Diagnosis of Pulmonary Tuberculosis in Children

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Abstract: Children account for a major proportion of the global tuberculosis disease burden, especially in endemic areas. Diagnosis of latent tuberculosis infection relies on immunodiagnostic methods, which include the tuberculin skin test and the Interferon-gamma release assays (IGRAs). IGRAs improve specificity in BCG vaccinated children and have been incorporated in several national guidelines especially in low incidence and high-resource settings. However, careful interpretation of this test should be taken especially in young children. Childhood pulmonary tuberculosis is under diagnosed, in part due to difficulties in obtaining microbiological confirmation. Other specimens include induced sputum and nasopharyngeal aspirated that simplify the sample collection without hospital admission. Nucleic amplification assays have similar sensitivity than culture but the results can be obtained in one day. The recent development of an integrated specimen processing and real-time PCR testing (GeneXpert MTB/ RIF system) allows the identification of *Mycobacterium tuberculosis* and also can detect rifampicin resistance, although additional confirmatory tests of resistance are recommended. Computed tomography (CT) scan is a useful tool in the symptomatic child with difficult diagnosis.

Keywords: Child, culture, diagnostic tests, induced sputum, nucleic acid amplification, pulmonary tuberculosis.

Globally, under diagnosis of childhood pulmonary tuberculosis (PTB) remains an obstacle to effective management. Children with tuberculosis usually have paucibacillary disease and contribute little to disease transmission within the community. Consequently the diagnosis and treatment of children with tuberculosis (TB) has not been considered a priority by TB control programmes until last years. Children carry a huge tuberculosis disease burden, particularly in endemic areas. In 2012, the TB disease burden in children was quantified, estimating there to be 490000 cases and 65000 deaths in 2011 [1]. Nevertheless, under-reporting of child TB cases is very common. The estimated number of children with TB equates to less than 6% of all incident cases, whereas estimates from tuberculosis endemic areas suggest proportions of 10–15% [2]. Furthermore, child TB data reported by national tuberculosis control programmes are often incomplete and hampered by restricted diagnostic access in most tuberculosis endemic areas. In these setting the accuracy and quality of non-microbiological diagnoses is weak [3].

The problems of TB infection are doubled in young children. There is a higher probability of progression to disease, with the possibility of severe and extrapulmonary forms. Furthermore, infected children make up a reservoir from which new future cases of the disease will arise. Therefore, to control TB, the diagnosis and the correct treatment of infected children and those with the disease is important. In developing countries the diagnosis of TB is frequently done in a child with symptoms, while in developed countries is often the result of contact studies of an adult with TB, in a child with few or no symptoms.

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DIAGNOSIS OF TUBERCULOSIS INFECTION

Tuberculin skin test (TST) has been used as the main tool in the diagnosis of latent tuberculosis infection (LTBI). However, TST has many drawbacks, such as the need for patients to return for test reading, as well as variability and subjectivity in test application and reading. False positive and false negative results of the TST are well known. TST has low specificity as the antigen used for the test (purified protein derivative, PPD), is a mixture of mycobacterial antigens also present in nontuberculous mycobacteria and in the Bacille Calmette Guérin (BCG) vaccine strains [4]. BCG vaccination significantly increases the likelihood of a positive TST in subjects without LTBI [5].

Identification in the *M. tuberculosis* genome of genes that are absent from BCG vaccine strains and nontuberculous mycobacteria, has allowed the development of more specific tests for *M. tuberculosis* infection. ESAT-6 and CFP-10 are deleted from BCG Region 1 (RD1), and are not present in most nontuberculous mycobacteria. These antigens are highly specific indicators of *M. tuberculosis* infection [6], and have enabled precise diagnosis in BCG vaccinated individuals [7,8].

New immune-based diagnostic tests have developed that measure ex-vivo interferon-gamma (IFN-γ) production by circulating T-lymphocytes when incubated in the presence of highly specific *M.*

tuberculosis antigens (ESAT-6, CFP-10 and TB7.7). There are two commercially available interferon gamma release assays (IGRAs) QuantiFERON®-TB (QFT; QuantiFERON®-TB Gold [QFT-G] and QuantiFERON®-TB Gold In-Tube [QFT-GIT], Cellestis, Carnegie, VIC, Australia) and T-SPOT.®TB (Oxford Immunotec, Oxford, UK). The QFT test incubates whole blood and measures IFN-γ production with an enzyme-linked immunosorbent assay (ELISA), while T-SPOT.TB measures the number of IFN-γ producing peripheral mononuclear cells.

A growing number of studies have compared the TST and IGRAs in the detection of *M. tuberculosis* infection and active TB in children. In the absence of a gold standard for infection, some studies have measured sensitivity in populations with active TB as a surrogate for *M. tuberculosis*-infected persons, while others have used *M. tuberculosis* exposure as a surrogate for infection [9,10]. A systematically reviewed and meta-analysis of the existing evidence on the accuracy of IGRAs compared to the TST for the detection of *M. tuberculosis* infection and diagnosis of active TB in children in settings with varying incidence of TB was published in 2011 [11]. Two small studies measured incident TB in children found weak positive predictive value. A school outbreak investigation in Japan assessed 313 children with TST and QFT tests [12]. QFT positive children and QFT-indeterminate/ TST-positive children received preventive chemotherapy. One year after the index case was reported, all children underwent chest radiography; no child developed active TB during the 3-year follow-up (positive predictive value 0%, 95%CI 0–35, negative predictive value 100%, 95%CI 0–1.5). German contact investigations assessed 168 children with QFT and completed approximately 2 years of follow-up [13]. Three of seven QFT positive children developed probable TB (PPV 43%, 95%CI 16–75), whereas none of the 161 QFT-GIT-negative children developed active TB (negative predictive value NPV 100%, 95%CI 0–3). These two studies suggested a high negative predictive value of IGRAs in the diagnosis of TB infection.

Recently, Nenadic´ prospectively evaluated the usefulness of IGRAs for diagnosis and treatment monitoring of children with LTBI and those with active TB [14]. IGRA was performed in 59 BCG vaccinated children (41 with LTBI and with 18 active TB) before and six months after the beginning of treatment. They found that there was no significant difference in IFN-γ concentrations between children with LTBI and active TB either before or after the treatment. Furthermore, difference between pre-treatment and post-treatment IFN-γ concentrations compared in both groups was not statistically significant. They concluded that the concentrations of IFN-γ did not differentiate children with LTBI and active TB and that IGRA is not useful for monitoring treatment of children with LTBI or active TB.

When it comes to the optimal application of IGRA results in LTBI diagnosis, there is some disagreement between the various national guidelines. A recent paper surveyed the literature and contacted experts to identify 33 guidelines and position papers from 25 countries and two supranational organizations [15]. Four approaches were found: (i) two-step approach of TST first, followed by IGRA either when the TST is negative (to increase sensitivity, mainly in immunocompromised individuals), or when the TST is positive (to increase specificity, mainly in bacillus Calmette–Guérin-vaccinated individuals); (ii) Either TST or IGRA, but not both; (iii) IGRA and TST together (to increase sensitivity); and (iv) IGRA only, replacing the TST. It was concluded that in high incidence and low-resource countries, the TST is still recommended because there is no strong evidence that IGRAs are superior to the TST in such settings, especially given the significantly higher costs associated with IGRAs. In low incidence and high-resource settings, the higher specificity of IGRAs and their logistical advantages seem to enhance their adoption and usage. However most of the current guidelines do not use objective, transparent methods to grade evidence and recommendations, and do not disclose conflicts of interests.

Careful consideration should be given about IGRAs. Indeterminate results occur more frequently in immunocompromised children and high-risk young children, especially those aged under 5 years. This could have substantial implications because false-negative results [16]. Furthermore, WHO recommendations advise against the use of IGRA assays in place of the TST given the cost, need for laboratory infrastructure and a blood specimen and the relatively lower sensitivity in high compared with low TB incidence settings [17]

DIAGNOSIS OF ACTIVE DISEASE

CLINICAL SAMPLES

The definitive diagnosis of tuberculosis disease is determined by isolating by culture and identifying *M. tuberculosis*. In children, the sensitivity of the culture is

low. On the one hand, the predominant forms of the disease are paucibacillary, and besides, clinical samples are not usually sputum, due to expectoration not being possible in many children. Traditionally, serial samples of gastric juice have been collected early in the morning, achieving a yield of 30-40% [18] and up to 80% in young infants and in cases with advanced endobronchial disease [19].

Induced sputum (IE) or nasopharyngeal aspirated (NPA) can be a valid alternative samples. Suctioning of the nasopharynx obtains upper respiratory tract secretions and the stimulation of cough reflex may include lower respiratory secretions. Early data suggested that the culture yield from NPA (24-30%) was similar to that of GA [20,21]. Subsequent studies showed variable performance [22,23].

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. In one study the yield from one IS sample was equivalent to three gastric lavage samples [24]. This has shifted clinical practice to include induced sputum as a diagnostic procedure in young children and infants with suspected pulmonary tuberculosis and some national guidelines recommend induced sputum as the sample of choice in children that are not able to expectorate [16].

Flexible bronchoscopy has proven to be a useful tool in many paediatric respiratory diseases. The sensitivity of culture from bronchoalveolar lavage of children with TB is lower than that from serial gastric aspirates so that routine bronchoscopy is not justified in attempting a microbiological diagnosis [25]. However bronchoscopy is able to detect endobronchial tuberculosis, serves as a guide for the use of corticosteroids therapy and excludes other infections particularly common in immunocompromised children.

Recently the diagnostic value of transbronchial needle aspiration biopsy in children with mediastinal lymphadenopathy was prospectively evaluated [26]. The biopsy was done in 28 children with subcarinal mediastinal lymph nodes assessed by CT. A definitive diagnosis was made in 15 children (54%), 13 of them with TB. In 7 children transbronchial needle aspiration biopsy was the only diagnostic sample (one of them with multidrug-resistant TB) and in 10 children the diagnosis was done in the endoscopy suite. No serious complications were reported.

The string test. Patients swallow a gelatine capsule containing a coiled nylon string which unravelled as the capsule descended to the stomach. After 1 - 4 hours the string is withdrawn and used for mycobacterial culture. The test was has been used in children and shown to be well tolerated by older children [27].

Lymph node aspiration. In children with respiratory symptoms and a palpable peripheral lymph node, fine needle aspiration and culture is a useful sample. The sensitivity can be higher than respiratory specimens (sensitivity of 60.8 vs. 39.2% respectively) [28]. The procedure may be performed safely on an outpatient basis in a resource limited setting.

LABORATORY TEST

Direct staining of the samples obtained provides a rapid probable diagnosis that enables specific treatment to be started; however, its sensitivity is very low. Even with concentration of specimens by centrifugation and the use of fluorescent microscopy, the sensitivity of smear microscopy for the diagnosis of childhood TB remains less than 15%, except in older children with adult-type disease [18,29].

Liquid culture systems with continuous monitoring for mycobacterial growth (such as MB/BacT [Biomerieux, Marcy l'Etoile, France], BACTEC 9000 [Becton Dickinson, NJ,USA] and the mycobacterial growth indicator tube [MGIT; Becton Dickinson]) are a significant advance over solid culture (Löwenstein Jensen). The time of detection is lower for liquid culture (13.2 vs. 25.8 days) [30] and the sensitivity is higher

The Microscopic Observation Drug Susceptibility Assay (MODS) is a potentially low-cost alternative [31]. The sample is directly inoculated into wells of a tissue culture plate containing liquid growth media and growth is determined by visual inspection using an inverted microscope. In one study in 96 children with suspected TB, the mean time to detection for MODS (8 days) was shorter than MGIT (13 days) but the sensitivity was slightly lower 43.8% vs. 48.5%, $p = 0.03$) [32].

Nucleic Amplification Assays

These assays are theoretically highly sensitive, able to detect very low copy numbers of nucleic acid, rapid (results typically available on the same day), and are relatively easy to automate. According to the various

studies published, the sensitivity of in-house polymerase chain reaction (PCR) in assessing samples of gastric aspirates from children with pulmonary tuberculosis varies between 40% and 83% [33-35]. The reasons for this variability are the lack of uniformity in the methodology of sample processing, the amplified target of *M. tuberculosis* and the way detection of amplified DNA is performed.

The yield of PCR is much higher than that of the smear, and provides a rapid test in a child with suspected tuberculosis. Moreover, and contrary to what happens with adults, the reported sensitivity of PCR has usually been slightly better than that of culture. This can be explained by the fact that only a small number of organisms is present in the samples from children and that a high proportion of mycobacteria may not be viable *in vitro*, as a result of the microbactericidal action of immune and inflammatory cells or the reduced viability attendant on processing and decontaminating specimens before culture.

The main clinical benefit of PCR in the diagnosis of childhood tuberculosis has been found in a group of patients who usually have negative smear and culture results: only hilar adenopathy on chest radiograph, no clinical symptoms and unidentified source case. In these cases, in contrast to traditional methods, PCR sensitivity does not seem to decrease significantly [33]. Another advantage of PCR can be seen in cases of severe TB, such as miliar TB, where a delay in the diagnosis can have fatal consequences.

PCR can detect nucleic acids from dead as well as live *M. tuberculosis* and therefore can be used to corroborate the clinical diagnosis of children undergoing specific therapy when the evolution seems to be unsatisfactory [33].

The specificity of PCR in children with tuberculosis has been inconsistent. Some of the published studies have found that positive PCR results are specific to tuberculosis [33,34], while others have reported false-positive results in children with non-tuberculous diseases and have obtained a specificity of 80–90% [35]. These false-positive results have been attributed to contamination with exogenous DNA or amplicon, and force each laboratory to take extreme measures to avoid contamination. Consequently, positive PCR results should always be interpreted carefully, taking into consideration the clinical and epidemiologic context of the child with suspected tuberculosis [36].

Commercial PCR kits have been developed (Amplicor, Roche Diagnostic Systems). The sensitivity of these test seem to be lower than that of “in-house” PCR techniques (44% versus 65%) and similar to that of culture [37]. Furthermore false-positive results were found with the commercial tests (specificity: 93%).

Other non respiratory samples have been evaluated. A small study using a PCR assay in stool samples demonstrated relatively poor sensitivity for detection of culture-proven cases (31-38%) [38]. PCR has also been performed on blood from children with TB. The sensitivity was 26.2%, but the test was also positive in 7.3% of children without TB and 26.2% of children characterized as having latent TB [39].

The development of real time PCR has been a significant advance. It detects the presence of amplified nucleic acid target in a closed system and reduces the risk of cross-contamination of samples by amplified DNA from previous samples and operator dependence. These tests have also been evaluated to detect the main mutations responsible of isoniazid and rifampicin resistance. The GeneXpert MTB/ RIF system amplifies a region of the *rpoB* gene of *M. tuberculosis* that give rise to 95% of rifampicin resistance. This system requires minimal manipulation of sample and operator training and allows to simultaneously detecting the presence of *M. tuberculosis* and rifampicin resistance. This test was evaluated in induced sputum of 452 children admitted for suspected TB (108 with HIV) [40]. The sensitivity was similar than culture (27.6% vs 32%) and the specificity was 99.8%. With line probe as a reference standard, MTB/RIF correctly identified all 70 rifampicin- susceptible cases and two rifampicin-resistant cases. However, 5 indeterminate results were reported with GeneXpert MTB/RIF (one case of rifampicin-resistant tuberculosis and four cases of rifampicin-sensitive tuberculosis).

A prospective study confirmed similar sensitivity of Xpert compared with culture in sputum and induced sputum of children with confirmed or clinical diagnosis of TB [41]. Also, Xpert has similar results in NPA samples and can be an alternative particularly in settings where IS and culture are not feasible [42].

A study in Zambia included 930 children admitted for suspected TB with a majority of gastric lavage samples collected (n=788) [43]. No significant difference was identified in the Xpert MTB/RIF assay between sputum and gastric lavage samples. Fifty two samples were analyzed with the MGIT drug-

susceptibility test and 2 of them were multidrug resistant. The Xpert MTB/RIF assay correctly identified the two multidrug-resistant samples (sensitivity 100% 95%CI 19.8–100). However the Xpert assay detected rifampicin resistance in a third child without TB and was considered a false positive. False positive results of Xpert for rifampicin resistance are well documented and the manufacturers of the assay have attempted to resolve this with the latest version (G4) [44]. Further studies of the use of the Xpert MTB/RIF assay for the diagnosis of multidrug-resistant tuberculosis are needed because false-positive results could expose children to unnecessary second-line treatment with toxic drugs. The WHO recommends additional confirmatory tests after detection of rifampicin resistance with the Xpert MTB/RIF assay [45].

IMAGING TECHNIQUES

Frequently in a child with minimal or no symptoms, the classification as TB infection or disease depends on the interpretation of the chest radiograph. Among them, the increase of the hilar and mediastinal lymp nodes is the most frequent finding. Tuberculous lymph nodes show up on the chest X-ray as an increase in density with generally blurred limits due to the adjacent pulmonary parenchymal being affected. In cases of lymphobronchial disease, bronchial compression can be seen, as hyper-clear areas due to valvular emphysema or as atelectasis. Different studies corroborate the difficulty and caution that must be used in interpreting a possible pulmonary lymph node on chest X-rays of children suspected of having TB. In one of these studies a wide intra- and inter-observer variability was observed in the viewing of lymph nodes when four pediatric pneumologists reviewed the X-rays of 100 children with a diagnosis of PTB or pneumonia [46]. Another study compared the sensitivity and specificity of anteroposterior and/or lateral chest X-rays, interpreted by pediatricians and primary care doctors, in detecting pulmonary lymph nodes in 100 children who were suspected of having PTB [47]. Taking CT as reference, the sensitivity of the chest X-ray was 67% and the specificity was 59%. Therefore, the interpretation of chest X-rays to detect tuberculosis lymph nodes is not without problems.

CT can help in children with suspected TB in the investigation of lung involvement, occult cavities and the assessment of nodular and reticulonodular forms. With aid of intravenous contrast, lymph nodes are observed with a rim on the peripheral ring and low density centre or with “ghost-like” enhancement [48,49]. It is useful in the symptomatic child with a normal or doubtful X-ray, since it specifies the extent of the disease and helps to check if the patient symptoms are associated with TB and also, in the assessment of complications (emphysema, atelectasis or bronchiectasis).

Magnetic resonance and chest ultrasound, in the hands of a radiology expert, can also detect mediastinal lymph nodes and their progress during treatment [50,51].

CT in Children with Tuberculosis Infection and with no Apparent Disease

In 1993 Delacourt published a study of 15 children with tuberculosis infection with no evidence of disease, with a positive tuberculin test, normal chest X-ray and a negative gastric juice culture [52]. A CT with intravenous contrast was performed on all of them, verifying an increase in the size of the lymph glands in 9 patients (60%). The lymph nodes were mainly detected in children less than 4 years old and in the right paratracheal chains and hilars.

Later, another group performed CT with intravenous contrast on 22 children with a positive tuberculin test, asymptomatic, normal chest X-ray and negative culture. In 14 of them (63%) lymph nodes, mainly in the paratracheal chains, were found that had been missed on the chest X-ray [53].

A recent study analyzed the usefulness of CT in the diagnosis of TB in a TB outbreak that affected 28 children younger than 4 years in a nursery in Spain [54]. Fourteen of the children had normal chest X-ray but 12 of them (8 without any clinical symptom) presented adenopathies >10 mm or infiltrates on CT.

It is difficult to know if these mild findings on CT that are not visible on chest X ray are indicative of active disease. In the period between 1920 and 1950, the availability of chest X-ray enabled descriptive studies to be performed on the natural history of TB without the influence of an effective treatment [55]. With these studies, it was documented that after the primary infection, 50-70 % of children had enlargement of hilar or mediastinal lymph nodes [56,57]. Serial radiological studies demonstrated that in 40% of cases the lymph nodes disappeared in the first 6 months and in 30% in the first year [58]. The spontaneous progression was favorable regardless of the size of the lymph nodes or there was a visible parenchymatous lesion [58]. On the other hand, age less than two years [56,57], as well as

persistent clinical symptoms were risk factors of the progression of the disease, while the absence of symptoms was indicative of a good containment of the germ [59].

In summary, in the asymptomatic child with tuberculosis infection and a normal chest X-ray, mediastinal lymph nodes are often seen on the CT. However, there is no evidence that correspond with the active disease, and the natural history of the disease suggests that they may be a part of the primary tuberculosis infection. The official national and international recommendations and opinions of prestigious authors do not recommend performing CT on the asymptomatic child, with a positive tuberculin test and with a normal chest X-ray, or to take a particular therapeutic path depending on their results [60].

CONCLUSION

IGRAs have incorporated in the diagnosis of LTBI, especially in low incidence and high-resource settings. The specificity of these tests is higher than TST in BCG vaccinated children. However undermined and false negative results have been reported in young children. Advances in the diagnosis of childhood PTB include alternative specimen and the development of new molecular tests. Induced sputum and nasopharyngeal aspirate have at least similar sensitivity that gastric aspirates and can be obtained without hospital admission. PCR based tests permit the diagnosis in one day. The GeneXpert MTB/ RIF system is -real-time PCR that identifies *M. tuberculosis* and also can detect rifampicin resistance. The sensitivity and specificity have been good in pediatric samples although additional confirmatory tests after detection of rifampicin resistance are recommended. CT scan is a useful tool in the symptomatic child with difficult diagnosis or complications. Some asymptomatic children with LTBI and normal chest X-ray present enlarged lymph nodes on the CT scan, but these findings seem to be related to the natural history of TB infection.

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