ISSN 2347-5579

Unique Journal of Medical and Dental Sciences

Available online: www.ujconline.net

Review Article

PATHWAYS TO BETTER DIAGNOSTICS FOR TUBERCULOSIS: A GLOBAL PRIORITY IN RESOURCE LIMITED HIGH BURDEN SETTINGS

Mane Abhay B[1*], Mahajan Akshay[2], (Air Cmde) Kevin Fernandez VSM[3]

[1]Professor, Department of Community Medicine, Smt. Kashibai Navale Medical College, Pune, Maharashtra, India-411041
[2]Post graduate student, Department of Community Medicine, Smt. Kashibai Navale Medical College, Pune, Maharashtra, India-411041
[3]Professor & HOD, Department of Community Medicine, Smt. Kashibai Navale Medical College, Pune, Maharashtra, India-411041

Received: 22-02-2014; Revised: 20-03-2014; Accepted: 17-04-2014

*Corresponding Author: **Dr. Mane Abhay B**
Department of Community Medicine, Smt. Kashibai Navale Medical College, Narhe, Pune, Maharashtra, India
Phone number: 8975008663 E-mail: drabmane@yahoo.co.in

ABSTRACT

The global burden of tuberculosis (TB) remains enormous due to lack of rapid and accurate diagnosis and case detection. It is also evident that improved diagnostic procedures will avert more TB cases. For the diagnosis of active TB, smear microscopy is a simple, low-cost technique, which can be implemented in health facilities with minimum infrastructure in resource-limited settings. However, smear microscopy has several limitations. The Current diagnostic techniques are either inadequate to detect TB cases with precision, or are time consuming, expensive, and require highly equipped laboratories which are not available in developing countries, where the disease is endemic. Lack of proper diagnosis costs patients and their families valuable time and money, delays treatment and leads to continued TB transmission. Hence it is a critical time to expand access to better diagnostic tests for control of the TB pandemic as part of a global effort. Recently many new techniques are available for the diagnosis of TB and also for the detection and identification of M. tuberculosis. However, because of their high costs, requirement of instruments and trained manpower, use of such techniques in the near future will be very limited in disease-endemic and resource limited countries such as India. This review describes some of those promising new technologies that may be implemented in resource limited settings.

Keywords: Smear microscopy; Sensitivity; Specificity; New diagnostic tools; Tuberculosis.

INTRODUCTION

Tuberculosis (TB) is curable, but current efforts to find, treat and cure everyone who gets ill with the disease are not sufficient. Of the 9 million people a year who get sick with TB, a third of them are "missed" by health systems. To reach the three million we need to aggressively scale up TB programmes and ensure access and coverage for all, especially for the most vulnerable groups in high burden countries[1]. Globally case detection rate have increased from 42% in 2000 to 66% in 2012. Of the 8.6 million (range, 8.3–9 million) incident cases of TB estimated to have occurred in 2012, only 5.7 million (66%, range 64–69%) were both detected and notified to national TB programmes (NTPs) or national surveillance systems[2]. This leaves a gap of about 3 million people with TB who were “missed”, either because they were not diagnosed or because they were diagnosed but not reported. Finding the “missed” cases is one of the biggest challenges in TB care and control today. Access to TB diagnosis needs to be urgently improved in countries where a large share of the “missed” cases is likely due to the lack of diagnosis rather than under-reporting. Hence the slogan for World Tuberculosis Day in 2014 is "Reach the 3 million". A single TB patient can infect 10 or more people in a year, if left untreated. In light of the problem of drug resistance, developing new diagnostic tools is essential.

India is the second most populous country in the World; it tops the world in Number of Tuberculosis (TB) cases occurring annually. As per WHO estimates, 2.3 million new TB cases occur annually in India, out of the Total 9 million cases. India accounted for 31% of the total cases missed globally[3]. Annually, Approximately 3.2 lakh deaths occur in India due to Tuberculosis or its Complications, i.e. 26 per 1, 00,000 population[4]. In India, 3 deaths occur due to TB every 5 minutes. But these deaths can be prevented with proper diagnosis and treatment, TB patients can be cured and the war against TB can be won. Undoubtedly, to achieve the Revised National TB Control Programme (RNTCP) Objectives, the most important part is the early diagnosis of TB by quality sputum smear microscopy and proper treatment. This paper highlights some of the recent advances in TB diagnostic

technologies and puts them into perspective for tuberculosis control in endemic and low resource settings.

Choosing a TB diagnostic Test:

An ideal diagnostic test should be highly sensitive & highly specific. Factors in selecting which test to use include the reason for testing, test availability, and cost. Deficiencies in current case-finding tools in disease endemic countries have made it difficult to ensure access to good diagnostics at all health service levels, leaving many patients undiagnosed. The various tools for the diagnosis of Pulmonary TB are:

1. Sputum Smear Microscopy
2. Chest X ray
3. PCR testing
4. Sputum Culture & DST for diagnosing MDR TB
   - Culture (L J) & DST (First line drugs-SIRE)
   - Liquid culture media (BACTEC MGIT 960)
5. Rapid Diagnostic Molecular Tests For detection of MDR TB
   - LPA-(Line probe assays) Molecular method for MTB complex
   - CBNAAT(Cartridge Based Nucleic Acid Amplification Test)–GeneXpert , for MTB complex

The tools for the diagnosis of Extra-pulmonary TB vary according to the symptoms and Systems involved that can be summarized as-

- FNAC & Direct Microscopy of Aspirate
- Biopsy/Excision Biopsy for HPE
- Fluid cytology/biochemistry/smear
- X-ray of the affected body part
- Ultrasonography
- Culture for M. Tuberculosis
- Animal Inoculation Studies

1. Sputum Smear Microscopy: It is still gold standard for TB diagnosis. It is most widely used and acceptable testing tool, for Smear Positive Pulmonary TB. It is Simple, inexpensive and requires minimal training. It has high specificity, high reliability with low inter-personal variation. It can be used for diagnosis, monitoring of treatment and to see the treatment outcome. It is the cheapest tool for TB diagnosis[5,6]. Fluorescent staining and examination under direct or indirect microscopy with or without LED lamps can also be used for smear examination. Where large numbers of smears are to be examined, smears stained with fluorescent stains like Auramine phenol or rhodamine is examined under ultraviolet light [6]. Though it is a gold standard, it fails to detect TB in multi drug resistant TB & extra-pulmonary cases, especially it is ineffective in diagnosis in children and HIV positive individuals. A single smear has a sensitivity of 22-43%, which goes up rapidly with examination of 2-3 smears over two days, up to 50-70% in patients with active TB [7, 8, 9].

2. Chest X-ray: It is more sensitive and less specific as there is high inter & intra reader variation of reporting. It can't be used alone for diagnosis of TB. It should always be preceded by repeat sputum smear microscopy for confirmation of diagnosis. No shadow is characteristic of TB. Even 10-15% culture positive TB cases remain undiagnosed by X-ray. 40% patients diagnosed to have TB by X-ray alone may not have Active TB infection. But it is highly useful for extra-pulmonary TB i.e. pleural effusion, pericardial effusion, mediastinal lymphadenopathy, miliary TB, etc. Many studies suggest that chest X-ray for diagnosis or follow-up of pulmonary tuberculosis cases with or without HIV co-infection is unreliable [7, 10].

3. PCR Tests: There are some blood tests which detect TB antigens from blood samples, e.g. QuantiFERON-TB Gold In-Tube test (QFT-GIT), T-SPOT TB test (T-Spot). There is no role for inaccurate and inconsistent diagnostics like serology (IgM, IgG, IgA antibodies against MTB antigens), various in-house or non-validated commercial PCR tests and BCG test. There is also no role for IGRA (Interferon Gamma Release Assay). In 2011, WHO had issued a Policy Statement to stop the use of serological tests for diagnosis of TB as well as to assess the treatment outcome. Hence the use of serological tests for TB diagnosis is not recommended [11, 12].

4. Solid Culture on LJ Medium & Drug Susceptibility Testing (DST) of First Line Drugs: Lowenstein Jansen is a standard solid medium. It is highly sensitive diagnostic technique as it detects as less as 10-100 bacilli per ml of sputum. It can take 3-8 weeks for the growth to occur. It can also be used for DST of first line anti-TB drugs i.e. Isoniazid, Rifampicin, Ethambutol & Streptomycin. Sensitivity of Culture to detect M. tuberculosis is 63%, which can be increased to 83% with second culture[5-7].

5. Liquid culture method -BACTEC MGIT (Mycobacteria Growth Indicator Tube) 960: In this method, Middle Brook liquid medium is used. It can be manual, Semi automatic or fully automatic, high capacity, radiometric, noninvasive instrument. It can incubate & monitor 960, 7 ml culture tubes at a time. Oxygen-quenching fluorescent sensor technology is used, in conjunction with unique on-board algorithms to determine the positivity of the culture tubes. The Liquid cultures give reports about Drug Resistant TB in 9-14 days (2 wks)[5-7].

6. Line Probe Assay: It is a molecular diagnostic test which can provide the DST results within 1-2 days for MTB Complex. It is Conventional PCR (Polymerase Chain Reaction) based test. The results are available within 24 hours. It is based on in-situ hybridization on nitrocellulose strips of specific genetic targets for resistance genes. Currently these are available for Isoniazid and Rifampicin[12].

7. CBNAAT-GeneXpert: It is Real-time PCR based Cartridge Based Nucleic Acid Amplification Test (CBNAAT). For CBNAAT test, sample has to be collected in Falcon Tube and transported to Intermediate Reference Laboratory (IRL), by maintaining Cold Chain. Preferred sample is sputum and minimum quantity required is 10 ml. Other samples like pleural fluid, CSF can also be used. It can be used for diagnosis of TB and DST for first line / second line Anti-TB drugs especially Rifampicin and Isoniazid resistance. The Results are available within 2 hours. Thus it is the fastest method available for TB diagnosis. It is available at all State level IRLs[14].

Conventional methods based on culture & DST are slow processes, during which period patients may be inappropriately and inadequately treated , and the drug resistant strains of Mycobacterium tuberculosis may continue to spread in the population. In contrast to this the GeneXpert is rapid, automated procedure, without human errors. It

provides a highly accurate diagnosis in a single test that identifies both the presence of TB and drug-resistance to Anti-TB medicines, this leads to early and proper treatment to the patients. Hence, it is recommended by WHO since 2010. The specificity of NAAT can be 98–100%, and sensitivity is greater than 95% in sputum smear positive cases, though it is only 50–60% in smear-negative. Recent amplification tests may have better sensitivity in smear-negative specimens also [7,11,14,15]. Talking hypothetically, a rapid and universally accessible test that is not affected by HIV status, with a sensitivity of 85 per cent, and a specificity of 97 per cent, has the potential to save 392,000 adjusted lives annually, or 22 per cent of the global TB deaths[16].

Table 1: Comparison of various TB Diagnostic tests

Sr. No.	Diagnostic Method	Duration for Result	Sensitivity	Specificity	Setting
1	Sputum Smear Microscopy	Immediate Results	50-70%	90-95%	Peripheral/Primary
2	Chest X-ray	Immediate Results	High	Low	Referral/Secondary
3	Solid Culture- LJ Medium	3-8 weeks	83%	Good	Referral/Tertiary
4	Liquid Culture- BACTEC	10-14 days	95%	-	Referral/Tertiary
5	Line Probe Assay	1-2 days	Poor	-	Referral Laboratory
6	CBNAAT- GeneXpert	Within 2 hrs.	95%	98-100%	

Currently FIND (Foundation for Innovative New Diagnostics) is developing a simple molecular case detection test based on loop-mediated isothermal amplification (LAMP) technology that permits visual detection of positive TB samples within two hours, at peripheral health centre's only[17]. So, in this era of MDR-TB & XDR-TB, proper and timely diagnosis of TB is as important as the appropriate & adequate treatment. Progress in India has a big influence on regional indicators. The region has about one third of the global burden of MDR-TB and major efforts are needed to increase detection and treatment of cases. As new diagnostic tools continue to emerge at a fast pace and international recommendations are modified as a result, which may give national level decision makers the information necessary to make important decisions related to the adoption of diagnostic tools and packages. Country-specific operational research and translation of findings into policy and practice must be accelerated to facilitate fast uptake of new tools and strategies for better diagnosis, treatment and prevention of all cases of TB. There is a clear need for development, introduction, and effective implementation of cost-effective new tools that contribute to improvement in patient-centered outcomes and public health. The future is brighter as several promising new tools have entered into evaluation stages. But the need is great, and important barriers remain in translating these technical diagnostic advances into meaningful and sustainable improvements in individual and public health in resource limited settings that are endemic for TB.

CONCLUSION

The rise in drug resistance among the TB bacilli and TB/HIV co-infection has highlighted the urgent need for more accurate and rapid diagnostic tests. The widely followed method of diagnosis by sputum microscopy fails to diagnose the majority of TB cases. It cannot detect drug-resistant or extra-pulmonary TB and also ineffective for diagnosis of TB in children and HIV-positive individuals. Hence the new diagnostic tests for tuberculosis offer the promising results of early and accurate diagnosis of active TB. These tests are very promising techniques not only for rapid diagnosis of disease but also for rapid diagnosis of drug resistance. If implemented, new diagnostic tests will make a bigger impact on interrupting TB transmission by early diagnosis with better sensitivity and specificity than existing tests. The challenges are to improve the accuracy, affordability and speed of new diagnostic tests, and also to make them accessible at all levels of the health system in resource limited settings like India.

ACKNOWLEDGEMENT

The authors are thankful to Dr A.V. Bhore, Dean, Smt. Kashibai Navale Medical College, Pune for his support.

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Source of support: Nil, Conflict of interest: None Declared