Abstract: Childhood tuberculosis accounts for a significant proportion of the global tuberculosis disease burden. However, tuberculosis in children is difficult to diagnose, because disease tends to be paucibacillary and sputum samples are often not easy to obtain. The diagnosis of tuberculosis in children is traditionally based on chest radiography, tuberculin skin testing, and mycobacterial staining/culture from appropriate samples. Newer diagnostic strategies have included improved bacteriologic and molecular methods, as well as new methods for sample collection from children. Recently, immune-based diagnostics, such as the interferon-gamma release assays, have been introduced for clinical use. These tests do not offer substantial improvements in sensitivity over tuberculin skin testing for the diagnosis of active disease but may be useful in excluding false-positive tuberculin skin tests. Further research is needed to develop better diagnostic tests for tuberculosis in children.

Key Words: tuberculosis, diagnosis, child

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The Diagnosis of Tuberculosis
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ESTABLISHED DIAGNOSTIC METHODS

Microscopy and Culture

Microscopic examination of respiratory samples for acid-fast bacilli using the Ziehl-Neelsen and fluochrome stains, such as the auramine and rhodamine, have been the standard and rapid diagnostic tools for tuberculosis (TB) diagnosis. Recent advances in light-emitting diode (LED) technology have widened the applicability of fluorescent microscopy. In adults and older children, sputum samples are often obtained with sensitivity from 34% to 80%. In younger children, who are unable to produce sputum, alternative methods of obtaining respiratory samples, such as gastric aspirates, are often used. However, microscopic yields may be <20% in children with probable TB. The detection rate on microscopy from other extrapulmonary samples, such as cerebrospinal fluid, are even lower because of the paucibacillary nature of disease at these sites.

Mycobacterial culture of respiratory samples has provided a more useful method of diagnosis in children with suspected pulmonary TB. Three consecutive daily morning gastric aspirates yield Mycobacterium tuberculosis in 30% to 50% of cases and may be as high as 70% in infants. Recently, sputum induction using nebulized hypertonic (3%-5%) saline has been used safely and effectively in young children. The culture yield from a single induced sputum sample has been shown to be equivalent to that of 3 cumulative gastric lavage samples. There are, however, some concerns regarding the risk of nosocomial transmission following sputum induction if adequate infection control procedures are not in place. Nasopharyngeal aspiration (NPA) has also been used to obtain respiratory samples, as the passage of a nasal cannula may elicit a cough reflex. The culture yield from NPA (19/64; 30%) was similar to that of gastric aspirates (24/64; 38%) among Peruvian children. However, subsequent studies have shown relatively poor yields from NPA samples compared with gastric aspirate. Since young children tend to swallow their sputum rather than expectorate it, mycobacterial culture of stool has been considered as an indirect way of analysis of respiratory secretions. However, studies in children have shown relatively poor recovery from stool, making this an insensitive method for mycobacterial culture. Furthermore, the major drawback of stool culture is the need for stringent decontamination procedures to prevent overgrowth of normal bowel flora, which may also kill or inhibit growth of mycobacteria further reducing the sensitivity.

Another novel method of sampling swallowed respiratory secretions is the string test. The string test was developed for the diagnosis of intestinal parasites such as giardiasis. This test involves swallowing a gelatin capsule containing a coiled nylon string, which unravels as the capsule descends into the stomach. After 4 hours, the string is withdrawn and cultured for mycobacteria. Although this test appears to have a better culture yield than sputum induction in adults with HIV infection (9% vs. 5%), it has not been studied in children other than a feasibility study where it appears to have been well tolerated. Furthermore, it may be of limited use in younger children who will be unable to swallow the capsule in the first place.
The culture yield from other body fluids or tissues from children with extrapulmonary TB is usually <50%. In children with palpable peripheral lymphadenopathy, fine needle aspiration and culture is a very useful adjunct to culture of respiratory specimens and may have a higher yield than such culture (sensitivity 60.8% vs. 39.2%, respectively). Recently, automated liquid culture systems with continuous monitoring for mycobacterial growth (such as BD BACTEC MGIT system or Biomerieux BacT/ALERT 3D) have been a significant advance over traditional solid culture (Lowenstein-Jensen media). In adult studies, these tests offer improved sensitivity (88% vs. 76%) and reduced detection time (13.2 vs. 25.8 days) compared with solid media. It is likely that these findings can be extrapolated to children with TB, although there is a paucity of pediatric data. Despite their higher cost and the laboratory infrastructure required, liquid culture has been recommended for all culture in resource-rich settings.

Newer culture-based methods, such as TK medium, use multiple dye indicators for the early detection of mycobacterial growth with the naked eye. The simple colorimetric system reduces turnaround times, but their accuracy and robustness in field conditions have not been reported. The Microscopic Observation Drug Susceptibility assay uses an inverted light microscope to rapidly detect mycobacterial growth in liquid growth media. It is an inexpensive method that has demonstrated excellent performance under field conditions (in both adults and children), being more sensitive than standard liquid broth or solid culture systems. The test is not widely available at present.

**Tuberculin Skin Test**

A positive tuberculin skin test (TST) reaction has been used as a hallmark of infection with *M. tuberculosis*, occurring within 3 to 6 weeks, but occasionally up to 3 months, and remaining positive lifelong, even after treatment.

The Mantoux test is the standard TST currently in use and involves the intradermal injection of 2 standardized tuberculin units of purified protein derivative solution. Subsequent induration, rather than erythema, is measured in millimeters after 48 to 72 hours. In some countries, such as the United Kingdom, with low TB incidence a TST is regarded as positive with induration of >5 mm in those without prior Bacille Calmette-Guérin (BCG) vaccination and >15 mm for those who have received BCG vaccination. The World Health Organization (WHO) guidelines differ slightly in that a positive TST is regarded as positive with induration >10 mm for those without prior BCG vaccination and >15 mm for those with BCG vaccination history. The US guidelines use a risk categorization based on epidemiologic and clinical factors: >5 mm (close contacts, TB disease, immunosuppression), >10 mm (increased risk of disseminated disease or increased exposure to TB disease), and >15 mm (children >4 years of age with no risk factors).

TST is prone to both false-negative and false-positive results. Up to 10% to 15% of otherwise immunocompetent children with culture-documented TB do not initially show TST reactivity. Host factors, such as young age, poor nutrition, immunosuppression, other viral infections (such as measles, varicella, and influenza), recent TB infection, and disseminated TB diseases, can further decrease TST reactivity. False-positive TST results may also occur following BCG vaccination and exposure to environmental nontuberculous mycobacteria. Skin reactivity can be boosted, probably through antigenic stimulation, by serial testing with TST in many children and adults who received BCG.

**Radiology**

Chest radiography is used widely for the detection of pulmonary TB, including detection of hilar lymphadenopathy, lung parenchymal changes, and miliary TB. Cavitary disease is uncommon in younger children but is often seen in adolescents, who may develop adult-type postprimary disease. Computed tomography imaging has been useful in demonstrating early pulmonary disease, such as cavitation, and intrathoracic hilar lymphadenopathy. Central nervous system disease, such as TB meningitis or tuberculoma, may also be identified on computed tomography imaging, where meningeal enhancement may be seen with contrast. Magnetic resonance imaging has been found to be useful for musculoskeletal TB, particularly involving bones and joints.

**ADVANCES IN DIAGNOSIS**

**Novel Culture Systems and Detection Methods**

Bacteriophage-based assays use bacteriophage viruses to infect and detect the presence of viable *M. tuberculosis* in clinical samples and culture isolates. Two main approaches have been developed: (1) to detect the presence of mycobacteria using either phage amplification and (2) to detect light produced by luciferase reporter phages after their infection of live *M. tuberculosis*. When the assays detect *M. tuberculosis* in drug-free samples, but fail to detect *M. tuberculosis* in drug-containing samples, the strains are classified as drug susceptible. In general, phage assays have a turn around time of 2 to 3 days and require a laboratory infrastructure similar to that required for standard cultures. There is currently only 1 commercially available kit, the FASTPlaque-TB (Biotec Laboratories, Ipswich, Suffolk, United Kingdom) assay, which can be used directly on sputum samples for diagnosis. A variant of this assay, the FASTPlaque-Response kit is designed to detect rifampicin resistance in sputum specimens, which has been used as a reliable marker for multidrug-resistant TB. However, no information exists on the utility of these tests in the diagnosis of childhood TB.

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 under 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 odor mixture. In a recent study using sputum samples from adult TB patients and non-TB patients, the E-nose had sensitivity of 68% and specificity of 69%. Further research is still required to improve sensitivity and specificity as well as its potential in the diagnosis of childhood TB.

**Molecular Diagnostics and Rapid Resistance Testing**

Nucleic acid amplification tests (NAATs) for the detection of mycobacterial DNA or RNA are increasingly being developed for clinical use. These tests are theoretically highly sensitive, able to detect very low copy numbers of nucleic acid, rapid, not requiring biosafety level 3 facilities and are relatively easy to automate. Commercial NAATs have been extensively evaluated in adults showing high specificity (85%–98%), high sensitivity for smear-positive TB (pooled estimate 96%) but poorer sensitivity for smear-negative TB (pooled estimate 66%). Sensitivity estimates are generally also lower in most paucibacillary forms of disease, including extrapulmonary, which represents most of childhood TB cases. Their performance in children has not been thoroughly evaluated; however, limited studies to date suggest that their performance in children is likely to be similar to that in smear-negative adults because of the paucibacillary nature of TB in children.

There have been several recent evaluations of NAATs performed on nonrespiratory samples to diagnosis respiratory disease. One study has reported the presence of small fragments of *M. tuberculosis* IS6110 DNA in urine (so-called transrenal DNA or tr-DNA) of 34 of 43 adults with TB but not in healthy controls. However, other studies have shown wide variations in performance (7%–100% sensitivity), and there are no data on...
the performance of these tests in children. A urinary test that could serve as a rapid and easy diagnostic test has advantages in the pediatric population.

NAATs have also been used for the rapid detection of rifampicin resistance directly from sputum samples. The Xpert MTB/RIF is a cartridge-based, automated diagnostic test that is rapid and simple to use and correctly identified 98% of bacteria that were resistant to rifampicin in a large study in adults. In December 2010, WHO endorsed this test for use in TB endemic countries and declared it a major milestone for global TB diagnosis.

As mentioned earlier in the text, young children swallow their sputum, and thus DNA of young children may be detected in stool. At present, there are limited data in children, although in several small studies, the sensitivity appears low (<40%) compared with adults (sensitivity 86%). Real-time polymerase chain reaction has increasingly become available for clinical use with the advantage of lower cross-contamination and as well as the ability to identify rifampicin resistance. The rpoB gene of Mycobacterium tuberculosis accounts for >95% of rifampicin resistance, and because rifampicin resistance is usually accompanied by isoniazid resistance (monoresistance is rare), this test is used as a marker for multidrug-resistant TB.

Line probe assays (LPAs) are NAATs that simultaneously detect infection with Mycobacterium tuberculosis and amplify regions of drug resistance. LPAs use strip technology, whereby amplified DNA is applied to strips containing probes specific for M. tuberculosis, isoniazid, and rifampicin resistance. The WHO has endorsed LPAs for culture and smear-positive clinical specimens as part of a larger commitment to target and implement new technology in high-burden countries.

Immunodiagnosis

Because of the limitations of TST, particularly cross-reactivity with BCG immunization and environmental mycobacteria, newer diagnostic tests have been developed based on in vitro T-cell-based interferon-γ release assays (IGRA), which measure interferon-γ production in response to stimulation to TB-specific antigens (ESAT-6, CFP10 and in QuantiFERON TB Gold TB 7.7). These antigens are present in M. tuberculosis complex but absent from all strains of Mycobacterium bovis BCG, and almost all environmental mycobacteria. Two IGRAsthe QuantiFERON-TB Gold assay (Cellestis Limited, Carnegie, Victoria, Australia) and the T-Spot.TB assay (Oxford Immunotec, Oxford, United Kingdom)—are currently available. Both tests measure interferon-γ release from T cells using enzyme-linked immunosorbent assay and enzyme-linked immunospot assay, respectively. IGRAsthe QuantiFERON-TB Gold assay and enzyme-linked immunospot assay, respectively. 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