

How to Optimize Current (Available) Diagnostic Tests

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Abstract Isolation of *Mycobacterium tuberculosis* is the gold standard in the diagnosis of childhood tuberculosis. However, it has inherent limitations due to paucibacillary nature of the disease in children and technical difficulties encountered in collection of appropriate sample. Thus, diagnosis is dependent on circumstantial evidence at best supported by conventional tests such as tuberculin test and chest radiograph. Several new tests are being developed but they lack ideal sensitivity and specificity. Hence, it is important to optimise use of current diagnostic tests. Clinical suspicion based on protocol developed by IAP is a pre-requisite of ordering tests and it is only then that proper interpretation is possible. Tuberculin skin test is still a useful screening test. It does help in establishing presence of infection though not necessarily disease. Attention must be paid to ideal test solution, proper technique and cautious interpretation. BCG test is not recommended. Miliary shadows and fibrocaceous cavitary lesions in chest radiograph are highly suggestive of tuberculosis in our epidemiology. CT scan is rarely necessary and is not cost and radiation-effective. It is ideal to attempt bacteriological examination in every suspected case of childhood tuberculosis. Most practical method is collection of gastric aspirate for smear and culture. It is possible to carry out this procedure in out-patient clinic. Better yield is likely with increasing expertise especially in extensive disease. Bronchoalveolar lavage is an invasive test and bacterial yield is comparable to that of gastric aspirate. Tissue collected for

histopathological examination must be submitted for bacteriological tests. PCR is not easily available. It has high sensitivity but lower specificity and thus, is not routinely recommended. Serology has no place in diagnosis of tuberculosis. Interferon gamma release assays are also now available. Sensitivity and specificity of Quantiferon Gold and T-spot tests have not been studied in children and hence are not recommended in routine practice. Instead of trying newer tests, it may be best to avail an expert advice in difficult cases.

Keywords Childhood tuberculosis · Diagnostic tests · Newer tests

Making a correct diagnosis of tuberculosis in children is extremely challenging because of difficulty in isolation of *Mycobacterium tuberculosis*—the gold standard in the diagnosis of tuberculosis. Lack of accurate, rapid and cost-effective diagnostic tests poses a huge obstacle in global tuberculosis control. While several new diagnostic tools are being developed and evaluated, it is important to validate accuracy, cost-effectiveness and impact of these tests in clinical practice. Thus, despite new inventions in diagnostic modalities, routine diagnostic tests remain useful and continue to be used in the diagnosis of childhood tuberculosis.

Pre-requisites of Testing for Tuberculosis

It is the clinical suspicion of tuberculosis that should be a starting point of ordering tests. Interpreting test results without correlation of clinical profile leads to erroneous judgement. As per the consensus statement on childhood tuberculosis of Indian Academy of Pediatrics, fever and / or

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cough for >2 wks with loss of weight and recent contact with an infectious case should arise suspicion of tuberculosis [1]. Clinical suspicion should be further supplemented by chest radiograph and broad-spectrum antibiotic trial for 7–10 days, if justified. In case of no response, tuberculosis is likely and hence should be further investigated.

Available Tests—Their Merits and Demerits

Tuberculin Skin Test (TST)

Century old TST till recently was the only means of diagnosis of TB infection in children. It continues to be used in the diagnosis of childhood tuberculosis as a first screening test in clinically suspected children. Best available test solution is purified protein derivative (PPD). Better test antigens are being looked at, such as early secreted antigenic target 6 (ESAT 6) and culture filtrate protein 10 (CFP 10). There have been issues about ideal concentration of PPD. With high prevalence of TB infection in the community, lower concentration of PPD would offer better interpretation. It is recommended to use either 1 or 2 tuberculin units (TU) for TST. However, up to 5 TU may be acceptable [1]. >5 TU PPD should not be used. Technique of the test is not easy and needs expertise to perform ideal test. Induration is measured by standard technique such as ball-point method. It was observed that most of the health workers did not know how to measure and document TST reaction [2].

TST has high specificity though poor sensitivity. Further, accuracy and reliability is in question because of technical difficulties. In our epidemiology, cut-off of 10 mms has been considered to represent natural infection with *Mycobacterium tuberculosis*. It is possible that cut-off of 10 mm may overdiagnose prevalence of infection and disease as TST reactions follow bell-shaped curve. On the other hand, higher cut-off may underdiagnose few diseased children. As TST is not the only tool for diagnosis, such an underdiagnosis may not come in the way. However, as of now, there is no evidence in favor of raising cut-off point for interpretation, it is considered best to continue with 10 mm as cut-off point for clinical use. Future studies may provide clear insights into this problem. It is well accepted that previous BCG vaccine does not influence interpretation of TST results [3–5]. Finally, TST as a single test is never employed in the diagnosis of childhood tuberculosis and hence the test should continue to be used as a primary screening tool. False negative results are seen in severe forms of the disease, in immunocompromised children including severe malnutrition and also during incubation period. False positive results may be ascribed to exposure to other mycobacteria from the environment.

BCG test is not recommended in the diagnosis of childhood tuberculosis [6].

Imaging—Chest Radiograph, CT Scan, USG

There are no pathognomonic radiological signs of tuberculosis. At best, some of the radiological lesions may favor diagnosis of pulmonary tuberculosis in a typical clinical setting. Such lesions include military shadows and fibrocaseous cavitory lesions. Hilar and paratracheal lymphadenopathy is not easy to make out as other lesions may look similar and moreover etiology may not be necessarily tuberculosis. In clinical practice, non-resolving lung shadows in spite of adequate antibiotic trial in a persistently symptomatic child should arouse suspicion of tuberculosis. In such a case, positive tuberculin test would support diagnosis of tuberculosis to a great extent. Ideally, diagnosis should be confirmed bacteriologically even in such a case. USG is useful in detecting pleural fluid though in tuberculosis, pleural effusion is a hypersensitivity phenomenon and amount of fluid is large enough to be made out on simple chest radiograph. Decubitus chest radiograph is a good alternative to USG. CT scan of chest is rarely necessary and it is not cost and radiation effective. It is well known that chest radiograph may be initially normal in pulmonary tuberculosis and CT scan may pick up a lesion [7]. However, it is best that expert opinion is sought in such a case as CT scan is also not diagnostic of tuberculosis.

CT scan of brain is an important diagnostic modality in CNS tuberculosis. It may reveal indirect evidence in terms of basal meningeal enhancement, hydrocephalus with or without periventricular ooze, infarcts and tuberculoma. Though normal CT scan of brain may not necessarily rule out CNS tuberculosis especially in early stage of the disease and in such a case, repeat CT scan may also be justified. Tuberculoma may not be easy to differentiate from neurocysticercus lesions.

Abdominal USG may aid in the diagnosis of abdominal tuberculosis. Though not specific, it does offer corroborative evidence in terms of echogenic thickened mesentery with enlarged necrotic lymph nodes >15 mm in size, dilated and matted bowel loops, thickened omentum and ascites. Barium follow-through may demonstrate intestinal lesion but is not specific of tuberculosis.

Bacteriology

This is the gold standard of diagnosis of tuberculosis and must be attempted in every child to the best possible extent. Even in a paucibacillary childhood lesions, it is possible to demonstrate acid fast bacilli though not always successful. Initial step is to obtain an appropriate specimen for

bacteriological examination. It could be sputum, gastric aspirate or bronchoalveolar lavage. Gastric aspirate is used in lieu of sputum in young children who cannot expectorate. At times, other fluids such as CSF or tissues such as lymph node, liver and bone marrow may be suitable to collect for examination. Using standard technique for collecting gastric aspirate is important as it is likely to improve bacterial yield [8]. Early morning sample is obtained before the child has had a chance to eat or ambulate because these activities dilute bronchial secretions accumulated during night. Stomach contents are aspirated through nasogastric tube and if small in amount <5 ml, 10–20 ml of sterile water is injected and added to the aspirate. Because tubercle bacilli do not tolerate acidity, neutralization of the sample should be immediately done with 10% sodium bicarbonate or 40% sodium phosphate. Sample should be delivered to the laboratory and processed within 4 hrs. If there is likely to be a delay in transporting the sample, it should be preserved in a refrigerator. This procedure is repeated on three consecutive mornings and can be accomplished in an outdoor setting—hospitalization may not be necessary [9].

Sensitivity of gastric aspirate varies a lot due to many variable factors. Thus it has been reported to be as low as 20% to as high as 70%. Specificity varies between 70% and 95%, positive predictive value between 70% and 80% and negative predictive value between 60% and 70% in various studies [10, 11].

Alternate specimen for isolation of mycobacterium is bronchoalveolar lavage. Reported yield of mycobacteria in bronchoalveolar lavage has been inferior to that of three gastric aspirate [12, 13]. Combining both the methods is likely to increase the yield from 17% to 34% [14]. Moreover bronchoalveolar lavage is an invasive procedure and not easily available. Mid-morning nasopharyngeal aspirate has been tried and found to yield similar results as that of gastric aspirate [15]. Sputum induction by nebulised hypertonic saline has been reported to induce a similar yield of mycobacteria as that of gastric washings [16]. Though sputum induction has been performed safely and effectively, there exists risk of nosocomial infection and skilled staff is necessary [17]. Homogenisation and decontamination is carried out in the laboratory. Liquefied specimen is then centrifuged and sediment is used for staining and microscopic examination and culture. Staining can be done with conventional Zeil-Nelson method or fluorescence. Evidence suggests that 5000–10000 bacilli per ml are necessary to be detected by staining and microscopy whereas 10–100 viable organisms are enough to yield positive culture [18]. Thus, in early stages of the disease, microscopic examination of stained specimen may be negative. Significant drawback of smear examination is the fact that they cannot differentiate *mycobacterium tuberculosis* from other mycobacteria and nocardia species.

On the other hand, culture has an advantage of differentiating *mycobacterium tuberculosis* from others and also detects drug sensitivity. However because mycobacterium.TB is a slow growing organism, conventional methods take 6–8 weeks for colonies to appear. To overcome this drawback, automated radiometric culture method is used—BACTEC. In this method, mycobacterial growth is usually detected within 8–15 days. Other methods of isolation are non-radiometric method—Septicheck and mycobacterial growth indicator tubes (MIGT).

Polymerase chain reaction (PCR)—nucleic acid amplification—allows direct identification of *mycobacterium tuberculosis* in clinical specimens. Although amplification techniques are promising tools for rapid diagnosis of tuberculosis, several caveats remain. Contamination of samples by products of previous amplification and the presence of inhibitors in the sample may lead to false-positive or false-negative results.

Sensitivity and specificity of PCR in smear positive cases exceed 95% but sensitivity of smear-negative cases varies from 40% to 70%. Thus, discordance between smear result and PCR techniques requires careful clinical appraisal and judgment. On the basis of culture results as a gold standard, sensitivity, specificity, positive predictive value and negative predictive value of PCR were 46%, 89%, 23% and 93% respectively [19]. Experienced clinician may help to optimize positive predictive value of PCR by appropriate selection of patients. High specificity of PCR may be combined with other tests that have high sensitivity, thus increasing diagnostic ability [20].

Interferon-Gamma Release Assays (IGRs)

Quantiferon-TB gold and T-spot are two blood tests recently available to aid diagnosis of tuberculosis. Quantiferon-TB gold test is an enzyme-linked immunoassay (ELISA) that basically detects presence of interferon gamma release protein (IFN-g) from blood of sensitized patients in response to stimulation with antigens such as early secreted antigenic target 6 (EAST 6) and culture filtrate protein 10 (CFP 10). These antigens are more specific than PPD used in skin test. Other advantage is the fact that no return visit is necessary as in case of TST, there is no boosting effect and it is unaffected by previous BCG vaccine and exposure to atypical mycobacteria. Test is as sensitive and more specific than tuberculin skin test. However sensitivity and specificity has not been well studied in children—especially <2 yrs of age [21]. Sensitivity of Quantiferon-gold test is slightly lower than T-spot test though it has higher specificity [22]. Studies have shown good correlation of T-spot test in culture positive patients. 90% of culture positive patients had

positive T-spot test. Remaining 10% of culture positive but T-spot negative patients were confirmed to have non-tuberculosis mycobacterial infection. However T-spot test may be negative in immunocompromised patients in spite of positive culture [23].

Serological Tests

Antibody profiling in blood or antigen detection in urine have been tried by many groups—mainly in adults. *Mycobacterium tuberculosis* has several antigens to which host would react with different antibodies. There is lack of understanding of correlation of antibody with type and stage of disease. Thus, no serodiagnostic test has adequate sensitivity and specificity and hence demonstration of one or few of the many antibodies does not help in diagnosis of tuberculosis. A review of serological tests has concluded that commercial antibody tests have no role in the diagnosis of tuberculosis [24]. Few more tests may aid in the diagnosis of localised organ involvement though they are non-specific. Histopathological examination of a biopsy specimen may reveal necrotic granuloma. CSF ADA is high in TBM. Various studies consider cut-off point between 7 and 11 IU/L but one should not consider test results in isolation [25].

While several new diagnostic tools are being developed and evaluated for TB, it is important that new tools are introduced for widespread use only after careful validation of accuracy, impact as well as cost-effectiveness in real-world settings. While there are large numbers of studies on the accuracy of TB diagnostic tests, there are few studies that are focused on cost and cost-effectiveness [26].

The evaluation of claims that a new diagnostic test is better than the current gold standard test is hindered by the lack of a perfect reference judge. However this problem may be sidestepped by focusing on the clinical consequences of the decision rather than on estimation of accuracy of the test. Consequences are best examined in cases with disagreement between current and new test [27]. Applying these criteria, no new test stands in full agreement with gold standard bacteriological test.

It is clear that except demonstration of mycobacterium tuberculosis in a specimen or tissue, there is no other test that can be fully conclusive of diagnosis. While smear and culture positivity is generally low, it is important to attempt bacteriological diagnosis in every child. With increasing attempts at bacteriological studies, few centers have developed expertise to achieve better yield. Especially in extensive tuberculosis, there is a good chance of proving the diagnosis. Gastric aspirate seems to be the method of choice though every tissue such as lymph node should also be subjected for smear and culture. Technique and process-

ing of gastric aspirate is vital for improving bacterial yield. It is also a cost-effective method that can be employed in outdoor setting.

In case of negative bacteriological results, diagnosis should rest on standard protocol as suggested by consensus statement on childhood tuberculosis of IAP working group [1]. Reasonably correct diagnosis can be achieved in majority children by following standard protocol. In case of uncertainty of diagnosis in spite of rational approach, expert opinion can be sought before trying newer diagnostic tests. As mentioned above, PCR and ELISA tests have inherent limitations for application in children. Besides, they are not freely available and may not be standardized.

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