Mini Review

Tuberculosis Diagnosis and Management: Recent Advances

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Abstract

Accurate and rapid diagnosis is crucial for starting effective treatment for tuberculosis (TB) and mitigating the transmission. Globally, nearly one-third of all TB cases remain undetected each year and consequently these are not reported. On top of that, the emergence of drug-resistant TB poses an added challenge. In the past 15 years, several advances have been made for improved diagnosis, including liquid culture and drug susceptibility, line probe assay for drug resistance detection, and cartridge-based nucleic acid amplification tests for rapid diagnosis of TB and drug resistance detection. However, some challenges remain, despite the clear edge of these new advances over the age-old conventional methods. Despite these advances, accurate, affordable, and accessible diagnosis of TB remains a challenge, especially in rural and difficult-to-reach settings, where the most desirable test would be a point-of-care triage test. Nevertheless, several attempts are being made in this direction, and in this article, we review these research advances that can help the TB elimination from India.

Keywords: GeneXpert, multiplex polymerase chain reaction, point of care test (POCT), tuberculosis, yield

Introduction

Tuberculosis (TB) is one of the world's ancient and most deadly infectious diseases and still kills millions of people every year. According to Houben and Dodd, approximately 26% of the population is estimated to be infected with *Mycobacterium tuberculosis* (MTB) worldwide,[1] however, the infection remains dormant due to host immunologic control in most of the individuals.[2,3] It is predictable that every year currently there are more than 10 million incidental cases of TB with 3 million deaths occurring worldwide.[4] It is also imposing a cost on our economy in terms of losses to our current and future outputs. The World Health Organization (WHO)[4] and several countries have committed to end TB by 2030 while some countries like India have proactively advanced this target to end TB by 2025. Although ending TB in the next 2–7 years is seemingly a herculean task, various stakeholders are working on various fronts.

The COVID-19 epidemic has taught us its negative as well as positive sides. On the one hand, TB case detection rates were compromised due to a shift of manpower and other resources in COVID-19 management, but it has also given us hope for speedy vaccine development for various infectious diseases including TB.[5] An effective vaccine against TB is extremely important and considering that a century-old Bacillus Calmette–Guérin remains the only vaccine and more than 32 vaccine candidates are in the pipeline of development;[5,6] however, we feel that no single approach is going to achieve the goal of ending TB.[7,8] In this review, we would like to invite attention toward the role of early and rapid diagnosis to start early treatment. Any delay in detecting an open pulmonary TB case will be tantamount to allowing transmission and high chances of treatment failure.

Though several advances in the diagnostic and management are being made and implemented by respective national TB control program implementation agencies (RAPiD AssessmenT RePoRT, 2018), and these are reviewed elsewhere by various authors,[9-18] in this article we will restrict only to highlighting the recent advancements made globally. All these diagnostic modalities must entertain concerns such as sensitivity, specificity, yield, predictive value, turnaround

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time (TAT), reproducibility, cost-effectiveness, safety, noncumbersomeness, robustness, and easy application for wider use.[18]

Advances in the Conventional Methods of Diagnosis

In the last two decades, several advancements have been made in the conventional methods of diagnosis, including the role of artificial intelligence and machine learning (AI-ML) in recording, and storing the patient details and transporting the data, a major component of digital health.

Radio-imaging modalities

The conventional chest X-ray (CxR) examination still remains the most commonly used method for screening, diagnose and to do follow-up of the treatment responses in patients with pulmonary TB (PTB) as well as extrapulmonary TB (EPTB). However, this century-old technology has limited specificity. Newer radiographic tools revolutionized the TB diagnosis using digital plates and digital radiography and more recently through computer-aided diagnosis (CAD).[11,12] In a recent study, newer versions of five commercial AI algorithms: the CAD4TB, InferRead DR, Lunit INSIGHT CXR, JF CXR-1, and qXR were compared to find their sensitivity and specificity. These advances helped precise and early identification of parenchymal lesions or mediastinal lymph node enlargements and to determine the disease activity in TB. All the five AI algorithms, as mentioned above, reduced the number of Xpert/MTB-rifampicin (RIF) tests by 50% while maintaining a sensitivity of more than 90%. However, AI algorithms performed poorly among the older age groups (>60 years) and people with a history of TB in past.[19]

More recently, serial pulmonary ^{18}F-2-fluoro-deoxy-D-glucose (^{18}F-FDG) positron emission tomography (PET) was compared with high-resolution computed tomography (CT), and the results were very promising. This noninvasive method was found useful to monitor the disease activity and responses to anti-TB treatment. It is known that ^{18}F-FDG accumulates in metabolically active cells, including the inflammatory cells including macrophages, neutrophils, and lymphocytes, are typically involved in active lesions of TB. Combining ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET with CT imaging (PET/CT) is an area of future interest in TB treatment monitoring. Although expensive, this technique could be useful for the management of patients with dreaded form of TB such as multidrug resistant (MDR) and extensively drug resistant (XDR). Data from multiple cohorts have demonstrated that PET glycolytic activity decreases in response to effective TB treatment and can predict treatment outcome.[20,21]

In the field of TB, India has made several breakthroughs, and Qure.ai® is another "breakthrough AI solution disrupting the conventional methods of radiodiagnosis" by enhancing the image accuracy and thereby improving the health outcomes, especially in the field of TB, with the assistance of ML tools. Qure.ai uses deep-learning technology to provide automated interpretation of radio-imaging platforms such as X-rays, CTs, and ultrasound scans enabling faster diagnosis and speedy treatment.[22]

The WHO, in the year 2021, recommended the use of CAD tools for TB screening among adults but did not include children in this recommendation due to low evidence of diagnostic accuracy among children. Efforts are underway by the Indian Council of Medical Research (ICMR), the Foundation for Innovative New Diagnostics (FIND), and Stop TB Partnership to compile a database of pediatric X-rays to develop CAD algorithms for children and to validate the accuracy of CAD. AI-ML tools have been developed jointly by ICMR, Department of Health Research and Institute of Plasma Research, Department of Atomic Energy, Government of India, for screening of PTB and other lung diseases using CxR images (personal communication). Once these tools and devices are validated on patients of TB, and other diseases, added with portable X-ray machines, can be game changer. The literature suggests that CAD systems could be highly useful tools for TB screening programs in remote areas in high TB prevalent places where physical access to expert radiologists may be a limiting factor. However, more large-scale prospective studies, preferably random clinical trials are needed to address long-awaited questions around the operational feasibility of the CAD systems.[11]

Sample collection, storage, transportation, and smear microscopy

Collection, storage, and transportation of clinical samples are of paramount importance. In the previous decade, various solid substrates such as glass slides, flinders technology associates (FTA) cards, and GenoCards have been evaluated for preserving the morphology and/or nucleic acids for molecular testing with as good results as from fresh samples.[23] Molecular testing has also been found highly useful in archived samples, where detection of MTB using conventional phenotypic methods was not possible earlier.[24]

Use of artificial intelligence and machine learning

The AI-ML has revolutionized the world and health section has not remained away from these technological advances. These technologies are now being tried in the field of medicine with exponential number of applications including the image analysis (as mentioned in the section "Radio-imaging modalities") or in the microscopy. In recent years, the "operator-independent sputum smear microscopy based on the ZEISS Axio Scan®" and other AI-ML-based systems have been evaluated to automatically detect and count the acid-fast bacteria with 97.06% sensitivity and 86.44% specificity.[25] However, such developments may not be very useful for resource-limited countries due to the high cost of the laboratory setup and because such devices may remain useful only for central laboratories.

Culture and drug susceptibility testing

The culture isolation of MTB remains a highly sensitive

diagnostic method that permits, theoretically as low as single mycobacterium but practically 10 colony-forming units (CFU) or live mycobacteria to grow to a visible colony on solid media or as turbid growth in liquid media. The positive mycobacterial growth means almost 100% specificity. In 2007, the WHO endorsed liquid cultures for the mycobacterial culture and drug susceptibility. Although there are at least three commercial liquid culture systems, the BACTEC MGIT 960 system still remains the gold standard for the diagnosis of TB and drug susceptibility testing (DST).[4,13,17-19,21,24] However, the culture-based diagnosis has important limitations of improved yet undesirably long TAT, requirement of trained personnel, and level 3 biosafety laboratory environment.[13,14,17-19,21] A number of noncommercialized culture-based methods have been reported for DST. These tests are useful in the early stages of mass screening and testing of new drugs, when the WHO-endorsed culture-based or molecular tests are not yet endorsed by the WHO. However, these tests require biosafety precaution and cannot be used in peripheral laboratories.[9,17]

Molecular Tools for Diagnosis

The discovery of the deoxyribonucleic acid (DNA) model by Watson & Crick in 1953 and later on the elucidation of nucleic acid transcription and translation into proteins, the recombinant DNA revolution followed in the 1970s and nucleic acid amplification technology in the 1980s. These discoveries allowed early diagnosis of TB. These methods are based on capturing the DNA and simultaneous detection of the pathogen the MTB and mutations in drug-resistance genes with high sensitivity and specificity. Recent advancements of molecular rapid diagnostic tests have reduced the turn around time (TA) significantly and thereby allowing the treating physician to initiate the treatment/preventive services. Several molecular tests have now been endorsed by the WHO for early detection of TB.

Polymerase chain reaction

A Nobel Prize-winning (1993) discovery of polymerase chain reaction (PCR) was made by Kary Mullis in 1983. This PCR technology became a boon for medical science in the early 1980s, and this technology has been used for the diagnosis of TB since the early 1990s.[26] In recent years, various forms of PCR have been developed including multiplex PCR (mPCR) that can detect genus and species-specific amplicons in a single tube [Figure 1].[27] The conventional PCR required agarose gel for the detection of amplified products, and this limitation was overcome by the development of real-time PCR (RT-PCR), in which the DNA amplification reaction could be monitored on the light emitting diode (LED) screen as the PCR runs on a real-time basis.[13-15,19,28] The RT-PCR could be used for amplifying the DNA but also the RNA using the reverse-transcription technology and for quantification of the pathogen load in the sample. There are a number of commercially available PCR kits.[29]

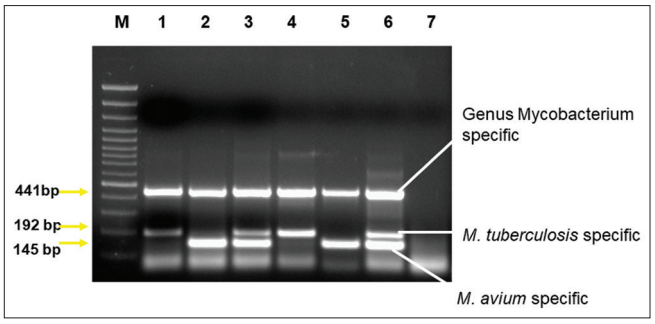

Figure 1: A multiplex polymerase chain reaction capable of detecting and identifying genus *Mycobacterium*, most common species *Mycobacterium tuberculosis* (MTB), and *Mycobacterium avium* intracellular complex. The top 441 base pair (bp) band indicates the presence of mycobacterium species in the samples, while the second and third bands of 192 and 145 bp indicate that species is either MTB, *M. avium* complex (MAC), or both. If only first band of 441 bp is positive but none of the remaining two, that will indicate other nontuberculous and non-MAC mycobacterial species[27]

GeneXpert®/*Mycobacterium tuberculosis*-rifampicin

Even the RT-PCR required separate processing of the sample for mycobacterial DNA extraction. This remains a major limitation of PCR. The laboratory of Professor Alland at New Jersey (USA), Cepheid Inc. (USA), and FIND, with financial support from the National Institutes of Health, USA, discovered a new technology known as cartridge-based nucleic acid amplification test (NAAT) or GeneXpert. Indeed, the GeneXpert system was originally developed by Cepheid™ Inc. (USA) for the detection of anthrax[29] and was deployed by the United States Postal Service in postal sorting facilities to detect anthrax in postal envelopes. The technology was first time applied and evaluated for the diagnosis of TB on clinical samples in 2010.[30] The technology was considered to be a game changer. The uniqueness of this system is sample processing and DNA amplification in a single closed cartridge, which no system could ever do before.

The GeneXpert MTB/RIF was endorsed by the WHO on December 8, 2010, after the publication of performance report of the test in the *New England Journal of Medicine*.[15] After this endorsement, the GeneXpert-MTB/RIF was rolled out by various nations including India in 2013 in the Revised National Tuberculosis Control Programme. Since then, more than 4000 systems have been installed in public and private laboratories and their usefulness has been unparalleled and exceptionally high for the diagnosis of PTB and RIF resistance detection[16] but low to very low (11%) in the pediatric PTB and in EPTB samples, especially in plural fluid,[31] the ascitic fluid,[32] cerebrospinal fluid,[33] yet higher than culture.

GeneXpert®/Mycobacterium tuberculosis-Rifampicin Ultra

Accepting some limitations, in 2017, the manufactures of GeneXpert/MTB-RIF included some additional genetic mutations and new two primers. The new system known as GeneXpert/MTB-RIF Ultra was shown to have a lower (16) CFU/ml detection limit for MTB as compared to 113 CFU/

ml in the GeneXpert MTB/RIF test.[34] Both GeneXpert MTB/RIF and GeneXpert MTB/RIF Ultra are recommended by the WHO for TB diagnosis.[4] Several comparative studies were conducted.[35,36] More studies are becoming available using various clinical samples.

GeneXpert® Mycobacterium tuberculosis/extensively drug resistant

This version of GeneXpert simplifies the TB DST with the use of increased multiplexing with 10-color GeneXpert® technology. The GeneXpert MTB/XDR launched in 2020 allows faster molecular DST. The GeneXpert MTB/XDR sets new standards by detecting mutations considered to be responsible for resistance against RIF, isoniazid (INH), fluoroquinolones (FLQs), second-line injectable drug (amikacin, kanamycin, and capreomycin), and ethionamide (ETH) in a single test run. In a recent study done at two sites with 100 sputum samples and 214 clinical isolates, its performance, as compared to sequencing, showed 94%–100% sensitivity and a 100% specificity for all drugs except for ETH.[37] Its sensitivity and specificity for drug resistance detection in comparison to phenotypic DST were 94.2% and 98.5% for INH, 93.2% and 98.0% for FLQs, 98.0% and 99.7% for ETH, and 86.1% and 98.9% for amikacin resistance.[38]

GeneXpert® Edge

Knowing that the GeneXpert® is not a point-of-care (POC) test because it requires laboratory infrastructure, including electricity supply. To overcome these limitations in 2018, Cepheid launched the GeneXpert® Edge (GX-Edge), which is a new-generation platform. This system is meant for health services with limited infrastructure. However, the GX-Edge is a single-slot platform that means only a single sample can be run per cycle, but it is powered by a re-chargable battery. Very little data are available on its feasibility and acceptability in the field.[39]

Cepheid GeneXpert® Mycobacterium tuberculosis-Host Response

The development of a nonsputum-based POC triage test, which is fast and accurate, remains an urgent need for initiating early treatment and case management. Very recently, Cepheid™ Inc. (USA) has developed a new fingerstick blood-based test named as Xpert MTB Host Response (MTB-HR) prototype. The system generates a "TB score" based on messenger RNA (mRNA) expression of 3 genes. Its sensitivity and specificity, irrespective of HIV status of the patient, were 87% and 94%, respectively. Considering this as low sensitivity for a triage test, when the sensitivity cutoff score was raised to 90%, the specificity fell down to 86%.[40] In a recent study, the MTB-HR could differentiate mRNA expression score in the culture-confirmed TB cases from those with unlikely TB but with an accuracy of only 59.8%. In microbiologically confirmed cases, it showed 41·6% sensitivity and 90·3% specificity. The sensitivity was even lower in children with HIV.[41] More data are expected from other countries.

TrueNat®

India can boast for the TrueNat MTB, MTB-Plus, and MTB-RIF Dx three versions of the assays developed by M/s Molbio Diagnostics (Goa, India). These tests can be used at the same health system level as GeneXpert MTB/RIF. In 2020, the WHO recommended the TrueNat MTB or MTB Plus assay as the initial diagnostic test for TB. The WHO also recommended these tests to be run along with the TrueNat MTB-RIF Dx for detection of RIF resistance on samples that are found positive in TrueNat-MTB or MTB-Plus results. After its endorsement by the WHO, the test has been evaluated extensively globally. In a recent study, the sensitivity of TrueNat MTB-Plus is found to be 91% as compared to GeneXpert (90%). Among the HIV-TB-coinfected participants, its sensitivity was reported as 85% compared to 81% for GeneXpert. The specificity of TrueNat MTB Plus was, however, slightly lower (96%) than GeneXpert (99%).[42] The manufacturers are working for further improvisation.

Line probe assays

In fact, the diagnosis of TB had remained one of the major puzzles scientists have faced. However, after decoding the DNA and development of gene sequencing technology by Sanger's laboratory, molecular biologists became hope and many historical landmarks were made in the late 1970s and 1980s. Methods for restriction enzyme digestion and southern hybridization methods are some of these early innovations, followed by PCR. In subsequent years, several molecular tests were developed that included amplification of mycobacterial 16S rRNA using an isothermal transcriptase-mediated amplification assay; Cobas Amplicor (Roche Diagnostic Systems Inc., USA); LCx MTB assay (Abbott Laboratories, USA), which uses the ligase chain reaction for amplification of the *pa*b gene; BD ProbeTec (Becton-Dickinson, USA) and finally combination of PCR based of the gene amplification followed by DNA hybridization commonly known as line probe assays. Many of these tests were commercialized. These include INNO-LiPA Mycobacteria version 2 and the GenoType assays. The INNO-LiPA Mycobacteria version 2 (INNOGENETICS N.V., Belgium) is based on the amplification of 16S-23S rRNA intragenic region which allows the identification of MTB complex species and 16 nontuberculous mycobacterial (NTM) species. The GenoType assays (Hain Lifescience, Germany) are based on mPCR. Indeed, the INNO-LiPA Rif. TB assay (also called LiPA, or line probe assay [LPA]) was developed in 1995 by INNOGENETICS and commercialized for the detection of MTB complex infection and the genetic mutations associated with drug resistance in MTB. These developments are extensively reviewed by Machado *et al*.[43]

Indeed, an evaluation study of LPA was carried out in Portugal and published in 1995.[44] The LPA was the first TB NAATs endorsed by the WHO but after several years of its successful evaluation and clinical application only in 2008. The assays identify mutations using reverse hybridization-based strip technology. At least 3 manufactures have commercialized the technology, and these include INNO-LiPA Rif. TB™

(Innogenetics NV, Ghent, Belgium), GenoType MTBDRplus™ (Hain Lifescience-Bruker, Nehren, Germany), and Nipro NTM MDRTB II™ (Osaka, Japan). New-generation LPAs have been developed with higher sensitivity, and newer versions covering more genetic mutations and more drugs have been made available. These include GenoType MTBDRsl version 2.0 (Hain Lifescience-Bruker) for second-line drugs. Genotype MTBDR*sl* V2.0 showed increased sensitivity for detection of fluoroquinolone (FLQ) and XDR-TB isolates over Genotype MTBDR*sl* V1.0.[45] Emerging novel mutations have limited the use of this assay as it does not have probes corresponding to novel mutations associated with MDR and XDR TB.

Loop-mediated isothermal amplification

Loop-mediated isothermal amplification (LAMP) is an isothermal PCR amplification technique that can be performed in a standard molecular biology setting. The LAMP-based TB-LAMP assay has been recommended by the WHO since 2016.[46] However, the technology requires biosafety laboratory and highly trained manpower. The technology compromises the specificity and therefore, can not recommended for routine use.

Next-generation sequencing

After having the full details of whole-genome sequence (WGS) of the MTB, targeted next-generation sequencing (NGS) allows us to sequence millions of fragments in each run simultaneously and recently this molecular tool has been found to be very useful in providing the profiles of drug resistance. The targeted NGS which have become available in the last few years can detect resistance to several known and unknown drugs concomitantly, it provides rapid results using patient samples directly, and the technology has the potential to gather new information on new genetic markers of resistance. The main use of NGS is in understanding the possible association of various known and unknown genetic mutations conferring drug resistance. The NGS provides an opportunity to test new drugs and novel molecules against MTB. The traditional WGS of MTB depends on bacterial culture, but the direct whole genome using the NGS platform can be done directly on the clinical samples obviating the process of bacterial culture.[9,47] However, the technology requires skilled manpower with experience in molecular biology as well as bioinformatics, besides the cost factor. Hence, this technology is best utilized by pooling the resources in a centralized laboratory.

Figure 2: A prototype of flow-through point-of-care test using 3 novel antigens (SS-1, SS-3, and SS-5). The reactivity and specificity of these antigens are depicted in rows A, B, C, and D. In row A are samples from microbiologically confirmed pulmonary tuberculosis patients while in row B are samples from microbiologically confirmed extrapulmonary tuberculosis patients showing two or more dots, indicating positive result. In row C samples are from healthy controls and in row D from disease controls, with no cross-reactivity. These devices can be used as triage test for mass screening by field workers or even by patients themselves to rule out tuberculosis.[51] TB: tuberculosis, PTB: pulmonary tuberculosis, EPTB: extrapulmonary tuberculosis, HC: healthy controls, DC: disease controls

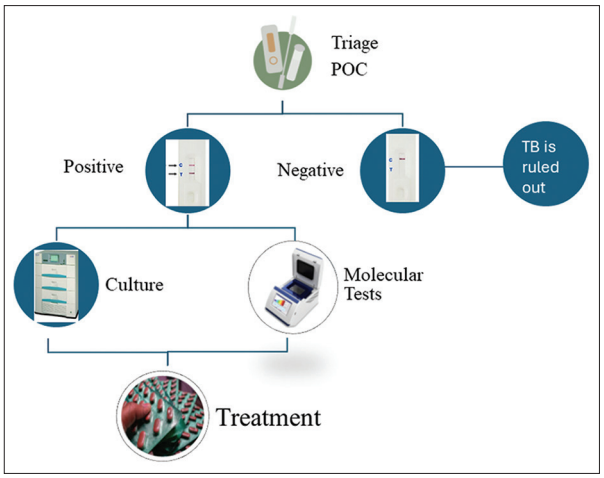

Figure 3: Proposed algorithm for using the immunochromatographic-based point-of-care test in triaging the tuberculosis (TB) screening and diagnosis, for community screening as well as in all suspected cases of all forms of TB. TB: Tuberculosis, POC: Point-of-care

Other Methods of Diagnosing and Screening the Population

Several immunological tests such as tuberculin skin test, T-spot, and QuantiFERON-TB Gold are well-known tests but primarily used to screen and for the purposes of ruling out the disease.[1,9,19,21] Nevertheless, some of these immunological tests have an important role in detecting the latent TB.[9,19,21,48]

For the diagnosis of TB, serological tests are not recommended.[49] Yet, preliminary results of innovative approaches, such as flow-through or lateral flow immunochromatographic tests, are encouraging.[50-53] Many of these tests are in the pipeline for public screening because these can be used as POC rapid tests, requiring no equipment, no electricity, and no skilled manpower [Figure 2]. We believe that the combination of serological triage tests and confirmatory molecular tests will help timely elimination of TB, if used rationally in an algorithmic manner [Figure 3].

Conclusions

A number of new technologies in the field of timely diagnosis of TB have been approved and endorsed by WHO in the last 2 decades, which has helped better management of active cases of TB. Yet, the number of undetected cases remains alarmingly high in some of the high TB burden countries, and this is a matter of concern, if we want to eliminate TB by 2030. For this, a comprehensive approach is required including the encouragement to the biomarkers' discovery.

Research quality and ethics statement

This study followed the applicable EQUATOR Network (http://www.equator-network.org/) guidelines, specifically the PRISMA guidelines, during the conduct of this research project.

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Conflicts of interest

There are no conflicts of interest.

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