Review Article

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Microbiological diagnosis of tuberculous meningitis: Phenotype to genotype

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Tuberculous meningitis (TBM) is a commonly encountered central nervous system infection. Characteristic clinical, imaging and cerebrospinal fluid parameters help clinicians to make a prompt presumptive diagnosis that enables them to start empirical anti-tuberculosis treatment. There are several close mimic to TBM, such as partially treated pyogenic meningitis, fungal meningitis, sarcoidosis, meningeal metastases and meningeal lymphomatosis. Microbiological confirmation instils a sense of confidence amongst treating physicians. With conventional phenotypic methods (cerebrospinal fluid microscopy and culture), in more than 50 per cent patients, microbiological confirmation is not achieved. Moreover, these methods take a long time before providing conclusive results. Negative result does not rule out *Mycobacterium tuberculosis* infection of the brain. Genotypic methods, such as IS*6110* polymerase chain reaction and automated Xpert *M. tuberculosis*/rifampicin (MTB/RIF) assay system improved the TBM diagnostics, as results are rapidly available. Xpert MTB/RIF assay, in addition, detects rifampicin resistance. Xpert MTB/RIF Ultra is advanced technology which has higher (60-70%) sensitivity and is being considered a game-changer in the diagnostics of TBM. A large number of TBM cases remain unconfirmed. The situation of TBM diagnostics will remain grim, if low-cost technologies are not widely available. Till then, physicians continue to rely on their clinical acumen to start empirical anti-tuberculosis treatment.

Key words *Mycobacterium tuberculosis* - polymerase chain reaction - rifampicin resistance - tuberculous meningitis - Xpert MTB/RIF assay

Introduction

Globally, tuberculosis stands amongst top 10 causes of untimely deaths. In 2018, tuberculosis (TB) caused an approximately 1.5 million deaths amongst human immunodeficiency virus (HIV)-negative people, in addition, there were 251,000 deaths among HIV-positive population. India is one of the top eight countries that accounts for nearly two-thirds of the new TB cases of the globe. In 2018, worldwide, there were an estimated 484,000 new rifampicin (RIF)-resistant tuberculosis cases[1]. India topped again as it harboured 24 per cent of global drug-resistant (DR)-TB burden. In India, in 2018, an estimated 27 lakh new TB cases were reported and 4.5 lakh cases had died. An estimated one million TB cases, in India, either remained undiagnosed or unreported[1,2].

Approximately, 21 per cent cases were of extrapulmonary tuberculosis (EPTB) that included meningeal tuberculosis as well[3]. The global burden of EPTB ranges from 8-24 per cent of all TB cases[4]. The exact prevalence of central nervous system (CNS) TB in India is not precisely known. As per a rough estimate, approximately one per cent of all cases of TB suffer from CNS-TB[5]. TBM is the most common type of CNS-TB. Report from a north Indian Centre, located in a very high TB burden region, noted that amongst 306 cases of CNS-TB, 214 (69.9%) had TBM. In 62 (20.26%) patients, TBM was a part of widely disseminated TB. Ten cases had MDR TBM[6]. Approximately, half of the CNS-TB cases either die or become severely disabled[7] as it is associated with five-times more risk of death[8].

TBM is diagnosed easily on clinical grounds, but it is difficult to confirm microbiologically. In roughly 50 per cent of cases a definitive microbiologic diagnosis is not achieved, and physician has to start empirical anti-tuberculosis treatment (ATT). Several infective and non-infective CNS disorders closely mimic TBM, such as partially treated pyogenic meningitis, fungal meningitis, sarcoidosis, meningeal metastases and meningeal lymphomatosis. All these conditions may pose a formidable diagnostic challenge in many patients.

TBM is a paucibacillary disease, and it is often difficult to isolate *Mycobacterium tuberculosis* (MTB) in cerebrospinal fluid (CSF) by conventional methods. Since the past decade, a sea change in the diagnostics of TBM has taken place. Genotypic tests have led to a rapid identification of MTB that to in a higher proportion of CSF specimens. Conventional drug susceptibility testing takes an unacceptably long time for definite results. In India, DR-TBM is also frequently encountered. Isoniazid (INH) mono-resistance is the most common form of drug resistance seen in TBM[9]. Multidrug-resistant TBM is potentially life-threatening and needs a prompt diagnosis. In isolated patients, extensive drug-resistant (XDR) TBM has also been identified[9,10]. This review focuses on the current global and Indian status of TBM diagnostics.

Phenotypic tests

Conventional tests, microscopy for acid-fast bacilli (AFB) and MTB culture in CSF, are the classical tests that are conventionally used to confirm bacteriological diagnosis. Ziehl-Neelsen (Z-N) staining of CSF sediments and microscopy, to detect (AFB), has long been the mainstay of the diagnosis of TBM. The sensitivity of microscopy is abysmally low. Yield from microscopy ranges from 10 to 20 per cent and can marginally be increased if a large volume of CSF is processed. Fluorescence microscopy is slightly more sensitive than conventional Z-N microscopy. However, fluorescence microscopy requires considerable technical expertise[9,10].

MTB culture has always been the gold standard for the diagnosis of TBM. It also detects drug resistance. MTB culture is performed on either a solid or a liquid medium. The yield of culture positivity is much higher for liquid culture system (BACTEC Mycobacteria Growth Indicator Tube system) than solid (Lowenstein-Jensen) culture. MTB culture, to yield a positive result, takes approximately 8-10 days in liquid medium and up to eight weeks on solid medium. The sensitivity of mycobacterial culture is around 40-50 per cent[9]. Liquid culture is slightly more sensitive test[9]. In a large Vietnamese study, that enrolled 545 TBM patients, with the objective to assess the role of corticosteroids, microbiological confirmation could be achieved in only 36 per cent cases. In this study, only conventional phenotypic tests were used[11] (Table I). In a reanalysis of four TBM studies done in Vietnam from 2004 to 2016, that included 1048 patients microbiological confirmation could be done in 50 per cent HIV-uninfected and 70 per cent HIV-infected TBM cases. In all four studies, Xpert MTB/RIF assay was one of the tests that had been used[23] (Table II).

In India, in majority of centres, conventional tests still remain the mainstay of tuberculous diagnostics. In a large number of Indian patients, diagnosis of TBM remains unconfirmed. In a tertiary care centre having all modern facilities, microbiological confirmation was possible in only in 36 per cent (43/118) of patients. In this study, culture was positive only in one patient, and Ziehl-Neelsen microscopy was positive in three cases only[34] (Table III).

Genotypic tests

Genotypic tests utilize transcription-mediated amplification of a portion of the genome-specific to the MTB complex, enabling identification the bacilli in cerebrospinal fluid. Genotypic tests also detect mutations in genes that are known to be associated with anti-tuberculosis drug resistance. Genotypic methods are rapid and have higher sensitivity and specificity (Table I).

Table I. Tests used for detection of *Mycobacterium tuberculosis* (MTB) in cerebrospinal fluid and identifying drug resistance

Phenotypic tests
Smear microscopy
Ziehl-Neelsen stains
AFB auramine O or auramine-rhodamine stains fluorescence microscopy
Culture
Solid culture L-J medium
Liquid culture system like Mycobacteria Growth Indicator Tube (BACTEC™ MGIT 960™ TB System)
(The liquid culture systems are more rapid than conventional solid culture system)
Genotypic tests
IS*6110* PCR[12]
IS*6110* is highly conserved part of *M. tuberculosis* complex genome and is specific for MTB complex
Simple PCR
Real-time PCR
Multiplex PCR
Multitargeted (LAMP)[13,14]
GeneXpert MTB/RIF assay[15,16]
Cartridge-based technology that simultaneously detects MTB complex and RIF resistance
GeneXpert Ultra (Ultra)[17,18]
Xpert MTB/RIF Ultra is a new technology for faster detection of MTB complex and RIF resistance
Molecular line probe assays[19]
Line probe assays are strip-based technology meant for rapid detection of MTB complex and RIF and INH resistance
Commercial line probe assay system
INNO-LiPA RIF TB (Innogenetics, Ghent Belgium)
The GenoType MTBDR (Hain Life Sciences, Gmbh, Nehren Germany)
Other tests
The IGRAs[20,21]
IGRAs are not recommended for diagnosis of tuberculous meningitis
Commercial IGRAs
An ELISPOT evaluates the release of IFN-γ from T lymphocytes following stimulation of the cells with MTB-specific antigens
QuantiFERON® TB Gold test (Cellestis Ltd., Carnegie, Victoria, Australia) and the T-SPOT® TB IGRAs (Oxford Immunotec, Oxford, United Kingdom) are other commercially available IGRAs
LAM lateral flow assay[22]
This test has a very low sensitivity in the CSF

L-J, Löwenstein-Jensen; PCR, polymerase chain reaction; LAMP, loop-mediated isothermal amplification; IGRAs, interferon-gamma release assays; ELISPOT, enzyme-linked immunospot test; IFN-γ, interferon-γ; LAM, lipoarabinomannan; CSF, cerebrospinal fluid; RIF, rifampicin; INH, Isoniazid; MTBDR, *Mycobacterium tuberculosis* drug-resistant; AFB, acid-fast bacilli

Polymerase chain reaction (*PCR*)

The PCR is a simple technology meant for rapid identification of MTB genome in the cerebrospinal fluid. In PCR methods, with the help of nucleic acid amplification technique, portions of MTB genome are amplified. PCR usually targets IS*6110* gene sequence, which is specific for MTB complex. Several other MTB DNA-specific sequences such as protein antigen B, MPB64 and 65 kDa are also targeted. Multiplex polymerase chain targets two genes simultaneously. Nested PCR technology is a two-step process. The first stage amplification is done with primers that focus on the target sequence. In the second step, the amplification process takes place on the products of the first amplification, with primers that bind to the target genome sequence. Nested PCR technique is more

Table II. Sensitivity of various phenotypic and genotypic tests and drug resistance pattern in tuberculous meningitis [studies reported from various countries (except India) published after 2010]*

Reference	Country	Number of patients	Type of study	Bacteriologically confirmed	Microscopy	Solid culture	Liquid culture	PCR	Line-probe assay	Xpert MTB/RIF	Xpert MTB/RIF Ultra	RIF/INH resistance	Lipoarabinomannan
Siddiqi *et al*, 2019[24]	Zambia	550; 474 (86.2%) with HIV	Prospective	19.1% (105/550)	NA	107 HIV-=12.3% HIV+=20.5%	NA	NA	NA	Overall 10% (55/550) with MTB in CSF HIV-=52.9% HIV+=94.2%	NA	11% (6/55)	Overall 4.2% (23/550) with MTB in CSF CSF HIV-=21.9% HIV+=94.2% Urine LAM HIV-=24.1% HIV+=6.1%
Heemskerk *et al*, 2018[25]	Vietnam, South Africa and Indonesia	618; 194 (31.3%) with HIV	Prospective	28.2% (174)	33.9% (129/380)	NA	31.8% (119/374)	NA	NA	25.1% (95/379)	NA	NA	NA
Metcalf *et al*, 2018[26]	Peru	37; 23 (62%) with HIV	Prospective	23% (8/37)	7%	NA	23% (7/37)	NA	NA	23% (8/37)	NA	38% (3/8)	NA
Chaidir *et al*, 2018[27]	Indonesia	1180; 203 (18.3%) with HIV	Prospective	42.2% (501)	12.2% (86/703)	46% (163/354)	48.8% (332/680)	64% (212/331)	NA	42% (73/174)	NA	NA	NA
Bahr *et al*, 2015[28]	Uganda	107; 105 (98.1%) with HIV	Prospective	17% (18) all with HIV	3.7% (4/107)	NA	11% (12/107)	NA	NA	16.8% (18/107)	0	0	NA
Solomons *et al*, 2015[29]	South Africa	55 children; 8 (14.5%) with HIV	Prospective	24% (13)	3.6% (2/55)	NA	22% (12/55)	NA	33% (18/55)	26% (14/55)	NA	inhA mutation in 1	NA
Patel *et al*, 2014[30]	South Africa	91; 78 (85.7%) with HIV)	Prospective	34% (31/91)	NA	NA	34% (31/91)	22% (20/91)	NA	21% (19/91)	NA	NA	NA
Nhu *et al*, 2014[31]	Vietnam	379	Retrospective	40% (151/379)	78.6% (143/182)	NA	66.5% (121/182)	NA	NA	59.3% (108/182)	NA	3.7% (4/109) by Xpert	NA
Patel *et al*, 2013[32]	South Africa	123; 108 (87.8%) with HIV	Prospective	48% (59/123)	5.7% (7/123)	NA	40% (49/123)	22.7% (28/123)	NA	19.5% (24/123)	NA	NA	NA
Chaidir *et al*, 2012[33]	Indonesia	207; 37 (17.9%) with HIV	Retrospective (stored samples)	50.7% (105/207)	1.4% (3/207)	NA	49.2% (102/207)	68% (140/207)	NA	NA	NA	NA	NA

*In 2010, World Health Organization approved Xpert MTB/RIF test for diagnostic use. HIV, human immunodeficiency virus; NA, not available; MTB/RIF, *Mycobacterium tuberculosis*/rifampicin

Table III. Sensitivity of various phenotypic and genotypic tests and drug resistance pattern in tuberculous meningitis (Indian data)

References	Number of patients	Bacteriologically confirmed	Sensitivity of different tests	Resistance pattern
Sharma *et al*, 2018[35]	180	80	Xpert=91 (50.5%) PCR=157 (87.2%)	RIF resistance=14
Manke *et al*, 2017[36]	20	7	PCR=12	NA
Rufai *et al*, 2017[37]	267	52 (19.5%)	Liquid culture=52 (19.5%) Xpert MTB/RIF=38 (14.2%)	RIF resistance=(10.5%)
Kumar *et al*, 2016[38]	698 CSF samples	176 (25.2%)	Liquid culture=176 (25.2%) ELISA=61 (34.7% of culture positive)	57 (32.4%) with 5 MDR
Bhatia *et al*, 2016[39]	34	-	Liquid culture=5 (14.7%) Xpert MTB/RIF=13 (38%)	NA
Gupta *et al*, 2015[19]	238	78	Microscopy=5 (2.1%) Liquid culture=78 (32.7%) PCR=30 (12.6%) Geno Type MTBDRplus Assay=31 (13%)	5 MDR 9 isoniazid
Panagariya *et al*, 2013[40]	50	11	Microscopy=2 (1%) Liquid culture=9 (18%) PCR=34 (68%)	NA
Haldar *et al*, 2012[41]	194	29	Liquid culture=29 (14.9%) PCR=66%	NA
Sharma *et al*, 2010[42]	70	10	Microscopy=1 (1.4%) Liquid culture=10 (14.3%) PCR=58 (82.8%)	NA

ELISA, enzyme-linked immunosorbent assay; MDR, multidrug-resistant tuberculosis

specific because it avoids non-specific amplification of the DNA template. Real-time PCR quantitatively assesses the amount of the PCR product in real time. These assays are highly accurate and reproducible.

A systematic review and meta-analysis demonstrated that commercial nucleic acid amplification tests had a sensitivity of 56 per cent and specificity of 98 per cent[12]. In the 35 studies with in-house developed tests, accuracy could not be established, because of a wide variability in test results. PCR assays are considered to have a confirmatory role in the diagnosis of TBM diagnosis, however, because of low sensitivity lack behind to rule out tuberculous meningitis with certainty[12]. Real-time PCR assay, with overall sensitivity of 86 per cent, in TBM is now preferred PCR technique[43].

Multi-targeted loop-mediated isothermal amplification (LAMP)

The LAMP is an isothermal DNA amplification method that uses two or three sets of primers to promptly multiply very small quantities of DNA. In this method, amplification procedures take place in a tube, which contains all the required material (buffer, target DNA, DNA polymerase and primers). The amplified DNA can be assessed with naked eyes. The biggest advantage of TB LAMP is that this technique needs minimal laboratory infrastructure and has limited biosafety hazards. In addition, LAMP is economical, that makes this test ideal for Indian conditions.

LAMP is currently being used in pulmonary TB. Its diagnostic role in EPTB is not clear. A meta-analysis showed a pooled sensitivity of 77 per cent and specificity of 99 per cent for LAMP in EPTB[13]. LAMP has also been employed in CSF specimens. This test had a sensitivity and specificity of 96 and 100 per cent respectively for confirmed (50 culture positive) TBM cases. The sensitivity in probable TBM was 82 per cent. The overall sensitivity was 88 per cent and the specificity was 100 per cent[14].

Xpert MTB/RIF assay

Xpert MTB/RIF assay (Cepheid, USA) is a fully automated, cartridge-based nucleic acid amplification technology, which simultaneous detects MTB bacilli and RIF resistance, directly in CSF specimens. GeneXpert MTB/RIF assay is a commercial, real-time PCR-based technique. Results of this assay

are available within two hours. This assay detects mutations responsible for RIF resistance. The WHO recommended the use of Xpert MTB/RIF assay in 2010. Since 2013, it has also been recommended for use in all forms of EPTB, including TBM[15]. Under the Revised National Tuberculosis Control Programme (RNTCP), Xpert MTB/RIF technology is now widely available in India[2].

Xpert MTB/RIF is being extensively used in Vietnam, Indonesia Uganda and South Africa. Even in developed counties, this is a frontline test for TBM. Xpert MTB/RIF results for 740 CSF specimens from 698 patients, in England, were compared with that of culture. The overall sensitivity of Xpert MTB/RIF test was 55 per cent and specificity was almost 100 per cent[16]. Value of Xpert MTB/RIF was assessed in two Indian studies. Rufai *et al*[37] noted a lower sensitivity of Xpert MTB/RIF than liquid culture. In a collection of 267 CSF specimens (52/19.5% culture positive), Xpert MTB/RIF assay was positive only in 38 (14.2%) specimens. In the second study, Sharma *et al*[35] demonstrated positive Xpert MTB/RIF test in 50 per cent (91/180) cases. Multiplex PCR technique demonstrated better sensitivity (157/180; 87.2%).

*Xpert MTB/*RIF Ultra

Xpert MTB/RIF Ultra is an advancement of Xpert MTB/RIF technology with greater sensitivity. Xpert MTB/RIF Ultra is able to detect MTB bacilli even if present in low numbers in clinical specimens. Technological advancement, in Xpert MTB/RIF Ultra, is basically in its cartridge. Ultra-cartridge accommodates a large volume of CSF. Two additional molecular targets have been added to the platform, to accurately identify MTB bacilli and RIF resistance[44]. The WHO recommends Xpert Ultra as the first diagnostic test in TBM[17].

Several studies have consistently demonstrated the value of ultra-technology in the diagnostics of TBM. A prospective cohort of 204 HIV-positive patients was evaluated for TBM. Uniform clinical case definition identified 51 patients, as probable/possible cases. Among these probable/definite cases, Xpert MTB/RIF Ultra had 76 per cent sensitivity compared with Xpert 56 per cent or culture 61 per cent. Xpert MTB/RIF Ultra was able to confirm nine TBM cases that were missed both by Xpert and culture[45]. In another study, Xpert MTB/RIF Ultra was evaluated in 129 HIV-positive patients of suspected TBM. Twenty three patients were classified as probable or definite tuberculous meningitis. Xpert Ultra sensitivity was 70 per cent compared with 43 per cent by Xpert and 43 per cent by culture. Eight patients were only positive by Xpert Ultra[46]. This technology is being considered a game-changer in the diagnostics of TBM; however, more data from HIV-negative patients are needed to further establish its value[18].

Line probe assay

The line probe assay is a strip-based technology that has sufficient sensitivity and specificity to promptly identify RIF and INH drug resistance in MTB isolates. The GenoType MTBDRplus test is one of the commercially available techniques that identifies mutations in *rpoB*, *katG* and *inhA* promoter genes. GenoType MTBDRplus line probe assay has almost no role in the identification of MTB and drug resistance, directly in CSF samples (Table IV).

A study from northern India evaluated GenoType MTBDRplus line probe assay for early identification of drug resistance in MTB isolates recovered from CSF samples of confirmed TBM patients[19]. Using BACTEC mycobacterial growth indicator tube drug sensitivity testing as a gold standard, the sensitivity and specificity of GenoType MTBDRplus line probe assay for INH resistance were 93 and 97 per cent, respectively. For RIF resistance, sensitivity and specificity figures were 80 and 98.8 per cent, respectively. The line probe assay could detect MTB only in 55 per cent of CSF confirmed TBM samples[19]. More data are needed to establish the precise role of the line probe assays in the diagnostics of TBM.

Metagenomic next-generation sequencing and pyrosequencing

Next-generation sequencing has great potential as a method for rapidly identifying MTB genome and diagnosing drug resistance pattern[49,50]. Next-generation sequencing is capable of identifying genomes of many bacteria, fungi, parasites and viruses and can be of great help in establishing the exact diagnosis, when other tests are unrewarding[51]. Pyrosequencing is a real-time technology for rapid sequencing of small segments of genomic DNA. This technique reliably detects mutations that confer antituberculosis drug resistance[52]. Under the RNTCP, two laboratories have been designated to perform whole genome sequencing and pyrosequencing[2].

Table IV. Differences between GenoType MTBDRplus line probe assay and Xpert MTB/RIF assay

Line probe assay	Xpert MTB/RIF
Mycobacterium tuberculosis isolates or AFB-positive samples	Directly performed on clinical samples Initial test for tuberculous meningitis
Diagnose MDR TB	Identifies *M. tuberculosis* and diagnoses drug resistance
Detects RIF and INH resistance	Detects RIF resistance only
It takes two to three days for positive results	It takes three to four hours for positive results
DNA strip technology	Cartridge based technology
It is a multiplex PCR in combination with reverse hybridization-based technique	It is an RT-PCR-based assay
Suitable for use at national/central reference laboratories	Xpert MTB/RIF suitable for public health settings as well
Commercial kits INNO-LiPA RIF TB (Innogenetics, Ghent, Belgium) GenoType MTBDRplus (Hain Life-Science, Nehren, Germany)	Xpert MTB/RIF (Cepheid Sunnyvale, USA)

RT-PCR, reverse transcription polymerase chain reaction
Source: Refs 47, 48

Other tests

Adenosine deaminase

Adenosine deaminase (ADA) estimation, in cerebrospinal fluid, has long been used, as a simple and reliable test for differentiating TBM from other meningitis[53,54]. ADA is produced in all human cells; lymphoid tissues, particularly, lymphocytes dominantly produce this enzyme. It eliminates a molecule called deoxyadenosine, which is detrimental to lymphocytes. ADA is released by T cells during cell-mediated immune reaction against MTB. In a recent meta-analysis, ADA estimation was found valuable in TBM diagnosis[55]. Sensitivity and specificity of ADA estimation were 89 and 91 per cent, respectively. The mean adenosine deaminase cerebrospinal fluid levels of ADA in CSF of TBM patients was 14.24 IU/l, non-tuberculous meningitis 7.92 IU/l and in other neurological diseases it was 2.32 IU/l. In pyogenic meningitis, mean ADA level was 8.22 IU/l[55]. The most appropriate cut-off value for ADA levels is not precisely known. At a cut-off level of 2.0 IU/l, many TBM cases are missed, and higher cut-off levels are associated with severely decreased sensitivity[56].

Interferon-gamma release assays (IGRAs)

Interferon-gamma release assays (IGRAs) capitalises estimation of interferon-gamma released from T lymphocytes from MTB-infected patients when stimulated with MTB-specific antigens. IGRAs are rapid tests developed to identify latent tuberculosis infection. IGRAs were developed as an alternative for tuberculin skin test, and are not meant to diagnose active tuberculosis[20].

IGRAs in TBM lack sufficient sensitivity and specificity to rely on. A meta-analysis observed that the pooled sensitivity of blood and CSF IGRAs were 78 and 77 per cent with 61 and 88 per cent specificity, respectively. Indeterminate results were noted in a large number of patients[21,57].

Lipoarabinomannan (LAM)

The LAM, a glycolipid, is a component of the mycobacterial cell wall. It has several immunomodulatory effects, like, interference with macrophage activation and antigen processing. LAM is released into the blood from metabolically active and disintegrating MTB. Urinary excretion is considered independent of the anatomical location of the infection. The detection of MTB LAM antigen in urine has also been used for the diagnosis of tuberculosis[21].

LAM assay has been evaluated as a rapid diagnostic test for TBM[23]. Unfortunately, LAM assay in the CSF lacks sufficient sensitivity and specificity. For example, in paediatric TBM patients LAM assay had a sensitivity of 4.8 per cent and a specificity of 93.1 per cent[58]. In adult TBM patients, the sensitivity of LAM assay was only 22 per cent, and urine LAM assay was 24 per cent[24]. The WHO currently does not recommend LAM assay for the diagnosis of any form of TB except, cases with HIV infection with low CD4 counts[22].

Methods to increase yield of diagnostic testing

Invasive pulmonary sampling

In patients with possible and probable TBM, an active search for TB with invasive pulmonary samples has shown to increase the number of microbiological confirmations in TBM. Pulmonary sampling can be obtained with the help of computed tomography thorax, followed by bronchoscopy and bronchoalveolar lavage[59].

Large cerebrospinal fluid volume

A CSF volume more than 6 ml is associated with significantly higher yield of almost all microbiological confirmation tests[60,61]. A meta-analysis analyzed the impact of CSF volume on the diagnostic performance of Xpert MTB/RIF assay[60]. Five studies reported the volume of CSF. Starting from the largest collection volume at 7 ml, the highest sensitivity was 85 per cent. If the CSF volume was 6 ml, the sensitivity decreased to 60 per cent[60].

Cerebrospinal fluid centrifugation

Centrifugation of CSF improves the sensitivity of all the tests. In a Cochrane review, pooled sensitivity in concentrated specimens was 74.8 per cent (15 studies, 2758 specimens) versus 66.2 per cent (12 studies, 905 specimens) in unconcentrated specimens. Pooled specificity in concentrated specimens was 98.3 versus 97.7 per cent in unconcentrated specimens[60].

Does improved diagnostics reduce mortality?

With the availability of better genotypic diagnostic tests, more number of suspected TBM can be microbiologically confirmed. Cresswell *et al*[61], in a retrospective evaluation, noted that significantly increased number of microbiologically-confirmed TBM cases led to a marginal decrease in in-hospital mortality. In this study, microbiological confirmation increased from 3 to 41 per cent that led to a decline in the in-hospital mortality from 57 to 41 per cent.

Conclusion

TBM continues to pose diagnostic challenges. Microbiological confirmation is not always possible. Latest genotypic tests are rapid and have additional capability to identify non-viable MTB bacilli. Unfortunately, genotypic tests do not have enough sensitivity to rule out meningeal tuberculosis. Xpert MTB/RIF test is now widely available in India but failed to demonstrate sufficient sensitivity in Indian patients. Xpert MTB/RIF Ultra, an advanced technology, though not available in India, is being considered a game-changer in the diagnosis of TBM. Physicians in India will have to rely on their clinical acumen till latest diagnostic technologies are available.

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References

1. World Health Organization. *Tuberculosis*; *Key facts*. Available from: *https://www.who.int/news-room/fact-sheets/detail/tuberculosis*, accessed on October 25, 2019.
2. National Health Mission. Central TB Division. *India TB report 2019*: *Revised National Tuberculosis Control Programme*. New Delhi: Ministry of Health & Family Welfare, Government of India; 2019. *Annual Report.* Available from: *https://tbcindia.gov.in/WriteReadData/India%20TB%20Report%202019.pdf,* accessed on June 25, 2019.
3. Sharma SK, Mohan A. Extrapulmonary tuberculosis. *Indian J Med Res* 2004; *120* : 316-53.
4. World Health Organization. *Global tuberculosis report 2018.* Geneva: WHO; 2018. *Available from: https://www.who.int/tb/publications/global_report/gtbr2018_main_text_28Feb2019.pdf*, accessed on June 25, 2019.
5. Global Health Advocates. Central TB Division. *Index TB Guidelines*: *Guidelines on extra-pulmonary tuberculosis for India*. Ministry of Health & Family Welfare, Government of India. Available from: *https://www.icmr.nic.in/sites/default/files/guidelines/Index-TB%20Guidelines%20-%20green%20colour%202594164.pdf*, accessed on June 25, 2019.
6. Ingole R, Garg RK, Malhotra HS, Jain A, Kumar N, Rizvi I, *et al.* Spectrum of central nervous system tuberculosis: An experience from a large tertiary care institution of India. *Indian J Tuberc* 2019; *66* : 49-57.
7. Graham SM, Donald PR. Death and disability: The outcomes of tuberculous meningitis. *Lancet Infect Dis* 2014; *14* : 902-4.
8. Nguyen DT, Agarwal S, Graviss EA. Trends of tuberculosis meningitis and associated mortality in Texas, 2010-2017, a large population-based analysis. *PLoS One* 2019; *14* : e0212729.
9. Garg RK, Rizvi I, Malhotra HS, Uniyal R, Kumar N. Management of complex tuberculosis cases: A focus on drug-resistant tuberculous meningitis. *Expert Rev Anti Infect Ther* 2018; *16* : 813-31.
10. Wilkinson RJ, Rohlwink U, Misra UK, van Crevel R, Mai NTH, Dooley KE, *et al.* Tuberculous meningitis. *Nat Rev Neurol* 2017; *13* : 581-98.
11. Thwaites GE, Nguyen DB, Nguyen HD, Hoang TQ, Do TT, Nguyen TC, *et al*. Dexamethasone for the treatment of tuberculous meningitis in adolescents and adults. *N Engl J Med* 2004; *351*: 1741-51.

12. Pai M, Flores LL, Pai N, Hubbard A, Riley LW, Colford JM Jr. Diagnostic accuracy of nucleic acid amplification tests for tuberculous meningitis: A systematic review and meta-analysis. *Lancet Infect Dis* 2003; *3* : 633-43.
13. Yu G, Shen Y, Zhong F, Ye B, Yang J, Chen G. Diagnostic accuracy of the loop-mediated isothermal amplification assay for extrapulmonary tuberculosis: A meta-analysis. *PLoS One* 2018; *13* : e0199290.
14. Modi M, Sharma K, Sharma M, Sharma A, Sharma N, Sharma S, *et al.* Multitargeted loop-mediated isothermal amplification for rapid diagnosis of tuberculous meningitis. *Int J Tuberc Lung Dis* 2016; *20* : 625-30.
15. World Health Organization. *Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/ RIF assays for the diagnosis of pulmonary and extrapulmonary TB in adults and children.* Policy update. WHO/HTM/TB/2013.16. Available from: *https://apps.who.int/iris/bitstream/handle/10665/112472/9789241506335_eng.pdf?sequence=1*, accessed on June 25, 2019.
16. Pink F, Brown TJ, Kranzer K, Drobniewski F. Evaluation of xpert MTB/RIF for detection of *Mycobacterium tuberculosis* in cerebrospinal fluid. *J Clin Microbiol* 2016; *54* : 809-11.
17. World Health Organization. Next-generation Xpert® MTB/RIF Ultra assay recommended by WHO. Available from: *http://www.who.int/tb/features_archive/Xpert-Ultra/en/*, accessed on June 25, 2019.
18. Khonga M, Nicol MP. Xpert MTB/RIF ultra: A Gamechanger for tuberculous meningitis*? Lancet Infect Dis* 2018; *18* : 6-8.
19. Gupta R, Thakur R, Gupta P, Jalan N, Kushwaha S, Gupta M, *et al.* Evaluation of geno type MTBDRplus line probe assay for early detection of drug resistance in tuberculous meningitis patients in India. *J Glob Infect Dis* 2015; *7* : 5-10.
20. Pai M, Behr M. Latent *Mycobacterium tuberculosis* infection and interferon-gamma release assays. *Microbiol Spectr* 2016; *4* : 5.
21. Yu J, Wang ZJ, Chen LH, Li HH. Diagnostic accuracy of interferon-gamma release assays for tuberculous meningitis: A meta-analysis. *Int J Tuberc Lung Dis* 2016; *20* : 494-9.
22. World Health Organization. *The use of lateral flow urine lipoarabinomannan assay* (*LF-LAM*) *for the diagnosis and screening of active tuberculosis in people living with HIV*. Policy update. Available from: *https://apps.who.int/iris/bitstream/handle/10665/193633/9789241509633_eng.pdf?sequence=1*, accessed on June 25, 2019.
23. Thao LTP, Wolbers M, Heemskerk AD, Thi Hoang Mai N, Thi Minh Ha D, Thi Hong Chau T, *et al.* Dynamic prediction of death in patients with tuberculous meningitis using time-updated Glasgow coma score and plasma sodium measurements. *Clin Infect Dis* 2019. pii: ciz262.
24. Siddiqi OK, Birbeck GL, Ghebremichael M, Mubanga E, Love S, Buback C, *et al*. A prospective cohort study on the performance of CSF Xpert MTB/RIF, CSF and Urine LAM lateral flow assay for the diagnosis of tuberculous meningitis in Zambia. *J Clin Microbiol* 2019. pii: JCM.00652-19.
25. Heemskerk AD, Donovan J, Thu DDA, Marais S, Chaidir L, Dung VTM, *et al.* Improving the microbiological diagnosis of tuberculous meningitis: A prospective, international, multicentre comparison of conventional and modified ziehl-neelsen stain, geneXpert, and culture of cerebrospinal fluid. *J Infect* 2018; *77* : 509-15.
26. Metcalf T, Soria J, Montano SM, Ticona E, Evans CA, Huaroto L, *et al.* Evaluation of the geneXpert MTB/RIF in patients with presumptive tuberculous meningitis. *PLoS One* 2018; *13* : e0198695.
27. Chaidir L, Annisa J, Dian S, Parwati I, Alisjahbana A, Purnama F, *et al.* Microbiological diagnosis of adult tuberculous meningitis in a ten-year cohort in Indonesia. *Diagn Microbiol Infect Dis* 2018; *91* : 42-6.
28. Bahr NC, Tugume L, Rajasingham R, Kiggundu R, Williams DA, Morawski B, *et al.* Improved diagnostic sensitivity for tuberculous meningitis with xpert(®) MTB/RIF of centrifuged CSF. *Int J Tuberc Lung Dis* 2015; *19* : 1209-15.
29. Solomons RS, Visser DH, Friedrich SO, Diacon AH, Hoek KG, Marais BJ, *et al.* Improved diagnosis of childhood tuberculous meningitis using more than one nucleic acid amplification test. *Int J Tuberc Lung Dis* 2015; *19* : 74-80.
30. Patel VB, Connolly C, Singh R, Lenders L, Matinyenya B, Theron G, *et al.* Comparison of amplicor and geneXpert MTB/RIF tests for diagnosis of tuberculous meningitis. *J Clin Microbiol* 2014; *52* : 3777-80.
31. Nhu NT, Heemskerk D, Thu do DA, Chau TT, Mai NT, Nghia HD, *et al.* Evaluation of geneXpert MTB/RIF for diagnosis of tuberculous meningitis. *J Clin Microbiol* 2014; *52* : 226-33.
32. Patel VB, Theron G, Lenders L, Matinyena B, Connolly C, Singh R, *et al.* Diagnostic accuracy of quantitative PCR (Xpert MTB/RIF) for tuberculous meningitis in a high burden setting: A prospective study. *PLoS Med* 2013; *10* : e1001536.
33. Chaidir L, Ganiem AR, Vander Zanden A, Muhsinin S, Kusumaningrum T, Kusumadewi I, *et al.* Comparison of real time IS6110-PCR, microscopy, and culture for diagnosis of tuberculous meningitis in a cohort of adult patients in Indonesia. *PLoS One* 2012; *7* : e52001.
34. Jha SK, Garg RK, Jain A, Malhotra HS, Verma R, Sharma PK. Definite (microbiologically confirmed) tuberculous meningitis: Predictors and prognostic impact. *Infection* 2015; *43* : 639-45.
35. Sharma K, Sharma M, Chaudhary L, Modi M, Goyal M, Sharma N, *et al.* Comparative evaluation of xpert MTB/RIF assay with multiplex polymerase chain reaction for the diagnosis of tuberculous meningitis. *Tuberculosis* (*Edinb*) 2018; *113* : 38-42.
36. Manke SD, Husain AA, Daginawala HF, Singh LK, Kashyap RS. Comparative diagnostic utility of IS6110 PCR assay in CSF and peripheral blood samples of tuberculous meningitis patients: A pilot study from central India. *J Clin Diagn Res* 2017; *11* : BC13-7.

37. Rufai SB, Singh A, Singh J, Kumar P, Sankar MM, Singh S, *et al.* Diagnostic usefulness of xpert MTB/RIF assay for detection of tuberculous meningitis using cerebrospinal fluid. *J Infect* 2017; *75* : 125-31.

38. Kumar K, Giribhattanavar P, Chandrashekar N, Patil S. Correlation of clinical, laboratory and drug susceptibility profiles in 176 patients with culture positive TBM in a tertiary neurocare centre. *Diagn Microbiol Infect Dis* 2016; *86* : 372-6.

39. Bhatia R, Dayal R, Jindal S, Agarwal D, Goyal A. GeneXpert for diagnosis of tubercular meningitis. *Indian J Pediatr* 2016; *83* : 1353-5.

40. Panagariya A, Sureka RK, Ralot T, Sharma B, Dubey P. Clinicodiagnostic features of tuberculous meningitis and the role of CSF PCR in early diagnosis: A study from North-West India. *J Indian Med Assoc* 2013; *111* : 309-12, 314.

41. Haldar S, Sankhyan N, Sharma N, Bansal A, Jain V, Gupta VK, *et al.* Detection of *Mycobacterium tuberculosis* glcB or hspX antigens or devR DNA impacts the rapid diagnosis of tuberculous meningitis in children. *PLoS One* 2012; *7* : e44630.

42. Sharma K, Sharma A, Singh M, Ray P, Dandora R, Sharma SK, *et al.* Evaluation of polymerase chain reaction using protein b primers for rapid diagnosis of tuberculous meningitis. *Neurol India* 2010; *58* : 727-31.

43. Gualberto FAS, Gonçalves MG, Fukasawa LO, Santos AMRD, Sacchi CT, Harrison LH, *et al.* Performance of nested RT-PCR on CSF for tuberculous meningitis diagnosis in HIV-infected patients. *Int J Tuberc Lung Dis* 2017; *21* : 1139-44.

44. Bahr NC, Meintjes G, Boulware DR. Inadequate diagnostics: The case to move beyond the bacilli for detection of meningitis due to *Mycobacterium tuberculosis*. *J Med Microbiol* 2019; *68* : 755-60.

45. Cresswell F, Tugume L, Bahr NC, Kwizera R, Bangdiwala AS, Musubire AK, *et al*. Xpert MTB/Rif Ultra for the diagnosis of HIV-associated tuberculous meningitis: A prospective validation (05/13/2019 18:27:11). Available from: *https://ssrn.com/abstract=3388827*, accessed on June 25, 2019.

46. Bahr NC, Nuwagira E, Evans EE, Cresswell FV, Bystrom PV, Byamukama A, *et al.* Diagnostic accuracy of xpert MTB/RIF ultra for tuberculous meningitis in HIV-infected adults: A prospective cohort study. *Lancet Infect Dis* 2018; *18* : 68-75.

47. World Health Organization. *The use of molecular line probe assays for the detection of resistance to isoniazid and rifampicin*. Policy update. Available from: *https://apps.who.int/iris/bitstream/handle/10665/250586/9789241511261-eng.pdf?sequence =1*, accessed on July 27, 2019.

48. World Health Organization. *Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children.* Policy update. Available from: *https://apps.who.int/iris/bitstream/handle/10665/250586/9789241511261-eng.pdf?sequence=1*, accessed on July 27, 2019.

49. Coker OO, Chaiprasert A, Ngamphiw C, Tongsima S, Regmi SM, Clark TG, *et al.* Genetic signatures of *Mycobacterium tuberculosis* Nonthaburi genotype revealed by whole genome analysis of isolates from tuberculous meningitis patients in Thailand. *PeerJ* 2016; *4* : e1905.

50. Wilson MR, O'Donovan BD, Gelfand JM, Sample HA, Chow FC, Betjemann JP, *et al.* Chronic meningitis investigated via metagenomic next-generation sequencing. *JAMA Neurol* 2018; *75* : 947-55.

51. Wilson MR, Sample HA, Zorn KC, Arevalo S, Yu G, Neuhaus J, *et al.* Clinical metagenomic sequencing for diagnosis of meningitis and encephalitis. *N Engl J Med* 2019; *380* : 2327-40.

52. Ajbani K, Kazi M, Naik S, Soman R, Shetty A, Rodrigues C. Utility of pyrosequencing for rapid detection of tubercular meningitis (TBM) and associated susceptibility directly from CSF specimens. *Tuberculosis (Edinb)* 2018; *111* : 54-6.

53. Rana SV, Singhal RK, Singh K, Kumar L. Adenosine deaminase levels in cerebrospinal fluid as a diagnostic test for tuberculous meningitis in children. *Indian J Clin Biochem* 2004; *19* : 5-9.

54. Raviraj, Henry RA, Rao GG. Determination and validation of a lower cut off value of cerebrospinal fluid adenosine deaminase (CSF-ADA) activity in diagnosis of tuberculous meningitis. *J Clin Diagn Res* 2017; *11* : OC22-4.

55. Pormohammad A, Riahi SM, Nasiri MJ, Fallah F, Aghazadeh M, Doustdar F, *et al.* Diagnostic test accuracy of adenosine deaminase for tuberculous meningitis: A systematic review and meta-analysis. *J Infect* 2017; *74* : 545-54.

56. Ekermans P, Dusé A, George J. The dubious value of cerebrospinal fluid adenosine deaminase measurement for the diagnosis of tuberculous meningitis. *BMC Infect Dis* 2017; *17* : 104.

57. Vidhate MR, Singh MK, Garg RK, Verma R, Shukla R, Goel MM, *et al.* Diagnostic and prognostic value of *Mycobacterium tuberculosis* complex specific interferon gamma release assay in patients with tuberculous meningitis. *J Infect* 2011; *62* : 400-3.

58. Blok N, Visser DH, Solomons R, Van Elsland SL, den Hertog AL, van Furth AM. Lipoarabinomannan enzyme-linked immunosorbent assay for early diagnosis of childhood tuberculous meningitis. *Int J Tuberc Lung Dis* 2014; *18* : 205-10.

59. Riste M, Hobden D, Pollard C, Scriven JE. Diagnosis of tuberculous meningitis with invasive pulmonary sampling. *Lancet Infect Dis* 2018; *18* : 25-6.

60. Kohli M, Schiller I, Dendukuri N, Dheda K, Denkinger CM, Schumacher SG, *et al.* Xpert® MTB/RIF assay for extrapulmonary tuberculosis and rifampicin resistance. *Cochrane Database Syst Rev* 2018; *8* : CD012768.

61. Cresswell FV, Bangdiwala AS, Bahr NC, Trautner E, Nuwagira E, Ellis J, *et al.* Can improved diagnostics reduce mortality from tuberculous meningitis? Findings from a 6.5-year cohort in Uganda. *Wellcome Open Res* 2018; *3* : 64.

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