosis usually have paucibacillary disease and contribute little to disease transmission within the community. Consequently the treatment of children with tuberculosis is often not considered a priority by tuberculosis control programmes. However, children carry a huge tuberculosis disease burden, particularly in endemic areas.[1 2] Accurate information on the global distribution of the childhood tuberculosis epidemic is scarce,[3] but of the estimated 8.3 million new cases of tuberculosis reported globally in 2000, 884 019 (11%) were children.[2] The extent of the childhood tuberculosis burden is well recognised, but not well quantified in many endemic areas.[4–6] A recent survey from Cape Town, South Africa, reported a calculated tuberculosis incidence of 407/100 000/year in children <13 years of age.[7]

In many countries, particularly in sub-Saharan Africa, the tuberculosis epidemic is fuelled by widespread immune compromise resulting from the HIV epidemic.[8] Although HIV-infected children are highly susceptible to develop active tuberculosis following *Mycobacterium tuberculosis* infection, most of the children diagnosed with active tuberculosis are not infected with HIV, even in countries where tuberculosis/HIV coinfection dominates in adults.[9 10] Another common misconception is that children develop mild forms of tuberculosis and that severe disease manifestations are the exception. It has been reported that tuberculosis accounts for 15% of all paediatric deaths in some Indian hospitals,[11] and a survey from Malawi reported a mortality of 17% in children diagnosed with tuberculosis.[12] The fact that tuberculosis rivals acute pneumonia as a major cause of death from respiratory disease in children from tuberculosis-endemic areas, irrespective of the child's HIV status, was conclusively shown in an autopsy study from Zambia.[9]

A recent community-based survey recorded the complete spectrum of tuberculosis disease manifestations in children, without the selection bias imposed by hospital-based recruitment, and confirmed that severe forms of childhood tuberculosis occur often in tuberculosis-endemic areas.[13] Only 48% of patients presented with uncomplicated hilar adenopathy, often regarded as the classical presentation of childhood tuberculosis, and the bulk of the disease burden (52.6%) was carried by children <3 years of age. However, despite the low cost and proved efficacy of standard treatment against tuberculosis, the access of children to treatment against tuberculosis remains poor in many endemic countries. This is demonstrated by the fact that the global drug facility (GDF), which provided quality assured adult anti-tuberculosis drugs to most resource-limited countries for many years, only made child-friendly drug formulations available from 2007.

THE DIAGNOSTIC CHALLENGE

The diagnosis of childhood tuberculosis is complicated by the absence of a practical gold standard.[14 15] Sputum microscopy, often the only diagnostic test available in endemic areas, is positive in <10–15% of children with probable tuberculosis,[16 17] and culture yields are also low (<30–40%).[16 17] For this reason, alternative strategies were developed to diagnose tuberculosis in children with sputum smear-negative disease; in non-endemic areas, the triad of (1) known contact with an adult index case, (2) a positive tuberculin skin test (TST) as evidence of latent tuberculosis infection (LTBI), and (3) suggestive signs on chest

Abbreviations: CXR, chest *x* ray; ELISPOT, enzyme-linked immunospot; FDA, Food and Drug Administration; LTBI, latent tuberculosis infection; MODS, microscopic observation drug susceptibility assay; NTM, non-tuberculous mycobacteria; TST, positive tuberculin skin test; WHO, World Health Organization

See end of article for authors' affiliations

Correspondence to: Dr B J Marais, Ukwanda Centre for Rural Health and the Department of Paediatrics and Child Health, Faculty of Health Sciences, Stellenbosch University, PO Box 19063, Tygerberg, Cape Town 7505, South Africa; bjmarais@sun.ac.za

Accepted 23 October 2006

x ray (CXR) is used in clinical practice.[18] This approach has also been recommended by the International Standards for Tuberculosis Care,[19] but the accuracy of the triad is greatly reduced in endemic areas, where most of the population acquire *M tuberculosis* infection during childhood and where transmission is not restricted to the household,[20 21] limiting the diagnostic contribution of both documented household exposure and a positive TST. Consequently, in endemic settings, the diagnosis of childhood tuberculosis depends mainly on clinical features and the subjective interpretation of the CXR.[22 23]

World Health Organization (WHO) guidelines categorise children as suspect, probable and confirmed cases of tuberculosis,[24] based mainly on documented exposure and/or infection, poorly defined symptoms and CXR interpretation. The application of these guidelines in tuberculosis endemic countries is additionally limited by the poor specificity of symptoms that are not well defined, the fact that chest radiography is mostly unavailable and the subjectivity of CXR interpretation. Hilar adenopathy is often regarded as the hallmark of primary tuberculosis,[25] but the natural history of disease shows that asymptomatic hilar adenopathy is a transient phenomenon in the majority (50–60%) of children following recent primary infection. Therefore, in the absence of suspicious symptoms, hilar adenopathy is more indicative of recent primary infection than active disease.[26–28] Despite these reservations, chest radiography remains the most widely used diagnostic test in clinical practice,[23 29] providing an accurate diagnosis in most of the children when interpreted by an experienced clinician. High-resolution computed tomography is the most sensitive tool currently available to detect hilar adenopathy and/or early cavitation.[28] Although it may be a useful test to consider in rare problem cases (if available), the natural history of disease shows why caution is particularly required when interpreting the relevance of these findings.

The natural history of disease also shows the importance of risk stratification. In an immune-competent child, age is the most important variable that determines the risk of developing disease following *M tuberculosis* infection.[26] Young children (<3 years of age) are at high risk,[27] but immuno compromised children remain at high risk irrespective of their age.[26 30] Owing to the frequency and rapidity with which disease progression occurs in high-risk children, an important diagnostic challenge in this group is to find a sensitive marker of infection, which will identify those who will benefit from preventive chemotherapy.

Table 1 provides a historical overview of the advances made in the diagnosis of tuberculosis since the discovery of *M tuberculosis* by Robert Koch in 1888. Table 2 summarises the problems and benefits experienced with traditional diagnostic modalities. Table 3 summarises recent advances, their potential application and their perceived problems and/or benefits.

ADVANCES IN SYMPTOM-BASED DIAGNOSIS

Screening for disease

Previous WHO guidelines regarded the TST and CXR as prerequisite tests for adequate screening of household contacts.[31] However, this limits access to preventive treatment in resource-limited settings where these tests are rarely available and where children are often exposed to tuberculosis at a young and vulnerable age. A study that compared the value of symptom-based screening with TST and CXR-based screening in child contacts[32] suggested that simple symptom-based screening may have considerable value in resource-limited settings. A report from Peru also showed that symptomatic household contacts are those at risk for active tuberculosis.[33] Symptom-based screening should drastically reduce the number of children who require further investigation, thereby facilitating the delivery of preventive chemotherapy to asymptomatic high-risk contacts, particularly in resource-limited settings. This recommendation has been included in the most recent WHO guidelines for National Tuberculosis Programmes on the management of tuberculosis in children.[34]

Diagnosing disease

Owing to the diagnostic limitations discussed, a variety of clinical scoring systems have been developed to diagnose active tuberculosis in children, but these scoring systems lack adequate validation.[35] Accurate symptom definition is essential to differentiate tuberculosis from other common conditions, as poorly defined symptoms (such as a cough of >3 weeks' duration) have little discriminatory power.[36] However, the use of well-defined symptoms with a persistent, non-remitting character considerably improves diagnostic accuracy.[37]

In a recent prospective, community-based study,[38] the presence of 3 symptoms at presentation ((1) persistent non-remitting cough of >2 weeks' duration, (2) documented failure to thrive during the preceding 3 months and (3) fatigue) provided good diagnostic accuracy (sensitivity 82.3%, specificity 90.2%, positive predictive value 82.3%) in immune-competent children ⩾3 years of age. In those with an uncertain diagnosis at presentation, clinical follow-up provided additional assistance to differentiate active tuberculosis from other common diseases. Previous reports from endemic areas also indicate that children usually present with symptoms indicative of active tuberculosis.[39] 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 extrathoracic manifestation of tuberculosis in children is cervical lymphadenitis. A simple clinical algorithm, identifying those children with persistent neck masses >2×2 cm, not responding to a course of oral antibiotics and without a visible local cause, provided good diagnostic accuracy in a tuberculosis-endemic area.[40] In addition, fine needle aspiration, using a small 23 G needle, proved to be a robust and simple technique providing excellent bacteriological yields. Fine needle aspiration remains under-used and may offer particular diagnostic value in settings where non-tuberculous causes of cervical adenopathy (neck masses) are more common.[40–43]

ADVANCES IN IMMUNE-BASED DIAGNOSIS

Serological tests

The development of antibody tests have been attempted for many years and their performance has been extensively reviewed,[44–46] but despite their long history, no assay is currently accurate enough to replace microscopy and culture. A major challenge with immunological diagnosis is the wide clinical spectrum of tuberculosis, ranging from LTBI to various manifestations of active disease.[44–46] In addition, the performance of antibody-based tests may be influenced by BCG vaccination, exposure to non-tuberculous mycobacteria (NTM) and HIV co-infection or other causes of immune compromise; all of which are particularly prevalent in tuberculosis-endemic areas.

Owing to these limitations, current efforts focus mainly on antigen detection. Antigen-capture ELISA assays for the detection of lipoarabinomannan in sputum and urine samples have shown promise in early trials,[47] but further work is necessary to determine their utility in clinical practice. Another innovative antigen-based method uses transdermal application of the MPB64 antigen. In early studies, the MPB64 skin patch test distinguished active tuberculosis from LTBI with 88–98% sensitivity and 100% specificity,[48 49] although the exact biological mechanism remains unclear. This test is currently being

Table 1 Timeline of the advances made in the diagnosis of tuberculosis

1880–1900	Robert Koch discovers that TB is caused by *M tuberculosis* Sputum smear microscopy using Ziehl–Neelsen staining *M tuberculosis* cultured on solid media (Lowenstein–Jensen slants) Tuberculin (purified protein derivative) isolated
1900–20	Rontgen discovers *x* rays: 1899 Tuberculin skin test developed: first used to diagnose *M bovis* in cows
1920–40	Use of attenuated *M bovis* BCG as TB vaccine: first given to a human (per os) in 1921 Sputum concentration using chemical flocculation Flourescent staining using auramine Chest radiography widely available after World War 1 Accurate diagnosis possible, but no treatment available Children kept in child sanatoria for prolonged periods of time
1940–60	Use of first drugs against tuberculosis Sanatoria closed down after institution of home-based care
1960–80	Effective treatment regimens developed Poorly validated symptom-based approaches used to diagnose childhood tuberculosis in resource limited settings
1980–90	HIV infection and disease first diagnosed Radiometric diagnosis of mycobacteria using liquid–broth media (BACTEC) PCR-based tests developed
1990–95	PCR-based tests applied to TB diagnosis TB declared a global health emergency by the WHO
1995–2000	Non-radiometric automated systems using liquid–broth media (MGIT) Development of novel T cell assays, measuring interferon-γ release stimulation with *M tuberculosis* specific antigens
2000–2005	Commercial T -cell assays available (T-SPOT.TB and QuantiFERON-TB Gold) Colorimetric culture system (TK medium) Bacteriophage-based tests (FAST plaque) Microscopic observation drug susceptibility assay (MODS) PCR-based drug resistance testing Revised symptom-based approaches Antigen-based tests and electronic ''nose'' in development

TB, tuberculosis; *M bovis*, *Mycobacterium bovis*; *M tuberculosis*, *Mycobacterium tuberculosis*; PCR, polymerase chain reaction; WHO, world health organisation.

developed commercially (Sequella, Rockville, Maryland, USA). Its ability to detect active tuberculosis in children has not been evaluated.

T cell assays

An alternative to the traditional TST recently emerged in the form of blood tests that measure interferon-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 segment of the *M tuberculosis* genome that is not shared with any of the BCG vaccine strains and most species of NTM, therefore being more specific to *M tuberculosis* than tuberculin (PPD). The two specific antigens used are early secreted protein 6 (ESAT-6) and culture filtrate protein 10 (CFP-10). Two assays are currently available as commercial kits: the T-SPOT.TB test (Oxford Immunotec, Oxford, UK), and the QuantiFERON-TB Gold (QFT-G; Cellestis, Carnegie, Australia) assay. The QFT-G assay is approved by the US Food and Drug Administration (FDA). The QFT-G In Tube, a simplified version of the QFT-G, which also includes a third antigen TB7.7 (Rv2654), has shown variable results in adult field studies.[50–52] It is currently not FDA approved. The T-SPOT.TB is licensed for use in Europe, Canada and other countries, but is still awaiting FDA approval. The use of T cell assays in children is currently limited by high cost, the relatively large volume of blood (4–5 ml) required, the non-availability of adequate laboratory infrastructure to perform enzyme-linked immunospot (ELISPOT) assays in particular, and the absence of conclusive evidence on which to base formal recommendations.

Previous reviews of these novel assays[53–57] showed higher specificity compared with the TST, and better correlation with *M tuberculosis* exposure gradients in low-incidence settings, suggesting improved sensitivity in detecting LTBI. However, given the lack of a gold standard test to detect LTBI, the sensitivity and specificity cannot be calculated with certainty. Recently, the US Centers for Disease Control and Prevention recommended that the QFT-G assay could be used in place of the TST for all indications, including screening of children.[58] However, there are limited data on the performance of the QFT-G assay in children. A recent study from Italy raised concerns about the diagnostic accuracy of the QFT-G assay in young children, as indeterminate results were recorded in 32% of children <5 years of age.[59] This observation was substantiated by an Australian study reporting that 17% of the QFT-G assays yielded indeterminate results in children, and the concordance between QFT-G and the TST was poor ($\kappa = 0.3$).[60] By contrast, a study among hospitalised children in rural India showed strong agreement ($\kappa = 0.73$) between the TST and QFT-G In Tube, but data were inadequate to estimate sensitivity among confirmed tuberculosis cases.[61]

Evidence for the ELISPOT assay indicate that it is a more sensitive marker of *M tuberculosis* infection than the TST, as reflected by better correlation with the degree of exposure following a school tuberculosis outbreak in the UK.[62] Improved sensitivity was also shown in HIV-infected children diagnosed

Table 2 Summary of traditional diagnostic approaches, their application and problems and/or benefits experienced

Diagnostic approach	Application	Problems/benefits	Validation
Tuberculosis culture using solid or liquid–broth media	Bacteriological confirmation of active tuberculosis	Slow turn-around time, too expensive for most poor countries Poor sensitivity in children	Accepted gold standard
Chest radiography	Diagnosis of probable active tuberculosis	Rarely available in endemic areas with limited resources Accurate disease classification essential Isolated hilar adenopthy may indicate recent primary infection and not disease	Marked inter and intra observer variability Reliable in expert hands and in presence of suspicious symptoms
Symptom-based approaches	Diagnosis of probable active tuberculosis	Poor defined symptoms are non-specific and have poor discriminatory power	Not well validated
Tuberculin skin test	Diagnosis of *M tuberculosis* infection	Rarely available in endemic areas with limited resources Does not differentiate latent tuberculosis infection (LTBI) from active disease Not specific for *M tuberculosis* infection Not sensitive in immunocompromised children Simple to use and less expensive than blood-based LTBI tests	Various cut-offs advised in different settings

Table 3 Summary of recent diagnostic advances, their potential application and perceived problems and/or benefits

Diagnostic approach	Application	Problems/Benefits	Validation
Symptom-based			
Symptom-based screening	Screening child contacts of adult tuberculosis cases	Simple, limited resources required Should improve access to preventive chemotherapy for asymptomatic high-risk contacts The use of well-defined symptoms and clinical follow-up provides reasonable diagnostic accuracy	Not well validated
Refined symptom-based diagnosis	Diagnosis of probable active tuberculosis	Simple, limited resources required Should improve access to chemotherapy in resource-limited settings Poor performance in HIV-infected children	Additional validation required
Immune-based			
Antibody-based assays	Diagnosis of probable active tuberculosis	Simple, point of care testing Variable accuracy and difficulty in distinguishing LTBI from active tuberculosis	Additional validation required
Antigen-based assays			
LAM detection assay	Diagnosis of probable active tuberculosis	Simple, point of care testing Limited clinical data on accuracy	Not well validated
MPB64 skin patch test	Diagnosis of probable active tuberculosis	Simple and easy to use Limited clinical data on accuracy, but initial data suggests it distinguishes LTBI from active tuberculosis	Not well validated
T cell assays	Diagnosis of LTBI; potentially a ''rule-out'' test for active disease	Limited data in children Inability to differentiate LTBI from active tuberculosis Large blood volumes required Very expensive Particular relevance in high-risk children, where LTBI treatment is warranted	Not well validated in children
Pathogen-based			
Colorimetric culture systems (eg, TK-Medium)	Bacteriological confirmation of active tuberculosis	Simple and feasible, limited resources required Potential for contamination in field conditions	Not well validated in children
Phage-based tests (eg, FASTPlaque-tuberculosis)	Diagnosis of active tuberculosis, and detection of rifampin resistance	Requires laboratory infrastructure Performs relatively poorly when used on clinical specimens	Not well validated in children
Microscopic observation drug susceptibility assay	Diagnosis of active tuberculosis, and detection of drug resistance	Simple and feasible, limited resources required	Not well validated in children
Electronic-nose technology	Diagnosis of active tuberculosis	Still in development	Never tested in children
PCR-based tests	Diagnosis of probable active tuberculosis, and detection of rifampin resistance	Rarely available in endemic areas Sensitivity tends to be poor in paucibacillary tuberculosis Specificity a concern in endemic areas, where LTBI is common Requires adequate quality control systems	Extensively evaluated, but evidence not in favour of widespread use

LAM, lipoarabinomannan; LTBI, latent tuberculosis infection; TST, tuberculin skin test.

with tuberculosis and in those with malnutrition, although the improved performance in malnourished children was not substantiated after correction for the HIV status of the child.[63] Despite its superior sensitivity, a study from South Africa reported that ELISPOT responses to ESAT-6 and CFP-10 were detectable in only two thirds of children with a clinical diagnosis of tuberculosis and in 83% with culture-confirmed disease.[64] In a recent household contact study conducted on Gambian children, the ELISPOT assay was slightly less sensitive than the TST in detecting *M tuberculosis* infection and neither test was confounded by prior BCG vaccination.[65] In a head-to-head comparison between the QuantiFERON-TB Gold and T-SPOT.TB assays, T-SPOT.TB gave considerably less indeterminate results, particularly in high-risk groups such as immuno compromised adults and young children.[59] Although the agreement between the various T cell assays and TST results are fairly high, the interpretation of discordant results remain problematic.

Overall, it appears that T cell assays are good at detecting *M tuberculosis* infection, but in the absence of symptoms or radiological signs, novel T cell assays, like the TST, fail to make the crucial distinction between LTBI and active disease. The main application of these assays in non-endemic areas, where disease elimination is a realistic target, would be the screening of groups with known or expected tuberculosis exposure to identify and treat everyone with LTBI. In tuberculosis-endemic areas where transmission is poorly controlled, tuberculosis exposure and infection are extremely common and the benefit of preventive treatment is reduced by the high likelihood of reinfection. However, the provision of preventive treatment remains a high priority in individuals who are at high risk of progressing to active disease after infection, such as young and/or immuno compromised children. In tuberculosis-endemic areas, the superior ability of the ELISPOT assay to detect *M tuberculosis* infection in high risk groups,[57 66] particularly in children infected with HIV, in whom the TST performs poorly,[67] seems to offer particular value. In this group, where the diagnostic dilemma is most pronounced, a sensitive test for *M tuberculosis* infection may also provide supportive evidence to establish or refute a diagnosis of active tuberculosis. Further research is needed, particularly in children from tuberculosis endemic areas, before formal recommendations on the use of T cell assays in these areas can be made.[68 69]

ADVANCES IN BACTERIOLOGY-BASED AND MOLECULAR DIAGNOSIS

Traditional methods

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.[70] In addition, the bacteriological yield in children with tuberculosis depends on

the specific intrathoracic manifestation of disease.[71] 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 (Beckton 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.[72] However, their high cost and the laboratory infrastructure required remains a major limitation.

In addition to poor bacteriological yield, the collection of bacteriological specimens is often problematic. Two to three fasting gastric aspirates collected on consecutive days and usually requiring hospital admission are routinely advised in children who cannot cough up sputum. The collection of a single hypertonic-saline induced sputum specimen reportedly provides the same yield as three gastric aspirate specimens.[17] Unfortunately, the safety and feasibility of this technique has not been studied outside the hospital setting. In addition, induced coughing may pose a transmission risk to healthcare workers, and also to other children, if the procedure is not performed in a separate, well-ventilated room and/or equipment is not adequately sterilised. 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 tuberculosis symptoms.[73] The string test showed superior sensitivity compared with induced sputum in this study population. In a more recent study, it was shown that the procedure is generally well tolerated by children, even in those as young as 4 years of age.[74]

Novel culture systems and detection methods

Major limitations of traditional culture methods are slow turn-around times, suboptimal sensitivity, and the excessive cost of using automated liquid broth systems. TK Medium (Salubris, Cambridge, Massachusetts, USA) is a novel colorimetric system that indicates growth of mycobacteria and allows for early positive identification, before visible bacterial colonies appear.[75] TK Medium also allows susceptibility testing for drug resistance, and can allow for differentiation between *M tuberculosis* and NTM. Although TK Medium promises to be a practical, low-cost, simple test, published evidence is limited and the test is currently not FDA-approved.[45] No data exist on its value in the diagnosis of childhood tuberculosis.

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.[76 77] 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 resistance in sputum specimens. The FASTPlaque-TB kits are currently not FDA approved, but are CE marked for use in Europe. No information exists on its utility in the diagnosis of childhood tuberculosis. The FASTPlaque-TB Response assay detects rifampicin resistance, a reliable marker of multi-drug resistant disease, with a fair degree of accuracy in adults, especially when used on culture isolates.[77]

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. In a recent study from Peru, MODS detected 94% of 1908 positive sputum cultures, whereas conventional LJ culture detected only 87%.[78] MODS also had a shorter time to culture positivity (average of 8 days) compared with Lowenstein–Jensen culture. Although MODS is a promising and inexpensive tool, limited information exists on its utility in children.

The potential of a gas sensor array electronic ''nose''; E-Nose 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 spiked sputa and sputum samples from patients with culture-confirmed tuberculosis and those without tuberculosis, after training of the neural network, the E-Nose correctly predicted 89% of culture-positive patients with a specificity of 91%.[79] Further applications of this test, including its potential value in the diagnosis of child tuberculosis, are under investigation.

POLYMERASE CHAIN REACTION BASED TESTS

Polymerase chain reaction (PCR)-based tests amplify nucleic acid regions that are specific to *M tuberculosis* complex. Available literature on PCR-based tests has been extensively reviewed,[80–83] showing highly variable results and limited utility in children.[84–86] Several recent meta-analyses[80 82 83 87] have shown that sensitivity estimates are low in paucibacillary forms of tuberculosis (extra-pulmonary tuberculosis and smear-negative pulmonary disease), which represents the vast majority of childhood tuberculosis cases. A negative test, therefore, does not rule out the diagnosis of tuberculosis. The same limitations apply to the use of PCR-based tests on cerebrospinal fluid samples for the diagnosis of tuberculosis meningitis.[83] Reduced specificity and the inability to differentiate clinically relevant disease is another concern, particularly in endemic areas where latent infection with *M tuberculosis* is common.

Overall, PCR-based tests have not lived up to their early promise, but efforts are under way to simplify testing protocols and increase their accuracy. However, PCR-based tests have definite value in routine species identification (confirming the presence of *M tuberculosis* complex), molecular epidemiology and the rapid detection of mutations associated with drug-resistance. With increased awareness of the emergent drug-resistant tuberculosis epidemic, the use of PCR to rapidly detect drug-resistant specimens may offer the most relevant application to date.

CONCLUSION

Many promising advances have been made in the development of novel tools to diagnose tuberculosis in adults,[45 52 88] 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. At present, the use of adequately validated symptom-based diagnostic approaches and improved access to chest radiography and anti-tuberculosis treatment seem to offer the most immediate benefit to children in tuberculosis-endemic countries with limited resources.[89] New T cell assays offer improved sensitivity and specificity, which may assist tuberculosis eradication efforts in non-endemic countries and the diagnosis of *M tuberculosis* infection in high-risk individuals.

Improving the provision of preventive treatment to high-risk children with exposure and/or LTBI and anti-tuberculosis treatment to those with active disease will drastically reduce the severe tuberculosis-related morbidity and mortality in children in endemic areas.[89] Ultimately, however, the burden of childhood tuberculosis reflects the level of epidemic control achieved within a particular community.[89] Current tuberculosis 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. There is a substantial body of evidence to suggest that community vulnerability, and community exposure in general, are strongly associated with poverty.[90] Breaking the cycle of poverty and achieving sustainable social upliftment seems to be essential elements in the struggle to contain the tuberculosis epidemic.[90] Several recent initiatives deal with these important issues, including the UN Millennium Developmental Goals,[91] the Global Plan to Stop TB 2006–15,[92] the International Standards for Tuberculosis Care,[19] and the new Stop TB strategy.[93] However, global political commitment is required to support these key initiatives.

Authors' affiliations

Ben J Marais, Ukwanda Centre for Rural Health and the Department of paediatrics and Child Health, Desmond Tutu TB Centre, Faculty of Health Sciences, Stellenbosch University, Cape Town, South Africa
Madhukar Pai, Department of Epidemiology & Biostatistics, McGill University, Montreal, Quebec, Canada

Competing interests: None.

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