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REVIEW

Routine investigations used in the diagnosis of childhood tuberculosis

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Abstract

World Health Organization approved the use of Xpert MTB/RIF Ultra (Ultra) in children due to quick turn-around time, improved yield over smear microscopy, and ability to detect rifampicin resistance despite culture being the gold standard. This study reviewed published literature on childhood tuberculosis diagnostic modalities.

For childhood tuberculosis (TB) diagnostic modalities, PubMed was searched using Boolean terms OR/AND between childhood tuberculosis and words such as diagnosis, polymerase chain reaction, molecular, histology, imaging, and cultures. All abstracts were read, after which selected articles that met this article's objectives were thoroughly reviewed and referenced appropriately.

Ultra is an important diagnostic method for confirming TB in children even though mycobacterial culture, other molecular, and histology tests are used in the diagnosis of pulmonary and extrapulmonary TB. Modalities such as imaging and immunologic testing support the diagnosis of microbiologically unconfirmed TB.

Despite advances in the diagnostic tools for tuberculosis in children, the sensitivity and specificity of such tests are still

relatively low. Clinical criteria of TB still play a role in deciding whether to treat children for TB.

Introduction

Globally, in 2021 there were approximately 9.9 million new cases of tuberculosis (TB), 11% of which were children. This drop from the 2020 global burden was attributed to the disruption of TB services from the covid19 pandemic.[1] However, the actual burden of TB in children is likely to be higher given the challenges in diagnosing childhood TB.[2]

Childhood tuberculosis typically presents with persistent or chronic cough greater than two weeks, fever unresponsive to conventional treatment, weight loss, failure to gain weight, failure to thrive, and fatigue or reduced playfulness or reduced activity. Generally, young children aged 0–4 years are the most vulnerable to the disease due to the vulnerability of their immune systems.[3] Immuno-suppression, commonly from human immunodeficiency virus (HIV) infection, multiplies the risk of progression from tuberculous infection to disease in children.[4] Severe malnutrition has a strong association with childhood TB.[5] Other risk factors known to be associated with TB infection in children include poverty, poor immunisation status (unvaccinated with BCG), low parental education, especially maternal education, overcrowding, high population density, contact with adult infectious TB cases, ingestion of unpasteurised milk, and chronic diseases.[6–8]

Childhood tuberculosis and HIV have overlapping clinical manifestations, leading to missed or late diagnosis. HIV infection increases the incidence of TB in children by a factor of around 8, increasing with the degree of immunosuppression; ART reduces TB risk by about 70%, with protection continuing to increase over 1–2 years.[9] Children living with HIV infection have an increased risk of TB-related morbidity and mortality.[10]

Children with TB present to health facilities just like any sick children. Children with respiratory symptoms are often misdiagnosed as pneumonia and offered multiple antibiotics. Those presenting with weight loss are managed with nutritional support by health care providers. The thought of HIV and TB often comes very late into managing such children leading to increased morbidity and mortality. The early diagnosis and initiation of treatment based on the susceptibility of the organism for children with tuberculosis can significantly reduce mortality in line with the aspirations of sustainable development goals (SDG) 3.[11–13]

Mycobacterium culture is the gold standard for diagnosing confirmed tuberculosis and identifying drug-susceptible bacilli, but it takes relatively long (2-6 weeks) for growth to be observed. Children have paucibacillary compared to multibacillary disease, contributing to low yield on cultures compared to adults. Microbiological confirmation of childhood tuberculosis is challenging due to the pathophysiology of childhood TB disease and for logistical reasons and, as a result, is uncommon.[14] Respiratory specimens are difficult to collect in young children, and the reported bacteriologic yield is low.[15] Nicol et al., in a study evaluating 452 children with a median age of 19.4 months that were admitted to hospital with suspected pulmonary tuberculosis (PTB) in 2009-2010 in Cape Town, found 27 children (6%) had a positive smear result, 70 children (16%) had a positive culture result, and 58 children (13%) had a positive Xpert MTB/RIF test (Xpert) result.[16] Microbiology laboratory capacity is lacking in many African countries, and diagnosis frequently relies on a combination of symptoms, signs, radiological findings, a tuberculosis contact history and tuberculin skin testing.[17]

Confirmed TB is defined as microbiological confirmation of *Mycobacterium tuberculosis* by either culture or Xpert MTB/RIF Ultra on at least one respiratory specimen.[18]

Unconfirmed TB is microbiological confirmation NOT obtained AND at least 2 of the following:

i. Presence of suggestive symptoms or signs of TB,
ii. Chest radiograph consistent with TB,
iii. A close TB contact or immunologic evidence of *M. tuberculosis* infection,
iv. Positive response to tuberculosis treatment.[18]

Pulmonary tuberculosis (PTB) is any bacteriologically confirmed or clinically diagnosed case of TB involving the lung parenchyma or tracheobronchial tree.

Extrapulmonary TB (EPTB) is any bacteriologically confirmed or clinically diagnosed TB involving organs other than the lungs. Tuberculous intrathoracic lymphadenopathy (mediastinal and/or hilar) or tuberculous pleural effusion, without radiographic abnormalities in the lungs, constitutes EPTB.19

Childhood TB is often paucibacillary; thus, TB is usually microbiologically unconfirmed in childhood practice.

The purpose of this article was to review the literature on advances in routine investigations for diagnosing tuberculosis in children, including pulmonary and extrapulmonary disease

Literature search

In March 2021, a peer-reviewed literature search was done in PubMed with key search terms related to routine investigations used to diagnose childhood tuberculosis. The search was a simple, non-systematic literature review. Box 1 shows the words inputted into the search.

Box 1: Literature Review Search Terms

tuberculosis, investigations AND diagnosis, polymerase chain reaction, molecular, histology, imaging, and cultures.

Routine investigations

I. Investigations that provide microbiological confirmation

Mycobacterial culture

Lowenstein-Jensen (LJ), as a solid culture medium, had been the gold standard for the diagnosis of TB for over a century. Its median time for positivity is four to six weeks. Commercial automated liquid culture methods, including the mycobacterial growth indicator tube (BACTEC MGIT 960 - Becton Dickinson USA), are widely used for routine TB diagnosis.[20]

The BACTEC MGIT liquid culture system has a shorter turn-around time than the conventional LJ method for smear-positive and negative clinical specimens with a median detection time of 2 weeks.[20] In a study in which smear-positive specimens were also cultured, the yield of positive results was 66.7% and 87.4% on LJ and MGIT methods, respectively. On smear-negative specimens, the yield was 13.4% and 17.4% on LJ and MGIT methods, respectively [21] Mycobacterial culture is superior to Ultra for diagnosing PTB in children because childhood TB is often paucibacillary.[22]

The microscopic-observation drug-susceptibility (MODS) assay is a liquid culture-based method for detecting living mycobacteria based on two well-known MTB characteristics. The growth in liquid medium is faster than that on solid medium and the microscopic visualisation of the unique cording of

MTB in liquid culture.[23] It is a low-cost, low-technology tool for high-performance detection of MTB and multidrug-resistant TB (MDR TB).[24] Compared to smear microscopy (28.2%), MODS (39.7%) was more sensitive to detecting MTB in children.[25]

Xpert MTB/RIF Ultra assay on a respiratory specimen

World Health Organization (WHO) in December 2010 approved the use of Xpert as a replacement for sputum smear microscopy, especially in settings with high rates of HIV-associated TB and MDR-TB.[26] It can detect *Mycobacterium tuberculosis complex* (MTBC) and simultaneously screens the β subunit of the mycobacterial ribonucleic acid (RNA) polymerase gene for the presence of mutations conferring rifampicin resistance.

More recently, the Xpert MTB/RIF Ultra (Ultra) assay was developed to overcome the limited sensitivity of Xpert in the detection of PTB, particularly in patients with a paucibacillary disease or HIV infection.[27] Dorman et al. in a study among adults with suspected TB who produced at least three sputum specimens in two days, the yield on Xpert and Ultra was 83% and 88%, respectively, on all culture-positive specimens. Among smear-negative, culture-positive specimens, the yield on Xpert and Ultra was 46% and 63%, respectively. Among HIV-infected patients with a culture-positive specimen, Xpert and Ultra yielded a positive result on 77% and 90%, respectively.[27]

A South African study published in 2018 investigated the comparative accuracy of Xpert and Ultra on induced sputum for diagnosing PTB in children. Among 76 children with a positive Xpert, Ultra or mycobacterial culture, Xpert detected 63.2%, Ultra 73.7% and culture 82.9%, (P = 0.117 for comparison of Xpert and Ultra).[22]

Xpert MTB/RIF Ultra assay on a stool specimen

Due to the challenges in collecting quality respiratory specimens for pulmonary tuberculosis diagnosis in children, researchers have been looking for alternative specimens that are easy to collect and process. Stool specimens are very easy to collect and could be a preferred alternative.

A study from Shanghai Public Health Clinical Centre evaluated the diagnostic efficacy of stool based Xpert MTB/RIF Ultra assay versus other assays for detecting paediatric PTB prospectively through a head-to-head comparative study. Samples were collected from children (< 15 years) with abnormal chest imaging (X-ray or CT scan) results for the following tests: Ultra on stool sample (Ultra-Stool), Ultra on respiratory tract sample (Ultra-RTS), Xpert *MTB*/RIF assay (Xpert) on RTS (Xpert-RTS), acid-fast bacilli smear on RTS (AFB-RTS), and *Mycobacterium tuberculosis* (*Mtb)* culture on RTS (Culture-RTS). Against a composite reference standard, Ultra-RTS demonstrated the highest sensitivity (52%) and specificity (100%). Ultra-Stool showed 84.1% concordance with Ultra-RTS, demonstrating 45.5% sensitivity and 94.7% specificity (kappa = 0.65, 95% CI= 0.51–0.79). The sensitivity of Ultra-Stool was similar to *Mtb* culture (45.5%, $p = 1.000$) and higher than AFB-RTS (27.3%, $p < 0.05$). Assay positivity was associated with age and infiltration range in chest imaging**.**[28]

Sputum smear microscopy

Sputum smear microscopy is widely used to detect TB. Light-emitting diodes (LED) were developed to offer fluorescence microscopy benefits over conventional Ziehl-Neelsen (ZN) microscopy.[29] The ZN stain is for acid-fast organisms like Mycobacterium, which has large amounts of lipid substances within their cell walls called mycolic acids. The lipoid capsule of the Mycobacterium takes up carbol- fuchsin and resists decolourisation with a dilute acid rinse**.** The organism stains as red bacilli under light microscopy. Conventional fluorescence microscopy has higher sensitivity than ZN. It is rapid, but uptake has been hampered by high cost due to expensive mercury vapour light sources, regular microscopy maintenance, and the requirement for a dark room. Light-emitting diode (LED) technology was developed to allow the benefits of fluorescent microscopy without the associated costs. It has some limitations, including low sensitivity, especially in HIV-positive individuals and children, and the inability to detect drug resistance.[30] With mycobacterial culture as the reference standard, Xpert identified twice as many cases (75·9%) as did smear microscopy (37·9%) in one study[16] whilst in a systematic review and meta-analysis, the sensitivity of Xpert was 36-44% more than microscopy.[31]

II. Investigations used in the diagnosis of microbiologically unconfirmed TB

Tuberculin skin testing (Mantoux test)

Short of demonstrating viable organisms in body tissues and fluids, the tuberculin skin test (TST) was the only method of detecting *Mycobacterium tuberculosis* (MTB) infection in an individual until the introduction of the interferon gamma-release assay (IGRA). Both tests are used to diagnose TB infection in individual patients and in epidemiological settings to measure the prevalence of tuberculous infection in populations.[32]

Tuberculin skin testing is performed by the Mantoux method. The Mantoux test is affordable, but it cannot distinguish actual TB infection from TB disease. Neither can it differentiate between *Mycobacterium tuberculosis* from environmental non-Mycobacterium tuberculosis nor the effect of BCG vaccination. Briefly, 0.1 mL of 2 tuberculin units of purified protein derivative is administered intradermally with a short bevel needle. The result of a Mantoux test is read in millimetres of induration 48-72 hours after injection. The diameter of the indurated area is measured across the forearm (perpendicular to the long axis).

The interpretation is dependent on the immune status of the child. In an immunodeficient child (e.g., HIV-infected not on ART or malnourished), a Mantoux test is considered positive when the transverse diameter of the skin induration reaction is ≥5 mm. In an immunocompetent child, a response ≥10mm is positive.[19] False-negative results following Mantoux testing could be due to HIV/AIDS, malnutrition, following immunisation with live vaccines such as measles and rubella, zinc deficiency, and recent TB infection. Exuberant reactions are usually caused by MTB infection but can result from nontuberculous mycobacterial infection and BCG vaccination.

Imaging modalities

Chest radiographic imaging is one of the oldest imaging techniques used for diagnosing respiratory conditions, including suspected pulmonary TB.

On chest radiography (CXR), a calcified Ghon focus or Ghon complex may be seen. Intrathoracic TB in infants and young children are frequently characterised by enlarged peripheral and hilar lymph nodes with resulting airway displacement, compression with/without lobar collapse consolidation.
Airspace disease with expansile lobar or diffuse TB bronchopneumonia can be seen on CXR. Lymph node disease is most common following primary infection before five years of age.[33] This, in addition to the small airway size, makes young children the most vulnerable group to develop lympho-bronchial TB.[34]

Children older than five years develop TB hypersensitive pleural effusion or pericardial effusion, which may develop after a recent primary infection.[35] TB lung abscess is seen as an irregularly shaped thick-walled cavity with an air-fluid level. TB empyema is characterised by a thick pleura ring with dense and irregular parietal and visceral pleura calcification.

In older children (>10 years), an adult-type cavitating disease often follows a recent primary infection.[36] Adolescents and young adults with TB commonly present with apical consolidation, cavitation, fibrosis, and atelectasis.[37] Tuberculosis of the spine (Pott's disease) typically involves the thoracic vertebrae and may be detected on CXR with or without gibbus and TB paraspinal abscesses[38]

Miliary TB is diagnosed by the presence of a diffuse miliary infiltrate on CXR. Post TB fibrosis or bronchiectasis changes are seen as lung scarring.

A classification proposed by Marais et al, has helped classify intrathoracic TB in children into domains namely: lymph node TB (lympho-bronchial TB), air-space parenchymal TB (consolidation/atelectasis/adult-type), miliary TB, and pleural TB.[39]

However, CXR is limited by its two-dimensional orientation and high inter-interpreter and intra-interpreter variability in identifying lymphadenopathy.[40,41] Poor image quality and co-infections in children living with HIV reduce the specificity of CXR as a diagnostic tool. In some studies specificity was < 50%.[42,43]

Imaging techniques other than chest radiographs are less widely available in African countries and require interpretation by radiologists. These techniques include ultrasonography (USG), computerised tomography (CT) and magnetic resonance imaging (MRI).

Ultrasonography findings of the abdomen suggestive of TB include lymph node enlargement greater than 1.5 cm and micro-abscesses in the liver and spleen with ascites as further supporting evidence. With additional history, USG findings as described above could suggest TB, especially in HIV-infected patients.[44] USG of the chest helps identify lymph nodes and effusions. Using the supra-sternal window, USG can detect lymph node abnormalities more frequently than radiography.[45]

Computerised tomography scans and magnetic resonance imaging (MRI) are imaging modalities that provide cross-sectional and three-dimensional spatial information to visualise multiple coexisting lesions, thus enhancing sensitivity and providing non-invasive monitoring for individuals.[46] The primary disadvantage to their routine use in developing countries context is cost.

Chest CT is superior for detecting lymphadenopathy, visualisation of airway compression, pneumonia, lymph node necrosis, and lung necrosis than CXR.[47,48]

CT scan of the brain helps assess features and complications of TB meningitis (TBM), including hydrocephalus, parenchymal enhancement of tuberculous granulomata, contrast enhancement of basal leptomeningeal lesions, cerebral infarction, and focal or diffuse brain oedema.[49] These advances notwithstanding, lateral skull X-rays are still helpful in demonstrating communicating hydrocephalus after intrathecal injection of air (air encephalograms).[50]

CT scanning is limited by exposure to radiation and the need for intravenous contrast to enhance visualisation.[47]

On MRI, necrotic tuberculosis lesions, including lymph nodes, show low T2 signal.[51] Three significant findings of spinal TB on MRI are endplate disruption, paravertebral soft tissue abscess, and increased signal intensity of intervertebral disc on T2-weighted image. It can identify abscesses, including extension into the psoas muscle and epidural space.[52] Some techniques require sedation in young children.

III. Investigations used in the diagnosis of microbiologically confirmed or microbiologically unconfirmed TB

Fine needle aspiration versus excision biopsy

Lymph nodes may be sampled by fine-needle aspiration biopsy (FNAB), providing diagnostic material for mycobacterial culture and drug susceptibility testing, cytology, as well as nucleic acid amplification testing (NAAT).[53]

About 30% of children with PTB also have an extrapulmonary disease, with tuberculous lymphadenitis as the most typical manifestation.[54] Tuberculous lymphadenitis is considered a local manifestation of the systemic disease, whereas lymphadenitis due to nontuberculous mycobacteria is truly localised disease.[55]

A prospective study in Cape Town, South Africa, showed that FNAB using a combination of cytomorphology, autofluorescence, and ZN staining in high-risk populations provided a rapid and definitive diagnosis of mycobacterial infection, allowing initiation of therapy pending culture and sensitivity testing.[56] In a prospective diagnostic study in adults using fine-needle aspirates (FNA) at

Groote Schuur Hospital, Cape Town, Ultra sensitivity was 75% using mycobacterial culture on FNA as reference.[57]

Even though excision biopsy is more sensitive than FNA (94% vs 80%) in diagnosing tuberculous cervical lymphadenopathy, the need for theatre space, anaesthesia and potential complications from the procedure such as surgical site bleeding, haematomas, and surgical site infections make it a lesser preferred option.[58]

Tissue histopathology

A key finding of *Mycobacterium tuberculosis*-infected tissue is necrotising granulomatous inflammation, composed of epithelioid histiocytes surrounding a central necrotic zone, and can be accompanied by a variable number of multinucleated giant cells and lymphocytes.[59]

A histological finding of chronic granulomatous inflammation (CGI) concomitant with caseating necrosis (CN) from a peripheral lymph node excision biopsy or tissues of any organ system can be strong evidence of active TB even though not confirmatory.[60] Other infectious (e.g. cat scratch disease) and non-infectious (e.g. sarcoidosis) conditions produce CGI.[61]

Diagnostics on extrapulmonary fluids

A definitive diagnosis is based on the discovery of *Mycobacterium tuberculosis* in any pleural, peritoneal (ascitic) fluid, or CSF cell smears, bacterial culture, or polymerase chain reaction (PCR).

In a study by Jeren et al., which retrospectively reviewed 84 cases in ten years, cerebrospinal fluid (CSF) in patients with TB meningitis in the first ten days showed cytological changes with neutrophils predominated (60% to 80%) then mononuclear cells, such as lymphocytes, lymphoid cells, monocytoid cells and macrophages, became predominant. Plasmocytes (20%) were found in 30% of these cases from the third week of the disease.[62]

The gold standard for diagnosing tuberculous pleural effusion (TPE) is the detection of *Mycobacterium tuberculosis* in pleural fluid or pleural biopsy specimens, either by microscopy or culture, the histological demonstration of caseating granulomas in the pleura along with acid-fast bacilli. Adenosine deaminase and interferon-γ in pleural fluid have been documented to be useful tests for the diagnosis of TPE.[63]

In cases of TB peritonitis, peritoneal fluid analysis typically shows an elevated lymphocyte count with lymphocyte predominance, serum-ascitic albumin gradient of <11 g/L, and high protein levels (>2.5 mg/dL).[64]

For abdominal tuberculosis, adenosine deaminase (ADA) helps make the diagnosis, specifically when levels are above ≥30 U/L.[65]

Other investigations

Table 1 summarises other tests less frequently used in the diagnostic workup of children with suspected TB.

Table 1. Other tests for TB diagnosis in children

LAMP[66]	A nucleic acid amplification method designed to amplify a specific DNA region under isothermal conditions	User friendly Less infrastructure needed More sensitive than sputum smear microscopy	Less sensitive than Xpert MTB/RIF Ultra
Urine-LAM[67]	Detects cell wall lipopolysaccharide lipoarabinomannan in urine	Commercially available as a POCT	More applicable in adult HIV patients with advanced disease
Immune response test			
ADA[68]	An enzyme involved in T-cell proliferation	Supportive for TB pleural effusion	Variable cut-off values indicating a significant result
IGRA[69]	Measures interferon (IFN)-gamma release in response to antigens present in *Mycobacterium tuberculosis*	Differentiates *Mycobacterium tuberculosis* infection from BCG	The test is performed using blood (invasive procedure) Expensive

LAMP-Loop-mediated isothermal amplification assay; LAM- Lipoarabinomannan; ADA- Adenosine Deaminase; IGRA-Interferon Gamma release assay; POCT-Point-of-care test; BCG- Bacillus Calmette-Guerin vaccine.

Limitations

The search was limited to PubMed and English language resources. This literature review and its conclusions may therefore be subject to publication bias.

Conclusion

This article reviewed investigations used in everyday clinical practice to support the diagnosis of childhood TB. All diagnostic modalities have limitations. Clinical criteria including the presence of symptoms consistent with childhood TB and a close contact history remain vital for establishing the diagnosis of childhood TB.

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