Pathology Section

Laboratory Diagnosis of Extra-pulmonary Tuberculosis (EPTB) in Resource-constrained Setting: State of the Art, Challenges and the Need

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ABSTRACT

During the last decade, remarkable progress has been made in the diagnostics of pulmonary tuberculosis; however, diagnostic challenges in extra-pulmonary tuberculosis (EPTB) remain to be addressed. Diagnosis of EPTB is difficult due to the pauci-bacillary nature of disease, the variable clinical presentation, and need for invasive procedures to secure appropriate sample, and lack of laboratory facilities in the resource-limited settings. A more accurate test to diagnose various forms of EPTB, which can easily be incorporated in the routine TB control programme, would contribute significantly towards improving EPTB case-detection and thus reducing the morbidity and mortality. In this overview, we describe the status of current conventional and newer methods available for laboratory diagnosis of EPTB and discuss the challenges in their implementation in the resource-limited settings, and suggestion for better EPTB diagnostic algorithms, which can be incorporated in the routine TB control programmes.

BACKGROUND

TB has been a major global public health problem from times immemorial. World Health Organization (WHO) estimates shows that globally there are 8.6 million incident cases of TB of which 80% are in 22 countries, with India ranked as the highest burden country [1]. EPTB constitutes about 15 to 20 % of all cases of TB. The annual global incidence of EPTB has been increasing in the last decade due to the changing TB control practices, spread of HIV (human immunodeficiency virus), the population growth and the cure of infectious cases of TB might have resulted in a relative rise of annual EPTB case detection. HIV pandemic further complicates the situation, as EPTB constitutes more than 50 % of all cases of TB in HIV-positive patients [2]. EPTB usually show atypical presentations in HIV infected person.

The diagnosis of EPTB poses a particular challenge for clinician because of the protean ways in which the disease presents. Diagnosis of this condition requires high clinical suspicion and special diagnostic procedures. EPTB diagnosis may not be difficult in a few cases with concomitant pulmonary involvement. However, patients with EPTB are more likely to have negative sputum smear results and many EPTB cases do not have direct lung involvement. EPTB diagnosis is much more lacking in rural health facilities that are used by about 70% of the population in developing countries [3]. Here, the diagnosis largely depends on unreliable methods such as ZN microscopy, histology, solid culture, and tuberculin test. Various National Tuberculosis Control Programmes thus have adopted clinical guidelines for diagnosis of EPTB, which alone can lead to over-diagnosis and treatment. Thus, clinical diagnosis may lead to diagnostic delays, misdiagnosis, resistant strains and increased mortality. Technical progress in diagnostics, however, has resulted in a number of improved tools; including some appropriate for low-income settings but the focus of improved tool is mainly pulmonary TB. Further, important work remains to integrate new diagnostic tools in control programs of high-burden countries to diagnose EPTB. In this overview, we describe 1) The status of available laboratory methods in resource-limited settings for diagnosis of EPTB, 2) the challenges of EPTB laboratory diagnosis and 3) the need for improvement of laboratory diagnosis of EPTB specifically in the TB endemic settings.

1. Current laboratory methods for diagnosis of EPTB

Accurate diagnosis of EPTB depends on the detection of mycobacteria by using (i) direct and (ii) indirect approaches. By the direct methods, the mycobacterium and its products are detected or demonstrated. These methods have low sensitivity due to the pauci-bacillary nature of majority of EPTB. The indirect evidence to diagnose EPTB depends on the measurement of the host's humoral and cellular response against mycobacterium as reviewed below. [Table/Fig-1] summarizes the currently used direct and indirect laboratory tests for the diagnosis of EPTB.

1.1 DIRECT METHODS

Tissue and aspirate Microscopy and Staining

Ziehl-Neelsen (ZN) stain helps to detect acid-fast bacilli (AFB) in tissues and smears. It is simple, cheap, and quick. Positive ZN test requires more than 10^6 bacteria/g tissue, so of limited diagnostic value 0-40% [4,5] in majority of pauci-bacillary EPTB samples. As for pulmonary TB the centrifugation of EPTB samples and fluorochrome staining with ultraviolet microscopy increases the sensitivity of microscopy by 10% of ZN staining [6]. Although limited data is available for its use in EPTB samples and for HIV-TB co-infected patients, evidence suggests that fluorescence microscopy may be promising in this population too. It, however, requires considerable technical expertise and is expensive. WHO evaluation showed that the diagnostic accuracy of light - emitting diode (LED) microscopy is comparable to that of conventional fluorescence microscopy with much less expense. Furthermore, neither the etiological diagnosis of *M. tuberculosis* nor the drug- resistance can be revealed with ZN microscopy.

Culture Methods

Isolation of *M. tuberculosis* from clinical samples by culture is the "gold standard" for a definitive diagnosis of EPTB. Culture methods are much more sensitive because fewer bacilli (10-100 bacilli/ml

Diagnostic Method	Sensitivity/ specificity	Advantages	limitations
Direct Methods			
Ziehl-Neelsen stain	Very low/ high	Simple, cheap, rapid, grading of disease	No species, viable bacteria & drug susceptibility detection
Fluorescent microscopy	Low/high	Slightly better sensitivity than Ziehl-Neelsen stain	Expensive &complex, no species, viable bacteria & drug susceptibility detection
Lowenstein-Jensen culture	Low/very high	Gold standard, species, viable bacteria & drug susceptibility detection	Complex, expensive, time-consuming, low sensitivity, need bio-safety facilities
BACTEC culture system	High/very high	rapid, automated, detect species, active disease, drug susceptibility	Expensive, radio-active hazard, technically demanding
Polymerase chain reaction	High/low	Rapid, mycobacteria species	Expensive, complex, high false-positive, cannot differentiate viable & dead bacteria
Xpert MTB/RIF*	High/high	rapid, automated, drug susceptibility	Low sensitivity in culture-negative EPTB†, expensive, cannot identify viable bacteria
Antigen based Serology	Low/low	Simple, quick	Low sensitivity and specificity
IHC & ICC‡	High/high	Simple, quick, robust, identify mycobacterial species	Cannot identify viable bacteria, drug sensitivity is not possible
Indirect Methods			
Histopathology	High/low	Differential diagnosis	Invasive, atypical lesion in HIV§ , required surgical facility, sinus tract & fistula formation
Cytology	High/low	simple, less expensive, rapid, outpatient diagnostic procedure	cannot distinguish TB‖ from other granulomas, atypical lesions in HIV & non tuberculous mycobacteria
Antibody based serology	Low/very low	Simple, less expensive	negative recommendation by WHO** in endemic countries
Skin tests	high/very low	Simple, less expensive, robust	High false-positive &-negative, cannot differentiate latent & active TB
Interferon- release assay	High/high	Assure latent tuberculosis in low endemic areas	No differentiation of latent & active disease Not of use in endemic countries (WHO)
Adenosine deaminase assay	High/low	Simple, less expensive, rapid	Specificity need evaluation

[Table/Fig-1]: Advantages and limitations of the currently available laboratory methods for diagnosis of extra-pulmonary tuberculosis in resource-constrained settings. *mycobacterium tuberculosis/Rifampicin, † extra-pulmonary tuberculosis, ‡ immunohistochemistry and immunocytochemistry, § human immunodeficiency virus, ‖ tuberculosis, ** world health organization

of concentrated material) can be detected [7] and provides the necessary isolates for conventional drug susceptibility test, and species identification. Most of the extra-pulmonary specimens need decontamination procedure that may be harmful to mycobacteria, thus culture methods are also not 100% sensitive. Culture with traditional methods (solid egg-based or agar-based media) are time-consuming (2–6 wk), complex, need skilled laboratory technicians and appropriate bio-safety conditions. Various modifications and newer, more rapid culture methods overcome some of these problems for EPTB samples. Liquid culture, as BACTEC System, increases the case yield by 10% over solid media. However, its high cost and the need for safe disposal of the radioactive waste precludes its use in peripheral laboratories. The non-radiometric technology as Mycobacteria growth indicator tube 960, continuously monitor bacterial growth using fluorescence. It is useful for early detection of mycobacterial growth, and drug sensitivity testing. The Septicheck AFB method is used for simultaneous detection of *M. tuberculosis*, atypical mycobacteria, and other respiratory pathogens. Other non-radiometric methods that differ little in their specifications are the ESP culture system II, MB/Bact T system, TK medium. However, the high technical expertise, and the cost of instrument precludes their use in peripheral laboratories in TB endemic countries. Similarly, microscopic observation of broth culture is a rapid detection method, relatively inexpensive to be suitable for endemic countries. The culture result needs to be tested for species identification to provide a definitive identification of *M. tuberculosis*. Rapid immunochromatographic assays (so-called strip speciation tests), molecular tests, and biochemical methods are recommended to identify species in short span of time. All liquid culture systems are also prone to contamination and require P2 bio-safety facilities. These systems should be used in combination with a solid medium and not as a stand-alone method.

Molecular Methods
In the past decade, Nucleic Acid Amplification techniques (NAAT) have led to the development of tests with high positive predictive value (98–99%) and relatively lower negative predictive value for

early detection of *M. tuberculosis* from various extra-pulmonary clinical specimens [8-15]. The advantages of amplification technique over culture are higher sensitivity, as it can detect as few as 1-10 organisms in clinical specimens at least in research conditions. These tests can be performed on the stored samples, and provide etiological diagnosis in short span of 6-8 h.

It is a diverse group of tests wherein *Mycobacterium* is amplified by either the polymerase chain reaction (PCR) technique; Transcription mediated amplification (TMA), or other forms of nucleic acid amplification methods. PCR techniques can be based on conventional DNA amplification, nested-PCR, or real-time PCR. PCR assays targeted for various genes (65 kDa, 38 kDa) or insertion sequences, (e.g. insertion element IS-6110 carried in multiple copies by most strains belonging to *M. tuberculosis*) are in routine use [5]. It is a suitable method for sensitive and fast detection of *M. tuberculosis* complex DNA in histological material including formalin-fixed tissues and dried scraped material [4,5,16]. This has major implications both in TB endemic and non-endemic areas especially when culture is negative or a fresh specimen is not available for further investigation and thus avoids over treatment and more invasive procedures. Ethanol-fixed aspirate smears can be stored at room temperature, and can easily be transported if molecular facilities are not available at the local health centre. However, the sensitivity of conventional PCR is low in pauci-bacillary EPTB and nested PCR or real time PCR is required to achieve a better sensitivity than AFB staining or culture [4,5,16].

Major hindrance in the operationalization of PCR as routine diagnostic test in high endemic settings is its extreme sensitivity to contamination generating high false positive results. Furthermore, it is unable to differentiate between viable and dead AFB, no drug susceptibility information can be available, false positive in patients with recent history of infection or adequate treatment. The effectiveness of PCR in diagnosing TB is also related to many demanding factors including - DNA concentration in the clinical sample, size of the target DNA sequence, repetitiveness of the amplified sequence, choice of primers, and expertise of the personnel conducting the

assay. A quality control study of seven laboratories worldwide found that false positives ranged from 00 to 77 % [17]. The American Thoracic Society has recommended the use of PCR as additional evidence in smear-positive clinical specimens, while in smear-negative cases it should be interpreted cautiously [18]. In 2007, NHS Health Technology Assessment Programme concluded that NAAT test accuracy is superior for respiratory samples as opposed to other specimens [12]. Currently no recommendations are made for use of PCR in the diagnosis of EPTB.

The development of the Xpert MTB/RIF automated molecular assay (Cepheid, CA, USA) for rapid diagnosis of TB and detection of Rifampicin resistance, a marker of MDR-TB, is a landmark event in TB research. The assay was developed, optimized, assessed, and endorsed specifically for the detection of pulmonary TB using sputum. More recently, however, assessments of the assay have extended to various extra-pulmonary clinical samples. The reported sensitivity of the assay for EPTB are highly heterogeneous, ranging from 25% to 96·6%, but exceeded 50% in all but one study [19]. However, lower sensitivities are reported for cerebrospinal, pleural, pericardial, peritoneal, and synovial fluid samples [20]. High costs, and lower sensitivity for smear-negative than for smear-positive EPTB samples limits its use in the diagnosis of EPTB in coming years [21].

Many other NAAT have been developed including the ligase chain reaction, TMA and Line Probe Assay. These systems are currently used for respiratory specimens to simultaneously detect mixed myco-bacterial and contaminated cultures. The use of these methods for extra-pulmonary specimens is still to be evaluated. Another molecular amplification method is the phage-based assay. A genetically engineered mycobacteriophage has been used for detecting viable *M. tuberculosis* directly in clinical specimens. The Phage-Tek MB assay is an inexpensive test, but has low sensitivity and specificity. Also in the developing countries, it is necessary to evaluate the availability of suitable infrastructure and trained personnel before adopting such sophisticated diagnostic techniques and for EPTB cases.

ANTIGEN DETECTION TESTS

Antigen-based Serological Tests
Free mycobacterial antigens may be detected in various body fluids at a minimum concentration of 3-20 ng/mL [22]. The commonly detected antigens from EPTB specimens are BCG, non-protein cell wall antigen, antigen 5, 14kDa, antigen A60, 45/47 kDa antigen, lipoarabinomannan (LAM), haemoglycolipid-lipid antigen and cord factor (trehalose-6, 6_dimycolate). However, none of these tests has shown a better sensitivity and specificity in pauci-bacillary EPTB which pose the main diagnostic challenge. An antigen assay based on the detection of mycobacterial LAM in urine has gained considerable attention as a point-of-care test. This is however, limited to the diagnosis of HIV-associated pulmonary TB, and is not applicable for pauci-bacillary EPTB. Recently a systematic review and meta-analysis of the antigen detection tests for the diagnosis of TB has concluded that more studies are needed to develop better antigen detection tests.

Immunohistochemistry (IHC) and immunocytochemistry (ICC) based antigen detection tests
In recent years, several reports have described immunostaining techniques as an alternative to conventional acid-fast staining [4,16]. The improvement in sensitivity may partly be attributed to the additional detection of degraded bacilli, unlike the ZN staining that requires intact bacilli. Different mycobacterial antigens have been detected in formalin fixed paraffin embedded tissue, tissue aspirate and body fluids e.g. BCG, MPT64, *M. tuberculosis* antigen 85, Antigen 5 (38kDa), LAM, ESAT6, HspX, Tb8.4, and the phospholipase C encoding A (PlcA) protein. Studies have shown that immunostaining can be applied to a wide range of extra-pulmonary

clinical specimens with a significantly high sensitivity (70-100%) and specificity (65-100%). It performs equally well in HIV co-infected TB cases with atypical histological features. Being robust, result available within one working day and insensitive to contamination immunostaining is suitable for high TB endemic settings. The main disadvantage of IHC/ICC is that invasive tissue samples collection and sample preparation is a pre-requisite.

1.2 INDIRECT METHODS

Histopathology
Histopathology is considered as the method of choice for the diagnosis of EPTB. Excision biopsy with routine histological analysis, ZN staining, and culture for *M. tuberculosis* plays a central role in the diagnosis. In endemic countries, identification of granulomatous inflammation with or without caseation and Langhan's giant cells on histology are considered and treated as TB. While *M. tuberculosis* is the most common causative agent of EPTB, the prevalence of non-tuberculous mycobacteria is increasing with or without HIV infection and ranges from 4% to 50% in different parts of world. Non-tuberculous mycobacterial lesions have similar histological features as tuberculous mycobacteria. As treatment is different for the two conditions, it is important to make a definitive diagnosis. The feasibility and acceptability of biopsy is limited for non-approachable sites of EPTB. The lack of biopsy facilities in peripheral health-care centers and its invasive nature limits its use in all cases of EPTB. Incision biopsy is also associated with sinus tract and fistula formation at times and therefore is contraindicated for the diagnosis of accessible lesions of EPTB. Presently, histopathology is reserved only for patients with high clinical suspicion and negative fine needle aspiration cytology (FNAC), which is performed as the first line investigation in nearly all accessible mass lesions.

Cytology
FNAC of mass lesions is a simple, less expensive, rapid, out-patient diagnostic procedure with high sensitivity and specificity for the diagnosis of EPTB [23]. FNAC is recommended as the initial diagnostic test in a TB suspected accessible mass lesion as the cytology criterion of TB has been well established. However, it is often difficult to distinguish tuberculous lesions from other granulomatous conditions, non-tuberculous mycobacteria and atypical lesions in advanced HIV disease on cytology. Similarly, doubtful cases on routine examination of body fluid arise because of poor specificity of cytology and biochemical markers, which leads to a difficult differentiation of lymphocyte-predominant "tuberculosis" cytology pattern from non-tuberculous lesions. The explicit diagnosis of TB on aspirated material and body fluids is based only on bacteriological confirmation, which can only be reached in 20-25% cases.

SEROLOGICAL TESTS

Antibody-based Serological Tests
Several serological tests based on the detection of antibody in various clinical specimens have been developed. These tests are well suited for resource-constrained settings as they are simple, inexpensive and ideal for point-of-care diagnosis. These tests can be used as supportive evidence along with conventional tests for diagnosis of EPTB at inaccessible body sites. Superoxide dismutase, a secretory protein of *M. tuberculosis*, has been evaluated for sero-diagnosis using an ELISA test. In low prevalence countries, this test has a good positive predictive value of 93-94%, but falls to 75% in high prevalent population [24]. The positive test may perhaps help in "rule in" the diagnosis, but a negative test cannot "rule out" a diagnosis of TB. As the currently available methods for purifying mycobacterium antigens are not reproducible, the results of antibody detection assays vary in different settings. Additional complexities include a delayed immune response due to delayed-type hypersensitivity in BCG vaccination, the persistence of an antibody even after the

sub-clinical or clinical disease subsides, and results affected by exposure of environmental non-tuberculous mycobacterium and in HIV co-infection. New purified antigens or monoclonal antibodies may provide better sensitivity and specificity but thus far, the various serological techniques have shown poor reproducibility and lack of specificity. WHO has issued negative recommendations about the use of sero-diagnostic tests for diagnosis of TB in low- and middle-income countries [25,26].

Cellular Immunity Mediated Test

Skin tests, widely used in screening for TB infection, are the prototype of cellular immunity mediated test. These measure the delayed type hypersensitivity response between 48-72 h after the intra-dermal introduction of tuberculin antigen shared by BCG or non-tuberculous mycobacterium. A positive tuberculin test may suggest active TB, past infection, BCG vaccination, or sensitization by environmental mycobacteria. A negative result may not necessarily exclude TB as false negatives can be seen in immunosuppressant conditions. Recently, various recombinant antigens as ESAT-6, CFP-10 and MPB 64 have been developed in order to improve the sensitivity and specificity of tuberculin tests. With MPB 64 patch test helps to distinguish environmental mycobacterium from *M. tuberculosis* [27]. Further studies are required, however, to show its potential relevance for the diagnosis of EPTB. In TB endemic zone, tuberculin test alone is not sufficient evidence to diagnose EPTB in adult patients.

Interferon-γ-release Assays

In the interferon-γ- release assays, mononuclear cells from peripheral blood are stimulated *in- vitro* using specific mycoacterial antigens such as ESAT-6 and CFP-10 and the production of interferon-γ is measured. This assay is commercially available as QuantiFERON-TB Gold (Cellestis, Australia) (QFT-TB) and T-SPOT TM.TB assay (Oxford Immunnotec, UK). These tests are used for the diagnosis of latent TB in many developed countries. The T-SPOTTM.TB assay is therefore more difficult to establish in a routine diagnostic laboratory whereas QFT-TB may be easily adapted for routine diagnosis. Pai and Menzies [28] have shown a pooled sensitivity of 75% for QFT-TB in patients with active TB including tuberculous pleuritis and the test offers a high specificity of 94% when tested against patients with non-tuberculous mycobacteria infection. The results are not confounded by BCG vaccination and therefore more specific than the TST. Emerging evidence indicates that enzyme linked immunospot assay is robust to hematologic malignancy-associated immunosuppression and some types of iatrogenic immunosuppression, including corticosteroids and azathioprine. The test also performs well in HIV co-infection, malnutrition, young children with either latent or active TB.

However, these assays cannot discriminate between latent infection and active disease, thereby limiting their use in high endemic countries. Simultaneous measurement of IL-2 and IFN-γ secretion by *M. tuberculosis*-specific T-cells correlates well with treatment so a next-generation T-cell based tests measuring dual cytokines may be promising in providing more clinical information. Negative recommendations were issued by WHO about the use of interferon-γ-release assays for diagnosis of TB or latent *M. tuberculosis* infection in low-income and middle-income countries. Furthermore, very few studies have evaluated the performance of these assays in EPTB [29,30]. There is also concern about the high rate of inconclusive results in immune-compromised individuals, including HIV positive patients, due to T-cell anergy.

Adenosine Deaminase Activity (ADA)

ADA is an enzyme involved in the conversion of adenosine to inosine. The cell differentiation of the immune system in humans, due to the interaction of the mycobacterium with the host factors, is the source of ADA activity. There are two iso-enzymes of ADA; ADA-1 and ADA-2. ADA-1 is produced by most cells, whereas ADA-2 is produced mainly by the monocytes and macrophages. ADA appears to be a useful test for early TB diagnosis in endemic and poor settings. A systematic review of ADA by the NHS Health Technology Assessment Program showed limited use of ADA tests for the diagnosis of pulmonary TB [12]. There is considerable evidence, however, to support its use for EPTB diagnosis with high sensitivity. Determination of the individual ADA iso-enzymes and ratio of the iso-enzymes could help in differentiating the various causes of increased ADA activity in body fluids especially in borderline ADA levels. ADA-2 is elevated in TB as compared to other infectious or malignant causes. The use of ADA iso-enzymes in diagnosis of EPTB should be further evaluated in large series.

Other Methods

Tuberculostearic acid, a component of *M. tuberculosis*, can be detected even in infinitesimal (femtomole) quantities by gas-liquid chromatography and is of use in diagnosis of EPTB [31]. However, the presence of organisms other than *M. tuberculosis* may lead to false positive results thus limiting its efficacy. Chromatographic methods appear promising therefore, but because of demanding infrastructure may not be widely available in the developing countries.

2. CHALLENGES IN THE LABORATORY DIAGNOSIS OF EPTB

Methodological Issues

The laboratory diagnosis of EPTB has always been a challenge. A high index of clinical suspicion is required for laboratory evaluation as the disease presents in various ways. Because of the involvement of obscure inaccessible sites, invasive procedures may have to be employed to obtain adequate sample amounts or volumes of body fluids/tissue for analysis. The feasibility and acceptability of biopsy is limited for non-approachable sites of EPTB. The pauci-bacillary nature of disease and non-uniform distribution of microorganisms may lead to false negative results for most of the laboratory procedures. Excision/aspiration biopsy of tissue with routine histological/cytological analysis showing granulomatous inflammation plays a central role in the diagnosis with its limitation of wide differential diagnoses. Histological features may be atypical at various extra-pulmonary sites and with concomitant immunosuppression. Thus, the explicit diagnosis of TB with direct evidences can only be reached in 20-25% cases [4,5]. In resource poor settings, however, indirect evidences are then the mainstay for the diagnosis. Diagnosing EPTB may be easy in those cases with concomitant pulmonary involvement. Patients with EPTB are, however, more likely to have negative sputum smear results and many EPTB cases do not have direct lung involvement. At present, histology, ZN staining and culture remain the most widely used tests in TB endemic countries.

Policy Issues

National TB control program in endemic settings does not directly include active diagnosis of EPTB as oppose to pulmonary TB. Thus, as only 5-70% of EPTB cases are associated pulmonary TB [32], lot many cases can easily be missed using the laboratory procedure applicable for diagnosis of pulmonary TB. The diagnosis of EPTB requires a complete work up of patient, invasive procedure with specialized equipment, highly trained experts, complete bio-safety measures, and a proper infrastructure. Unfortunately, the laboratory infrastructure in resource-constrained settings is still relatively weak and very few centers have all these facilities. The bacteriological confirmation of cases relies only on smear microscopy. Facilities to perform culture and drug sensitivity testing are centralised at the National Reference Laboratory. Approximately 70-80% of patients are diagnosed in private sector at the first symptom with a very high prohibitive cost [33]. The situation leads to avoidance for the early work up and considerable delay in starting the treatment. The

operational research to develop affordable diagnostic resources, which is available at all the centers in poor countries, is lacking. A limited generation of market profit in poor countries is also a hindrance for manufacturing agencies to develop new diagnostic tests for diagnosing EPTB cases.

Diagnostic Issues in HIV-EPTB Co-infection

Properly characterized symptoms together with a complete medical history prompt a primary health-care worker to refer the TB patient to a specialized TB centre, however, as EPTB presents in protean ways and more so, in HIV co-infected patients, it is a great challenge for the health-care providers to suspect these cases promptly. HIV can lead to anergy and thus false negative tuberculin skin test, which is available at primary and district health care level, further complicates the situation. Not only conventional tests such as microscopy is highly insensitive in HIV patients, but also newer advanced tests as PCR-based assays for example Line probe assay and gene X-pert MTB showed a reduced sensitivity in smear negative cases. The development of a diagnostic indicator that can be of use in smear-negative HIV-infected individuals is restricted because of the incomplete knowledge of the biology of EPTB disease itself and its interaction with HIV. Further, there is poor synergy between national HIV/AIDS and TB programmes. Above all the EPTB diagnosis faces challenges, not only related to pauci-bacillary nature of disease and co-infection with HIV, but also in terms of accessibility to health services and health systems responsiveness to the patient's needs.

3. Need of the Time in Resource-constrained Settings

The rapid and accurate diagnosis of all forms of TB is the cornerstone of the global TB control. However, it has been acknowledged that the TB laboratory capacity available worldwide is insufficient to address the diagnostic challenges related to EPTB, HIV- associated TB and drug- resistant TB. In endemic countries, WHO and National TB Control Program are giving priority to expand laboratory network and strengthen laboratory services. WHO has acknowledged the need to facilitate the rapid intake of evidence-based new diagnostic approaches into routine practice. The Foundation for Innovative New Diagnostics (FIND) has also motivated the development and evaluation of new diagnostic tools for resource-limited settings. The pipeline of new diagnostic tools has increased fast in the last decade. There is a high-priority for determining the feasibility of using low-cost, rapid, easy to interpret, and with minimum infrastructure non-commercial diagnostic methods for detection of pauci-bacillary and multidrug-resistant TB cases in resource constrained settings. To fulfil the purpose, the Global Laboratory Initiative (GLI) has been created [25]. The GLI aimed to expand access to quality assured TB diagnostic services within integrated laboratory systems by accelerating laboratory strengthening, helping development of laboratory norms and standards, and training and retention strategies of human resources.

New diagnostic methods and algorithm have the potential to improve the laboratory-based diagnosis of EPTB. Various assays for example immunochemistry assay, [4,5,16] have been validated in the research settings with reproducible results and warrants its operationalization as a routine diagnostic test. The availability of new guideline and tools such as Standards for Reporting of Diagnostics Accuracy (STARD) initiative and the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) can facilitate the implementation of such tests. There is limited evidence, however, on the effectiveness and the impact of new diagnostics after programmatic implementation. Recently, an Impact Assessment Framework (IAF), an instrument that aims to summarize the evidence not only in terms of test´s accuracy but also taking into account its overall impact has been developed. As a consequence of IAF in TB diagnostics, there is a need of evidences demonstrating the impact of new diagnostic-intervention in patient-important outcomes and their performance in routine use. Thus operational and implementation researches are needed to confirm diagnostic method's performance characteristics in diverse settings in order to determine the optimal indications and algorithms for its use to detect EPTB cases in resource-limited countries. This approach is relatively new in the TB research field and might be particularly useful to assess the routine applicability of innovative diagnostic test. The main goal is to increase EPTB case detection through new and improved diagnostics at point-of-care level.

Operational research for EPTB case finding must also go beyond efficacy evaluations to include effectiveness studies that should focus on alternatives, which can be applied and sustained in resource constrained TB endemic settings. The studies should aim to develop, validate and evaluate the effectiveness of the context-relevant algorithms comprising of combinations of selected existing and new diagnostics for detection and characterisation of the various forms of EPTB in various populations (e.g. in children and HIV) and at all health-care levels. It is important to assess the baseline laboratory preparedness and identify perceived needs, barriers, and facilitators for adoption of new TB diagnostic tools. Additionally, there is also a need to ascertain the factors affecting the acceptability and implementation of these new interventions.

An inventory of currently available diagnostics, infrastructure including physical condition and human resources available should be assessed. The laboratory-based evaluation of a method in terms of technical feasibility and accuracy for screening and diagnostic algorithms in different epidemiological settings, the cost-effectiveness and the cost-benefit in different programmatic settings should be assessed. The assessment should also take into account the quality assurance protocols being used (or needed) and the impact of the proposed TB diagnostic algorithms on outcomes relevant to the patients, the health care providers and the health care systems in resource limited settings. Newer diagnostic methods such as automated NAAT are potentially cost saving from both patient as well as health system perspective owing to fewer physician consultations and investigations and reduced use of antibiotics and visit-cost. Such methods must be decentralized to scale up its use in endemic settings.

CONCLUSION

Diagnosis of EPTB poses a major challenge to health care facilities and the research community in resource-constrained settings. The more sophisticated molecular tools for diagnosis and drug susceptibility testing are not widely available as in developed countries. Ideally, a diagnostic test for EPTB should be accurate, fast, easy-to-implement, sustainable and affordable. Acknowledging the need to facilitate a timely transition from the research laboratories to the programmatic level implementation, WHO has speeded-up the intake of new technologies that are evidence based. The evidence is provided by diagnostic accuracy evaluations, where accuracy performance has been used as a surrogate of patient-important outcomes.

In addition, operational research is needed for EPTB diagnostic methods that will provide valuable information to improve the health care services. More evidence is required to assess the operational impact of non-commercial, low-cost, rapid diagnostic methods in field conditions. In addition, prior to implementation of any new diagnostic tool or algorithm in a resource-constrained setting it is necessary to assess – 1) the impact of such implementation in terms of infrastructure, human resources, sample referral system and financial investment required, 2) the level of laboratory preparedness and perceived needs, barriers and facilitators for introduction of new diagnostic tools, 3) the preparation, implementation and performance of combinations of conventional and new diagnostic techniques for the rapid detection of EPTB cases, and 4) the impact on outcomes relevant to the patients, the health care providers and the health care systems.

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