Diagnosis of pulmonary tuberculosis in children – what's new?

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The global burden of paediatric tuberculosis (TB) has been underappreciated. Control programmes, focused on adult infectious cases, have largely based case detection and reporting on sputum smear results.[1] However, evidence suggests that in developing countries, where most disease occurs, childhood TB constitutes a large proportion of the TB caseload, contributing approximately 15 - 20% of all cases. The burden in children and impact on child health has been under-recognised, partly because of difficulties in confirming the diagnosis. Diagnostic confirmation may be difficult because of many factors including nonspecific clinical signs, coexisting malnutrition, variable interpretation of chest radiographs, paucibacillary disease, difficulty in obtaining specimens for culture and relatively low rates of bacteriological confirmation. As a result diagnosis in children has relied mainly on clinical case definitions, tuberculin skin testing and chest radiography.[2] Diagnostic uncertainty has been compounded by the HIV epidemic in which chronic lung disease, anergy, coexisiting malnutrition and nonspecific clinical and radiological signs make definitive diagnosis even more challenging.

The consequences of undiagnosed or untreated paediatric TB are especially serious as children are more likely to develop miliary or severe disease. Furthermore cases of childhood TB frequently reflect an undiagnosed adult infectious source case; therefore the occurrence of TB in children frequently indicates failure of a TB control programme. Moreover, definitive diagnosis and microbiological confirmation have become increasingly important in the era of multidrug-resistant TB (MDR) and extremely extensively drug-resistant TB (XDR).

This review considers currently available and new diagnostic methods for pulmonary TB (PTB). Current methods have relied predominantly on clinical case definitions, tuberculin skin testing and chest radiography. Newer methods include improved specimens and microbiological methods, immune testing, especially gamma-interferon assays, phage-based tests, polymerase chain reaction and antigen detection.

Clinical diagnosis

Clinical symptoms and signs in children may be nonspecific for TB. Although a number of scoring systems for children have

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been developed, these lack diagnostic accuracy particularly for young, malnourished or HIV-infected children.[3] Scoring systems usually use a combination of clinical features (chronic cough, weight loss or failure to thrive, history of a close contact) in combination with tuberculin skin test results and/or chest radiology findings. A review of existing scoring systems found that 5 of 17 had been adapted for use in HIV-infected children, while only 1 had been specifically developed for use in such children.[3] Defining symptoms more accurately may be useful to increase the reliability of diagnosis, as addressed in the accompanying article in this supplement.[4]

Tuberculin skin testing

Tuberculin skin testing using purified protein derivative (PPD) can be useful for confirming TB infection. However, this test is technique dependent, requires standardised application and interpretation, and a positive result depends on an adequate immune response.[5] The commonest causes of false-negative results in South African children include severe malnutrition, severe TB disease and HIV infection (Table I).[5] Conversely, false-positive results can occur as a result of non-tuberculous mycobacteria, BCG or improper application or interpretation (Table I).

Radiological diagnosis

Chest X-ray changes are frequently nonspecific for TB. However, certain patterns such as miliary disease, hilar adenopathy with airway compression and cavitary disease are associated with TB pulmonary disease. An atlas of diagnostic radiology for childhood TB is freely available through the website of the International Union Against Tuberculosis and Lung Disease (IUATLD).[6] A further difficulty is the potential for wide intra- and inter-observer variation in interpretation of chest X-ray findings, especially the presence of enlarged lymph nodes.[7]

Computed tomography (CT) of the chest is more reliable for detecting adenopathy and pulmonary disease but has much higher radiation exposure, is more expensive and requires specialised expertise.

Microbiological diagnosis

Microbiological confirmation of childhood TB, unlike in adults, has not been routinely attempted particularly in primary care. This is partly due to the paucibacillary nature of childhood TB, with most children being smear negative. Microbiological confirmation currently depends on obtaining an adequate sample for acid fast staining, culture and sensitivity.

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Table I. Causes of false-positive or false-negative tuberculin skin tests (TSTs) in children[5]

False-negative TST	False-positive TST
Improper placing/interpretation	Improper interpretation
HIV infection	BCG vaccination
Malnutrition or low-protein states	Non-tuberculous mycobacteria
Severe TB	
Improper storage of tuberculin	
Viral infections	
Bacterial infections	
Live viral vaccines (within 6 weeks)	
Immunodeficiencies (other than HIV)	
Neonates	

Samples

A number of specimens have been used for confirmation of PTB including gastric aspirates (GL), induced sputum (IS), bronchoalveolar lavage (BAL) or ear swabs (in the presence of a chronically discharging ear). The yield from GL has been consistently reported to be higher than that from BAL. In contrast, IS is more effective than GL, even in infants.[8] In a study of young children (median age 13 months) with suspected PTB, the yield from a single IS was equivalent to that from 3 GLs while more cases were identified with 3 sputa compared with 3 GLs.[9] In addition, almost 40% of children who were culture positive on sputum were also smear positive, enabling rapid diagnosis of PTB.[8] IS has obvious advantages over GL as the procedure is quick, does not require hospitalisation of the child, is relatively easy to perform, safe and effective; however precautions to prevent nosocomial transmission must be taken.[8] Wider use of IS as a diagnostic procedure in children is warranted, especially in the era of MDR and XDR TB when bacteriological confirmation is increasingly important.

Fine-needle aspiration of a superficial lymph node (in lymphadenitis), bone-marrow aspiration (in miliary disease) and tissue biopsy of lung (especially in chronic lung disease where other investigations have not yielded another organism or cause) may be useful for detecting *Mycobacterium tuberculosis*. In addition, the string test, in which gastric contents adherent to a gelatine capsule (which is swallowed, taped *in situ* and then removed after a few hours) are obtained, has been reported to be well tolerated in older children.

Culture methods

Lowenstein-Jensen medium has usually been used for culture of *M. tuberculosis*; results take approximately 6 weeks. More recently, liquid culture medium (MGIT), in which an indicator dye changes colour with growth, provided culture results more quickly, within 2 - 4 weeks. However, susceptibility testing required a further 3 weeks. New culture methods include the use of TK medium, which changes colour with growth, allowing for culture results within 2 weeks. This is a promising, inexpensive but understudied method.

Recently microscopic observation drug susceptibility (MODS) has been reported to be a sensitive and rapid culture method with results in 7 days.[10] MODS uses an inverted light microscope to detect mycobacterial growth in liquid medium. Specimens are inoculated onto a microtitre plate with or without antibiotics and examined daily for growth. A study of over 3 000 specimens comparing MODS, liquid medium and Lowenstein-Jensen found a similar culture positive rate (11%) for all.[10] However, MODS provided both culture and susceptibility results within 7 days, which was significantly quicker than the others (13 and 22 days for liquid medium and 26 and 68 days for Lowenstein-Jensen).[10] This technique is very promising but is labour intensive.

Interferon assays

These *in vitro* tests measure gamma-interferon production by T cells. The principle of these assays is that sensitised T cells produce interferon when they encounter mycobacterial antigens. Initial assays used purified protein derivative (PPD), which contains a mixture of antigens shared by many mycobacterial species. However, new assays use *M. tuberculosis*-specific antigens such as ESAT-6 and CFP-10. Two commercial assays are available and utilise ELISA or Elispot technology. Quantiferon-TB measures interferon production in whole blood; results are expressed as IFN pdxn (pg/ml). T SPOT-TB uses peripheral mononuclear cells to detect the number of T cells producing interferon; results are expressed as the number of spot-forming cells.

Generally interferon assays have been reported to have higher sensitivity and specificity compared with tuberculin skin testing.[10] However, there is relatively little information in young children and HIV-infected children. One study of children with suspected TB in rural KwaZulu-Natal reported that the sensitivity of the Elispot interferon assays (83%) was significantly higher than PPD testing (63%).[11] This occurred in the subgroup of young children under 3 years (85% v. 51%), HIV-infected children (73% v. 36%) and malnourished children (78% v. 44%).[11] Combining ELISPOT and PPD improved the diagnostic sensitivity to 91%, so additional accuracy was achieved by using both.

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Concerns about interferon assays include the inability to distinguish latent from active disease, the relative lack of studies in children, their applicability in high-incidence HIV and TB settings and discordance with PPD results. Use of assays is also limited by their cost and the need for laboratory infrastructure. Nevertheless, these assays are promising, more sensitive than tuberculin skin testing, and an important addition to improving diagnostic accuracy for *M. tuberculosis.*

Phage-based tests

These use bacteriophages to infect *M. tuberculosis* and can also detect resistance. They provide rapid results within 2 - 3 days but are less sensitive than culture.[12] Phage-based assays have not been validated in children and require laboratory expertise.

Polymerase chain reaction (PCR)

PCR for *M. tuberculosis* has produced variable and disappointing results to date. Studies report poor sensitivity in paucibacillary disease and in extrapulmonary TB but good specificity. In smear-negative disease sensitivity is around 50 - 60% but specificity approaches 99%.[13] In general PCR is less sensitive than culture and is limited by cost and need for laboratory expertise and infrastructure.

Antigen-based tests

These detect TB-specific antigens such as lipoarabinomannan (LAM). The LAM assay in urine or sputum using ELISA test has been reported to have a sensitivity of 80%.[14] However, there are limited data on their accuracy and they have not been well validated in children.

Antibody-based tests

The available serological assays are highly variable in their sensitivity and specificity. Serological assays are limited as antibody responses are variable, they do not distinguish latent from active disease and their reliability may be influenced by other factors like HIV, BCG and non-tuberculous mycobacteria (NTM).[15] In the future, a useful serological test will need to assay a number of antibodies, not a single antibody.

Conclusion

Diagnosis of TB in children remains challenging, especially in the era of HIV. Diagnosis cannot rely on a single factor, but on a constellation of findings and tests. Careful and optimal technique should be used for existing tests. Improved immune diagnostic tests and microbiological methods are valuable additions in diagnosis. Greater efforts at microbiological confirmation should to be made in children, including in primary care settings. Simpler, cost-effective, faster and more accurate diagnostic methods for TB are urgently needed for children in developing countries.

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