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REVIEW

Diagnosis of tuberculous meningitis: challenges and promises

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Abstract

Tuberculosis (TB) which is caused by *Mycobacterium tuberculosis* infects primarily the lungs but it also affects other parts of the body. Tuberculous meningitis (TBM) is the most severe form of TB and has the highest mortality and morbidity rate compared to other forms of TB. It is common in young children and HIV-infected patients, but is also seen in adults. Despite anti-tuberculosis treatment, TBM is still a major cause of death and neurological sequelae as treatment given to the patients is often delayed. Early diagnosis is challenging due to the non-specific symptoms of TBM and the low number of tubercle bacilli in cerebrospinal fluid (CSF). Until now, there is no established diagnostic method that can rapidly detect *M. tuberculos*is in TBM patients with high sensitivity and specificity. The emergence of drug resistant *M. tuberculosis* strains further complicates the diagnosis and treatment regimen of TBM. This review summarizes challenges of the currently used diagnostic methods and the potential future use of molecular diagnostic methods for TBM.

Keywords: tuberculous meningitis, diagnosis, culture, smear, PCR, GeneXpert

INTRODUCTION

Tuberculosis (TB) is a major global health problem which causes 1.4 million deaths per year.[1] TB is caused by the acid fast bacterium, *Mycobacterium tuberculosis* which primarily affects the lungs but it can also affect other parts of the body such as brain, liver, kidneys, genitals, intestine and spine. Tuberculous meningitis (TBM) is infection of the meninges, the membrane that covers the brain and represents roughly 1% of all TB diseases. It is the most severe form of TB as it causes death or severe neurological defects in more than half of those affected, despite the advancements in available antituberculosis treatment.[2,3] Age was considered the most important determinant of TBM development before HIV co-infection, in which TBM was most commonly seen in children aged between 0-4 years in populations with high TB prevalence while in populations with low TB prevalence, most cases of TBM occur in adults.[4,5] The clinical features of the TBM are hydrocephalus and vasculitis.[6] Hydrocephalus is more common in children compared to adults while vasculitis that develops due to the inflammatory process is the most serious consequence of TBM.[4,7,8] According to the British Medical Research Council (MRC), TBM can be classified based on its severity, as stage I (mild cases), stage II (moderately advanced cases) and stage III (severe cases).[9] Early clinical diagnosis of TBM is challenging since this disease presents with non-specific symptoms and low number of tubercle bacilli in cerebrospinal fluid (CSF). Diagnosis and treatment of TBM become more challenging due to the emergence of HIV and drug-resistant strains of *M. tuberculosis*. This review highlights the current challenges faced in the diagnosis of TBM and the potential use of newer molecular diagnostic methods.

DIAGNOSIS OF TBM

Early diagnosis and treatment of TBM are the most important factors in determining the outcome of the disease.[10] Early diagnosis is difficult as TBM shows non-specific clinical symptoms such as fever, headache, vomitting and cough. Persistence of these symptoms is the only factor that differentiates TBM from other common diseases such as influenza.[11] It is necessary to recognize TBM in early stage from non-specific symptoms rather than from the

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classic signs of meningitis. Numerous studies have been conducted in search of more rapid, sensitive and specific methods of diagnosis for TBM and these methods include definite microbiological confirmation such as culture, smear, PCR and supportive diagnostic methods such as radiographic assessments, cytology analysis, antibody and antigen detection, and GC-MS.[2]

Microbiological assays are slow and not sensitive to detect *M. tuberculosis* in CSF

The identification of *M. tuberculosis* in CSF by culture is the gold standard and various culture techniques have been evaluated for their performance and each have different advantages and disadvantages. Culture on solid medium such as Lowenstein-Jensen (LJ) enables examination of colony morphology and it takes long time for the result to be obtained, which is about 28 - 50 days.[12-14] Results can be obtained faster in commercially available automated systems such as Bactec MGIT 960 (Becton Dickinson, Sparks, MD, USA), radiometric Bactec 460 (Becton Dickinson, Heidelberg, Germany), MB Bact (Organon Teknika, Boxtel, Netherlands) and ESP II (Difco Laboratories, Detroit, MI, USA) and they are not suitable to use for routine diagnosis due to their high cost.[13,14] Microscopically Observed Drug Susceptibility assay (MODS) that uses Middlebrook 7H9 liquid medium offers a lower cost and a more rapid diagnosis as the early stage of characteristic cord formation of *M. tuberculosis* can be visualized under the microscope.[14-16] This assay allows incorporation of drug susceptibility testing (DST) simultaneously with the culture thus permit a faster detection of drug-resistant *M. tuberculosis*.[15] Although diagnosis based on culture is the reference standard, results are obtained only after 2-8 weeks of incubation which is too slow to aid in clinical decision-making.[17] Overall, the sensitivity of culture to detect *M. tuberculosis* in CSF sample is low and range from 40-60% as culture is less sensitive in paucibacilliary conditions.[17,18] Moreover, it also requires appropriate biological hazard containment facilities and aseptic technique that limits its use.[19] The DST for the identification of drug-resistance by culture methods detects bacterial growth in the presence of antibiotics.[20] The critical concentration of drug that used in DST varies from drug to drug and usually depends on the culture medium used.[21] While DST for isoniazid (INH), rifampin (RIF), fluoroquinolones and some of the second-line injectable drugs is reported to be reliable in solid- and liquid-media systems, DST for other drugs such as ethambutol (EMB), pyrazinamide (PZA) and other second-line drugs are much more complex and has not been standardized.[20]

Smear microscopy with traditional Ziehl-Neelsen stain is a rapid and practical method for routine analysis due to its low cost, and high predictive value.[22] The sensitivity of Ziehl-Neelsen stain in detecting acid-fast bacilli (AFB) in CSF is generally low and range from 10-60%.[17] Moreover, large volume (10-15ml) of CSF is required for a more sensitive result but it is difficult to obtain in children who have a low total volume of CSF.[23] A simple modification of Ziehl-Neelsen combined with cytospin and Triton X-100 permeabilization which enables the identification of both extracellular and intracellular *M. tuberculosis* shows improved detection of AFB in CSF samples.[22,24] However, this technique needs to be replicated in larger studies for reliability before it can be accepted as an established diagnostic method.

Cytological examination of the CSF in TBM show a characteristic mononuclear lymphocytic-predominant pleiocytosis in 60-85% of the patients, in which the total white counts range between 100 and 500 cells/µl, with elevated protein levels, typically between 100 and 500 mg/dL and low glucose level, usually less than 45mg/dL.[25-29] These moderate changes in CSF mimic the profile of a large list of nontuberculous and bacterial infections that affects the CNS making cytology not specific for the diagnosis for TBM.[2]

Molecular analysis is more rapid and superior for TBM diagnosis

Nucleic acid amplification technique (NAAT) such as polymerase chain reaction (PCR) for detection of mycobacterial DNA has been reported to be more rapid, sensitive and specific.[30] Studies show the sensitivity of PCR assay for TBM range from 31% to 100% and specificity from 66% to 100%. The paucibacilliary nature and presence of amplification inhibitor in CSF specimen are the main challenges of applying the PCR method to detect *M. tuberculosis*.[3] Moreover, the cell wall of *M. tuberculosis* is made of an impermeable complex structure that makes the lysis of the cell difficult and thus result in poor quality and low yield of nucleic acids when simple and common nucleic acid isolation procedures are used.[31] Physical methods of lysis

such as shock treatment (freezing and heating) or Triton X-100 treatment combined with any other DNA extraction procedures are shown to improve the yield of nucleic acids isolated from *M. tuberculosis* for PCR preparation.[31,32]

Several *M. tuberculosis* DNA-specific sequences such as IS6110 insertion sequence, protein antigen B, MPB64 and 65kDa have been evaluated by NAA assays.[18,33,34] The IS6110 insertion sequence was mostly used in earlier PCR based studies due to its repetitive nature and had been shown to have superior amplification efficiency in many of the studies in which the overall sensitivity and specificity ranged between 32-100% and 38-100% respectively.[33,35-45] Absence of the IS6110 sequence in some *M. tuberculosis* isolates had been reported in few studies.[33,46] MPB64 gene is regarded the most specific sequence for PCR assay in the detection of *M. tuberculosis* and had been used as a target sequence in many studies.[33,47-57] The sensitivity and specificity of PCR assay targeting MPB64 gene in these studies showed a relatively good result, ranging 53-100% and 85-100% respectively. Most of the PCR based studies to diagnose TBM have used single gene target for amplification and it may cause false negative results as the target gene might be absent in some of *M. tuberculosis* isolates. To overcome this problem, multiplex PCR that target and amplify several genes simultaneously can be used. The sensitivity and specificity of multiplex PCR using protein b, MPB64, and IS6110 primers for confirmed TBM cases was shown to be 94.4% and 100% respectively.[18]

Various modifications of the conventional standard PCR assay have been tried for the diagnosis of TBM.[3] Nested PCR, a modified version of PCR technique designed to increase the amplification efficiency and to reduce the level of nonspecific PCR products had been shown to have higher sensitivity and specificity than the conventional PCR.[3] Studies have reported that both sensitivity and specificity of the nested PCR ranged from 90-100% and this method was able to detect as low as 1-10 copies/2 µl of purified *M. tuberculosis* DNA.[38,49,54] The sensitivity of nested PCR was reported to be approximately 1000-10,000 times higher than the conventional standard PCR assay.[49,54] A novel PCR method, Wide-Range Quantitative Nested Real-Time PCR (WR-QNRT-PCR) assay combines the high sensitivity of nested PCR with the accurate quantification of real-time (TaqMan) PCR.[58] Application of WR-QNRT-PCR assay shows high sensitivity (95.8%) and specificity (100%) for clinically suspected TBM patients.[57] Although nested PCR and WR-QNRT-PCR assays have high sensitivity and specificity in detecting of *M. tuberculosis*, these assays have rarely been used for TBM diagnosis due to complicated, laborious and time-consuming procedures which render them not practical for routine clinical analysis.[3] Rapid molecular methods that can identify specific mutations responsible for drug resistance such as direct-sequencing and commercial assays are reported to have high sensitivity and specificity.[59,60] The application of molecular methods is not universally used as some require high cost and sophisticated laboratory infrastructure. Moreover, the numbers of genes that can be analyzed remains limited.[61]

GeneXpert MTB/RIF System is promising and needs validation

The GeneXpert MTB/RIF System (Cepheid, Sunnyvale, CA, USA) is a fully automated, single use closed-cartridge-based real-time PCR that performs sample decontamination, sonication, automated nucleic acid amplification, and fluorescence-based quantitative PCR.[62,63] It is designed to detect MTB and rifampicin susceptibility simultaneously within two hours with high accuracy for the detection of pulmonary TB (sensitivity 89%, specificity 99%) and rifampicin resistance (sensitivity 95%, specificity 98%).[64] GeneXpert MTB/RIF had been approved by WHO for *M. tuberculosis* detection in sputum while its diagnosis value for the detection of non-respiratory TB is uncertain.[17,30,63,65,66] A systemic meta-analysis by Denkinger and colleagues show that the sensitivity of GeneXpert for extra-pulmonary TB varied widely across different sample types in which the detection rate for TBM was only moderate.[66] Studies with extrapulmonary TB samples have been reported promising in smear-positive samples compared to smear-negative specimens (sensitivity 96–100% vs. 37–90% and specificity 98–100%).[67-71] It is reported by Nhu and colleagues that the sensitivity of GeneXpert MTB/RIF for diagnosing TBM was lower than smear and culture (59.3% Vs 78.6% and 66.5%).[63] Larger studies to assess the usefulness of GeneXpert MTB/RIF for diagnosis of TBM is required.

Biochemical analysis is not very specific for TBM

Antibody detection methods such as enzyme-linked immunosorbent assay (ELISA), enzyme-linked immunospot (ELISPOT), immuno-fluorescent assay (IFA) and passive hemagglutination assay are rapid to detect *M. tuberculosis*-specific antibodies in CSF with varying sensitivity and specificity.[2] Antibody detection is limited by its inability to differentiate acute infection from previous infection and problems with cross-reactivity.[2,72] The sensitivity and specificity of *M. tuberculosis*-specific antigen detection directly in CSF are shown to be in the range of 35-95% and 95-100% respectively.[2] However, antibody and antigen detection are not recommended as routine tests to diagnose TBM.

Adenosine deaminase (ADA) measurement is simple, inexpensive and rapid and studies show that its level is significantly elevated in TBM patients compared to the non-TBM infectious meningitis and thus it can be used as a supportive diagnostic finding for TBM.[73-76] The sensitivity and specificity of ADA measurement for diagnosis of TBM ranged from 60-90% and 80-90% respectively.[17] ADA assay is not recommended for routine analysis since the assay has not been standardized and the cut-off level that defines a positive result has not been determined.[77,78] Also, ADA test is not useful in distinguishing HIV-infected TBM patients from other clinically similar neurological illnesses.[78,79]

Gas chromatography mass spectrometry (GC-MS) measurement of tuberculostearic acid (TSA), which is a component of *M. tuberculosis* cell wall in CSF of TBM patients showed a high sensitivity ranging between 80-100%.[80-83] However, the clinical use of this technique is limited due to expensive equipment and complexity of sample preparation protocol.[2,84]

TBM IS TREATABLE AND PREVENTABLE

The optimal antibiotic therapy for TBM has not yet been definitely established since recommendations by different societies and expert groups vary. For patients with drug sensitive TB, the treatment consists of 2 months of daily INH, RIF, PZA, and either streptomycin (SM) or EMB, followed by 7-10 months of INH and RIF as maintenance therapy.[85-87] INH is the most critical and important drug due to its excellent penetration into the CSF and high bactericidal activity.[23,88] RIF penetrate the CSF less freely, however, it is important in treating patients infected with RIF-susceptible strains.[23] PZA is also excellent in penetrating the CSF and is considered as a key drug in shortening the treatment period for drug-susceptible TB.[89] SM and EMB could be used for short periods or when needed as both can produce significant toxicity with long-term use.[89] *M. tuberculosis* is able to undergo spontaneous mutation, which results in resistant mutants and are not killed during the treatment. Multidrug-resistant tuberculosis (MDR-TB) is caused by the strains resistant to at least two of the most potent first-line anti-TB drugs such as INH and RIF.[90,91] XDR-TB is caused by *M. tuberculosis* strains that are resistant to not only INH and RIF but also to at least three of the six classes of second-line anti-TB drugs such as fluoroquinolones, aminoglycosides, polypeptides, thioamides, cycloserine and para-aminosalicylic acid.[61,92] Drug resistance in TBM pose a greater problem due to variability of each drug to cross the blood brain barrier (BBB) to achieve sufficient intracerebral concentrations to kill the bacilli.[93] It has been reported that the time to sterilize CSF infected with INH-resistant strain is longer compared with the INH-susceptible strain.[94]

Most of the neurologic sequelae of TBM are considered due to the excessive host-inflammatory response to the presence of *M. tuberculosis,* causing tissue injury and brain oedema.[95] Thus, the use of dexamethasone as adjunctive corticosteroid therapy improves the patients probability of survival and has been shown to be most beneficial when administered at the mild stage or when there is increased intracranial pressure, cerebral oedema and spinal block.[6,9,96-98]

The role of surgery in TBM has been considered as an important treatment in dealing with serious complication of hydrocephalus, tuberculoma and brain abscess. Patients with communicating hydrocephalus are treated with furosemide and acetazolamide in addition to the standard antituberculosis therapy while patients with non-communicating hydrocephalus are treated with ventriculoperitoneal shunt placement (VPS) and endoscopic third ventriculostomy (ETV).[2,23,99]

Currently, BCG is the only licensed vaccine for TB and is usually administered to infants. It is able to protect young children aged below 5 years against extrapulmonary TB.[100] The efficacy

TABLE 1: Laboratory diagnostic methods for tuberculous meningitis

Diagnosis method		Time	Sensitivity	Specificity	Reference
I. Microbiological analysis	Culture	2 - 8 weeks	40 - 60%		17
	Smear	1-2 days	10 - 60%		17
II. Molecular analysis	PCR	1-2 days	31 - 100%	66 - 100%	3
	IS6110	1-2 days	32 - 100%	38 - 100%	33, 35-45
	MPB64 gene	1-2 days	53 - 100%	85 - 100%	33, 47-57
	Multiplex PCR: Protein b, MPB64, IS6110	1-2 days	94.4%	100%	18
	Nested PCR	1-2 days	90 - 100%	90-100%	38, 49, 54
	Wide-Range Quantitative Nested Real-Time PCR (WR-QNRT-PCR)	1-2 days	95.8%	100%	57
	GeneXpert MTB/RIF	1-2 days	96 -100% (smear +) 37 - 90% (Smear -)	98 - 100%	67-71
III. Biochemical analysis	*M. tuberculosis* antigen detection	1-2 days	35 - 95%	95 - 100%	2
	Adenosine deaminase (ADA)	1-2 days	60 - 90%	80-90%	17
	Tuberculostearic acid (TSA)	1-2 days	80 - 100%		80-83

of BCG to protect against TBM is reported to be 75-85% although it varies tremendously in the protection of adults from pulmonary TB.[2,101] Several studies reported that the beneficial effect of the BCG against TBM might be due to the nutritional status and BCG just delays the onset of TBM.[102,103] Significant efforts have been made in recent years to develop newer vaccines particularly to boost neonatal BCG vaccination such as MVA85A, Aeras-402, M72F, H56, ID93, Hybrid-4 which are all still in trial phase and intensive studies are being carried out to evaluate their efficacy.[101]

CONCLUSION

The diagnosis and treatment of TBM poses a challenge since there are no rapid, sensitive and specific methods for detecting the few tubercle bacilli in CSF. The emergence of drug resistant *M. tuberculosis* strains and HIV further complicates diagnosis and treatment regimens of TBM, thus resulting in high mortality and morbidity. Efforts are required to develop a more rapid, sensitive and specific method for diagnosis of TBM and to search for new drugs that are less toxic and have high penetration in CSF, and to develop newer vaccines that can protect against *M. tuberculosis* infection. Table 1 summarises the laboratory diagnostic methods. Molecular methods particularly multiplex PCR and GeneXpert show promise for the future diagnosis of TBM while combining microbiological methods with molecular techniques will be beneficial for rapid detection and treatment of TBM.

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