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New specimens and laboratory diagnostics for childhood pulmonary TB: progress and prospects

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EDUCATIONAL AIMS

- To describe current state-of-the-art for diagnosis of pulmonary tuberculosis in children
- To discuss the advantages and limitations of various specimen types for diagnosis of pulmonary tuberculosis in children
- To discuss the role and limitations of nucleic acid amplification assays in the diagnosis of pulmonary tuberculosis in children

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SUMMARY

Childhood pulmonary TB (PTB) is under diagnosed, in part due to difficulties in obtaining microbiological confirmation. However, given the poor specificity of clinical diagnosis, microbiological confirmation and drug susceptibility testing is important in guiding appropriate therapy especially in the context of drug resistant TB. Confirmation is often possible, even in infants and young children, if adequate specimens are collected. Culture yield varies with the severity of illness, specimen type and culture method. Induced sputum is recognised as a safe procedure with a high diagnostic yield. Advances include optimised protocols for smear microscopy and modified culture techniques, such as the Microscopic Observation Drug Susceptibility Assay. Detection of *Mycobacterium tuberculosis* nucleic acid in respiratory specimens has high specificity but relatively poor sensitivity, particularly for smear negative disease. The recent development of an integrated specimen processing and real-time PCR testing platform for *M. tuberculosis* and rifampicin resistance is an important advance that requires evaluation in childhood TB.

INTRODUCTION

Globally, under diagnosis of childhood pulmonary tuberculosis (PTB) remains an obstacle to effective management. TB control programmes still focus predominantly on the diagnosis and cure of cases of smear-positive adult TB, as these are the major drivers of TB transmission. Whilst adult TB cases are often easily recognizable, due to typical radiological features and a positive sputum smear, childhood TB is frequently more difficult to diagnose. The clinical and radiological features of childhood TB are often non-specific and subject to variable interpretation.[1] Structured diagnostic scoring systems based on clinical and radiological findings and tuberculin skin testing (TST) show high variability in case yield and very poor agreement.[2]

The diagnosis is even more problematic in HIV-infected children, since clinical and radiological features overlap with other infections and anergy to the TST is common.[3] Clinical scoring systems have not been adequately evaluated in HIV-infected children, but it is likely that their performance will be even poorer in this patient population.

Microbiologic confirmation of PTB is still rarely attempted in children, especially in primary care settings, in contrast to adults where this is the accepted standard of care. This is due to the incorrect perception that respiratory specimens are difficult or impossible to obtain in children, the lack of infrastructure or trained staff to obtain such specimens and the lack of policy regarding microbiologic confirmation in children. However, even when samples can be obtained, since disease is typically paucibacillary, the yield of direct acid-fast smear microscopy is very low[4] and prolonged mycobacterial culture is required. As a result,

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Table 1
Summary of new specimens and laboratory diagnostic tests for pulmonary TB in children

Diagnostic test	Description	Major findings
Specimen		
Induced sputum	Samples lower respiratory tract secretions; nebulisation with hypertonic saline followed by suction or expectoration	Obtainable in most children and safe 1 IS provides same yield as 3 GLs in hospitalised children Incremental yield with a 2nd IS specimen[4]
Nasopharyngeal aspirate	Suctioning of the nasopharynx to sample upper respiratory tract secretions; stimulation of cough reflex may include lower respiratory secretions	Relatively non-invasive procedure; variable performance, but some studies suggest similar yield to culture of IS[9]
Laboratory test		
Optimised smear microscopy	Chemical processing and centrifugation Fluorescent microscopy	Increases sensitivity by 18%[18] Increases sensitivity by 10%[19]
Automated liquid culture	Liquid culture medium with continuous monitoring for bacterial growth	Higher sensitivity than solid medium and shorter time to detection[22]
MODS	Inoculation into multiple wells of liquid culture with or without INH or rifampicin – growth determined by visualisation with inverted microscope	Simultaneous detection of drug resistance May be more rapid than automated liquid culture systems[24]
Nucleic acid amplification tests	Identification of gene sequences specific to *M. tuberculosis* and specific for drug resistance	High specificity of commercial assays[25] Lower sensitivity especially in smear negative samples
Urine LAM	Detection of *M. tuberculosis* glycolipid by ELISA	Low sensitivity, higher in disseminated disease in severely immunosuppressed adults[40] Not yet evaluated in children

microbiological confirmation may be delayed by weeks. This has important implications for a disease that may progress rapidly in young children, with associated morbidity and mortality. Extrapulmonary TB is common in young children and poses particular challenges for specimen collection and culture.

The major advantages of obtaining microbiological confirmation are the ability to make a definitive diagnosis and to perform drug susceptibility testing to exclude drug-resistant TB. In the era of increasing multidrug resistant (MDR) and extensively drug resistant (XDR) TB this information becomes critical in order to guide appropriate therapy.

However, mycobacterial culture is frequently negative in children with clinically diagnosed PTB, particularly amongst less ill patients in a primary care setting.[5] This may represent the poor specificity of clinical diagnosis or alternatively, the impaired sensitivity of culture for childhood TB. This presents a fundamental problem in assessing the performance of any novel diagnostic test or clinical algorithm as there is no reference standard which is both highly sensitive and specific to which results can be compared. A further implication is that clinicians are often wary to discontinue TB therapy when a negative culture result is obtained.

This review will address the progress made in recent years in obtaining laboratory confirmation of PTB in children. The diagnosis of latent TB infection and the use of serological tests and interferon-gamma release assays are considered elsewhere in this edition.

OBTAINING REPRESENTATIVE SPECIMENS FROM THE LOWER RESPIRATORY TRACT OF CHILDREN

Microbiological confirmation of TB in young children is not routinely attempted in many high burden settings due to the difficulty in obtaining samples and the poor performance of smear microscopy. However, if facilities for mycobacterial culture and drug susceptibility testing are available, such confirmation is invaluable.

Since young children are frequently unable to expectorate, additional procedures are often required to obtain samples from the lower respiratory tract. For many years the collection of three consecutive early morning gastric lavage (GL) or gastric aspirate (GA) samples has been the accepted method for attempting microbiological confirmation. However GL is unpleasant, relatively invasive, requires trained staff and hospitalization for an overnight fast, although it may be performed in an outpatient setting.[6] This procedure is not feasible in many high burden countries; moreover the yield for *M. tuberculosis* has been disappointingly low. More recently, a number of less invasive alternative methods have been proposed, including induced sputum (IS), nasopharyngeal aspiration (NPA) and the string test [Table 1].

Sputum induction

Sputum induction does not require overnight hospitalization and can be performed in an out-patient setting. The technique involves administration of an inhaled bronchodilator followed by nebulised hypertonic (3%-5%) saline and then nasopharyngeal aspiration or expectoration of mucus from the lower respiratory tract.[4] IS has been successfully used for diagnosis of childhood TB in several countries in the developing world (South Africa, Kenya, Uganda, Tanzania, India, Colombia). In an early study of 149 children (median age 9 months) hospitalised with acute pneumonia, samples were successfully obtained from 95% of enrolled patients, of whom 10% had a positive culture.[7] The yield from a single IS sample (10%) was greater than that of sequential GL samples (6%). These results were subsequently confirmed in a larger study of children with suspected PTB admitted to the same paediatric referral hospital (250 children, median age 13 months) which demonstrated a significantly higher cumulative yield for 3 IS samples (87%) compared with 3 GL samples (65%, $p = 0.018$).[4,8] The yield from one IS sample was equivalent to three GL samples.[4] This has shifted clinical practice to include induced sputum as a diagnostic procedure in young children and infants with suspected PTB.

The yield of mycobacterial culture is likely to vary with the patient population (primary care versus referral hospital) and severity of illness. Whilst positive cultures were obtained from 3 GL and 3 IS samples in 25% of children admitted to a paediatric referral hospital,[4] only 10% of children admitted with suspected TB or with a TB contact in a community-based study had positive cultures (from 2 GL and 2 IS samples).[5] In this community-based study, the yield of a single IS and GL sample were equivalent (38% and 42% of patients with a positive culture respectively), again highlighting that the severity of illness may impact on diagnostic