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Short Commentary
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NAAT: A New Ray of Hope in the Early Diagnosis of EPTB

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Introduction

India is the home of world's largest tuberculosis (TB) burden, accounting for around 21% of the TB incidence globally. While pulmonary involvement is the most common presentation, tuberculosis can potentially affect any organ or system of the body.

Extrapulmonary tuberculosis (EPTB) is one of the important problems in day to day clinical practice. In India, EPTB constitutes 10-15% of total TB cases which primarily involve the pleura, lymph nodes, gastrointestinal tract, central nervous system, genitourinary system, bones/spine, and other organs with a significant case mortality rate (25 to 50%). In such situation, not only early diagnosis and treatment is very crucial but also may save many lives [1].

Extrapulmonary Tuberculosis: A Diagnostic Challenge

EPTB is notoriously difficult to diagnose due to the frequent uncharacteristic clinical presentation with vague symptoms and signs, simulating other chronic clinical conditions including neoplastic and inflammatory disorder, which often results in either a postponement or lack of treatment. Consequently, a high index of clinical suspicion is essential to clinch the diagnosis early, and frequently, multiple diagnostic procedures are required for the affirmation of the diagnosis. Moreover, the paucibacillary character of EPTB is a big hurdle in establishing the diagnosis. Existing investigations for the diagnosis of EPTB are limited in accuracy, and often require invasive procedures (e.g. lymph node or pleural biopsy) and demanding to perform in peripheral healthcare setups as these procedures require special expertise. Smear microscopy with Z N staining, most frequently used tests to diagnose TB, lacks sensitivity for EPTB. Although culture and histopathology are more precise but are either have long waiting time or not widely available in settings with limited resources like India, where the meager diagnostic infrastructure significantly worsen the crisis. In these circumstances the misdiagnosis or underdiagnosis of EPTB may lead to under or overtreatment [2].

Newer Molecular Modalities for EPTB Diagnosis

Due to many known limitations of conventional diagnostic tests; Nucleic Acid Amplification Techniques (NAAT) have emerged to enable clinicians for early recognition of M. tuberculosis from a variety of extra-pulmonary clinical samples, with very good positive predictive value (PPV) (around 99%) and comparatively lesser negative predictive value [3,4]. The advantage of this technique is very superior sensitivity, as it can pick out as low as 1-10 organisms from a clinical samples in a petite duration of 6-8 hours wherein the mycobacterium tuberculosis complex is identified by the unique target nucleic acid regions which are augmented by either the polymerase chain reaction (PCR) technique; Transcription mediated amplification (TMA), or various other methods of nucleic acid amplification. PCR methods can be based on conventional DNA amplification, nested-PCR, or real-time PCR.

However, EPTB being a pauci-bacillary disease, the sensitivity of conventional PCR is very poor and to attain a improved sensitivity than universal methods of AFB staining or culture, nested PCR or real time PCR is required [5]. Currently many of the commercial NAATs for TB are available in the market including the GenProbe Amplified M. tuberculosis Direct® (AMTD) test (GenProbe Inc., San Diego, CA, USA), BD-ProbeTec ET® test (BD Diagnostics, Sparks, MD, USA), Roche Amplicor® MTB and Cobas Amplicor® tests (Roche Molecular Diagnostics, Pleasanton, CA, USA), and Eiken Loopmediated Isothermal Amplification® (LAMP) test (Eiken Chemical Co., Tokyo, Japan). Recently a fully automated cartridge-based NAAT (CBNAAT), "the GeneXpert® System" (Cepheid, Sunnyvale, CA, USA) which uses real-time PCR to detect target DNA by amplifying it, automatically executes all the required steps starting from sample preparation to detection of TB by DNA amplification; has been developed and widely available in India. The contraption of the GeneXpert MTB/RIF automated molecular assay is being considered a milestone in TB research which is useful not only in rapid TB diagnosis but also in the detection of Rifampicin resistance, an indicator of MDR-TB.

Role of NAAT in EPTB Diagnosis: A New Era of Possibilities

Several studies have been performed to assess utility of NAATs in diagnosis of EPTB including lymphadenitis, pleuritis and meningitis. The best available current data on these tests suggests that although NAATs cannot substitute the usual tests like microscopy, culture and histopathology for EPTB, and they should be utilized and interpreted in conjunction with available clinical data and conventional tests. As NAATs have very good specificity and PPV, thus crucial in ruling in TB while relatively low sensitivity and negative predictive value in all forms of extra-pulmonary TB, thus a negative NAAT result is solely not capable to rule out possibility of TB. Thus, in a patient with a high clinical suspicion of tuberculosis with a negative NAAT result should further be investigated [3,6,7].

The sensitivity of these assays for EPTB is highly variable in different studies, ranging from 25% to 96%. 8 Samples from lymph node biopsies or fine-needle aspiration (FNA) on CBNAAT testing has pooled sensitivity around 84.9% (95% CI, 72.1–92.4%); the pooled specificity of 92.5% (95% CI, 80.3-97.4%). If fresh sample is used then a slightly higher sensitivity and a lower specificity is observed than frozen samples. On the other hand, lower sensitivities have been observed for pleural, peritoneal, cerebrospinal, pericardial, and synovial fluid samples [8,9].

While in the assessment of drug (rifampicin) resistance, the negative predictive value of Xpert MTB/ RIF24 is more than 99% both in areas

with a low or high prevalence of rifampicin resistance that means, a negative result virtually rules out the possibility of rifampicin resistance and no further investigation is needed to confirm the negative results.

The reported PPV of Xpert MTB/RIF in rifampicin susceptibility testing exceeds 90% in areas with prevalence of rifampicin resistance more than 15%. In areas with low rifampicin resistance prevalence, the PPV is low and ranges from 71% to 84% in areas with rifampicin resistance between 5-10%, and further diminishes to < 70% where the prevalence rifampicin resistance falls below 5%. Thus, the PPV can be greatly enhanced by engaging in a vigilant risk assessment for individual patients and by using targeted testing [10].

Some other NAAT have also been developed like the ligase chain reaction, TMA and Line Probe Assay and are currently being used for respiratory specimens. Their use in extra-pulmonary samples is still to be assessed.

Limitations of NAATs

Major obstacle in the implementation of PCR based NAATs as routine diagnostic test in high endemic areas is its extremely high sensitivity to contamination leading to high false positive results, thus, to make sure dependability and trim down the risk of contamination, NAAT testing should be done in laboratories with proper quality assurance systems in place. Besides, it is also incapable to distinguish between live and dead AFB, may give false positive result in patients with history of recent infection or previous treatment and should not be used in patients who are being treated currently. The efficiency of PCR based NAATs in diagnosis of TB is also affected by many other factors including - concentration of DNA material in the clinical sample, size of the target DNA sequence, choice of primers, repetitiveness of the amplified sequence, and expertise of the technician conducting the assay [11]. A key concern in commercially available NAATS in developing countries is the price and feasibility as commercial NAAT kits are expensive (up to INR 2500 to 3000 per test), but are now provided for free by RNTCP (revised national tuberculosis control program of India) to many of district tuberculosis centers.

WHO Recommendations for Use of NAATs (Gene Xpert MTB/RIF) in the Diagnosis of EPTB and Detection of Rifampicin Resistance

In both adults and pediatric patients, Xpert MTB /RIF should be preferred to microscopy and AFB culture as the initial confirmatory diagnostic test for CSF specimens from patients with TB meningitis (strong recommendation due to the urgency of rapid diagnosis, very low-quality evidence). While Xpert MTB/RIF may be used in place of usual diagnostic tests like microscopy, culture or histopathology for testing other specific non-respiratory specimens (like lymph nodes and other tissues) from patients having extrapulmonary TB (conditional recommendation, very low-quality evidence).

Furthermore, Individuals with suspected EPTB but with a single negative result from previous Xpert MTB/RIF should undergo other diagnostic testing for confirmation of EPTB, and in patients with a high clinical suspicion for TB (especially in children), should straightforward be treated even if an Xpert MTB/RIF result is negative or if the test is not available.

A pleural biopsy specimen is the preferred sample for the mycobacterial confirmation of pleural TB as pleural fluid is considered an inadequate sample regardless of the method used for NAATs. The reported sensitivity of Xpert MTB/RIF in pleural fluid samples is very low. Nonetheless, any individual, if found positive by Xpert MTB/RIF test from pleural fluid, should be treated considering a case of pleural TB and those with a negative result from Xpert MTB/RIF should undergo further tests. Microscopy and culture are essential tests not only for monitoring of the therapy but also for performing drug sensitivity for anti-TB agents other than rifampicin.

These recommendations are not applicable to samples of stool, urine or blood, due to lack of robust data on the effectiveness of Xpert MTB/RIF for these specimens [12].

Conclusion

Cartridge-based NAATs are very crucial in ruling in but suboptimal in ruling out, EPTB. As compared to conventional tests for diagnosis of EPTB, CBNAAT is not only highly sensitive and specific, helpful in rapid diagnosis which has a great impact on patients' outcome but simultaneously detects rifampicin (RIF) resistance.

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