Chapter 20

Progress on Diagnosis of Tuberculous Meningitis

Yi-yi Wang and Bing-di Xie

Abstract

Central nervous system (CNS) disease caused by Mycobacterium tuberculosis (MTB) is highly devastating. Tuberculous meningitis (TBM) is the most common form of CNS tuberculosis (TB). Rapid, sensitive, and affordable diagnostic tests are not available. Ziehl–Neelsen (ZN) stain has a very low sensitivity in cases of TBM, the sensitivity rates is of about 10–20%. The detection rate can be improved by taking large volume CSF samples (>6 ml) and prolonged slide examination (30 min). Culture of MTB from the CSF is slow and insufficiently sensitive. The sensitivity is different, which varies from 36% to 81.8%. The microscopic observation drug susceptibility (MODS) assay was recommended by the World Health Organization in 2011. The sensitivity is 65%, which is more sensitive and faster than CSF smear. Commercial PCR assays were found to be insensitive at detecting MTB in CSF samples. Many research provided the value of ADA on the TBM diagnosis. Interferon-gamma release assays (IGRAs) are not recommended for diagnosis of active TB disease. Imaging is essential in diagnosis and showing complications of CNS TB. Thwaites criteria and the Lancet consensus scoring system (LCSS) were developed to improve the diagnosis of TBM. Clinicians will continue to make judgment based on clinical examination, inflammatory CSF examinations, imaging studies, and scoring systems.

Key words Tuberculous meningitis, Diagnosis, Progress, Scoring system

1 Introduction

According to WHO global tuberculosis report of 2015, the tuberculosis (TB) remained one of the top ten causes of death worldwide in 2015. There were an estimated 1.4 million TB deaths in 2015. Central nervous system (CNS) disease caused by Mycobacterium tuberculosis (Mtb) is highly devastating [1]. CNS tuberculosis accounts for approximately 1–5% of all cases of tuberculosis [2, 3]. The types of CNS TB involve intracranial TB and intraspinal TB. The types of intracranial TB involve TB meningitis (TBM), complications of TBM, sequel of TBM, and parenchymal TB. Complications of TBM include hydrocephalus, tuberculous vasculitis, and cranial nerve involvement. The types of parenchymal TB involve tuberculomas, tuberculous abscess, tuberculous cerebritis, and tuberculous encephalopathy [4]. Tuberculous
meningitis is the most common presentation among CNS tubercu-
losis, which remains a formidable diagnostic challenge [5]. Mortal-
ity and long-term disability remain unacceptably high [6]. Despite
antituberculosis chemotherapy, according to literature report, mor-
tality of TBM is high, which varies from 10% to 36.5% [7–10].

Rapid, sensitive, and affordable diagnostic tests are not avail-
able. What is the progress on the diagnosis of tuberculous menin-
gitis? The purpose of this review is to discuss recent advances and
describe the utility and limitations of current diagnostic methods
for TBM (Table 1).

2 Microscopy

2.1 Ziehl–Neelsen (ZN) Stain

Detection of acid-fast bacilli (AFB) in patient samples using
Ziehl–Neelsen (ZN) staining is the most practical and universally
adopted test for diagnosing TB. The ZN stain, also known as the
acid-fast stain, was first described by two German doctors: the
bacteriologist Franz Ziehl (1859–1926) and the pathologist Frie-
drich Neelsen (1854–1898). It is a special bacteriological stain used
to identify acid-fast organisms, mainly Mycobacterium tuberculosis.
The CSF of most patients with TBM contains only 10^0–10^2 organ-
isms/ml, yet approximately 10^4 organisms/ml are required for
reliable detection with ZN stains [23]. The limit of detection on
microscopy is 100 mycobacteria/ml [24]. Although the sensitivity
of ZN stain in different studies varies considerably (0–87%) [3], it
has a very low sensitivity in cases of TBM; the sensitivity rate is of

The detection rate of smear microscopy in TBM can be
improved by taking large volume CSF samples (>6 ml) and pro-
longed slide examination (30 min). For example, Thwaites et al.
have increased the positive rate to 58% with a prolonged slide
examination (median 10 min) [12]. However, these criteria are
rarely achieved in practice.

In high-income countries, fluorescence microscopy is the stan-
dard diagnostic method in ZN stain, which has improved the
sensitivity of microscopy over conventional ZN staining (by approximates 10% in sputum) and significantly decreased the
time required to examine each slide [25]. The equipment and bulbs
of fluorescent microscopy using fluorochrome dye (auramine-O or
auramine-rhodamine) are more expensive [25]. The development
of light-emitting diode (LED) fluorescent microscopy (FM) is less
expensive than mercury vapor fluorescence microscopes and is now
recommended by the World Health Organization [26]. Using
mycobacterial culture as a reference standard, the sensitivity of
LED-FM is higher than conventional fluorescence microscope in
the sputum, other respiratory samples, and extrapulmonary sam-
ple samples. In extrapulmonary samples, the sensitivity of LED-FM is 50%
(95% CI 23.0–77.0), which is higher than conventional fluorescence microscope with 35.7% (95% CI 12.8–64.9). Specificity was very similar between conventional fluorescence microscope and LED-FM [26]. However, until now, the evidence is insufficient.

### Table 1

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Characteristic</th>
<th>Category</th>
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</thead>
<tbody>
<tr>
<td><strong>Microscopy</strong></td>
<td></td>
<td>Special to identify MTB</td>
<td>The most practical and universally adopted test</td>
<td>1. Large volume CSF samples (&gt;6 ml)</td>
</tr>
<tr>
<td><strong>Culture of Mtb</strong></td>
<td>36–81.8% [12–15]</td>
<td>Special to identify MTB</td>
<td>Slow and insufficient sensitive</td>
<td>1. Solid culture medium</td>
</tr>
<tr>
<td><strong>MODS</strong></td>
<td>65% [16]</td>
<td>Special to identify MTB</td>
<td>Sensitive and faster</td>
<td>2. Liquid culture medium</td>
</tr>
<tr>
<td><strong>NAAT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The Xpert MTB/RIF assay</td>
<td>27–86% [18, 19]</td>
<td>95% [20]</td>
<td></td>
<td>2. In-house</td>
</tr>
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<td><strong>IGRAs</strong></td>
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<tr>
<td><strong>ADA</strong></td>
<td>29.9–79% [14, 22]</td>
<td>91% [22]</td>
<td>1. ADA values from 1 to 4 U/l helped to exclude TBM</td>
<td>2. T-SPOT.TB</td>
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<td></td>
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<td>2. Values between 4 and 8 U/l were insufficient to confirm or exclude the diagnosis of TBM</td>
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<td>3. Values &gt;8 U/l improved the diagnosis of TBM</td>
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</table>

*Mtb* mycobacteria, *MODS* the microscopic observation drug susceptibility, *NAATs* nucleic acid amplification tests, *IGRAs* interferon-gamma release assays, *QFT-IT* QuantiFERON-TB© Gold In Tube, *ADA* adenosine deaminase
2.2 Culture of Mycobacterium tuberculosis

Culture of *Mycobacterium tuberculosis* from the CSF of TBM patients is slow and insufficiently sensitive. The sensitivity is different, which varies from 36% to 81.8% [12–15]. A further increase of sensitivity was observed when the solid and liquid culture medium were used at the same time.

Traditionally, solid culture media for culture of Mtb are kept for up to 8 weeks before a negative result is reported to the physician. Reduced turnaround times have been achieved using broth-based culture compared with solid media for the isolation of Mtb (13 days vs. 26 days) [27]. Most incubation protocols still require a maximum of 6 weeks [28]. Culture of Mtb is used as a “rule-in” test not a “rule-out” diagnostic test. The research from India suggested that for CSF samples, both liquid and solid culture media should be used for optimal detection and should be incubated for longer period (up to 8–10 weeks) than routine culture [29]. Another research attempted to shorten the incubation time of mycobacterial cultures. The study from Swiss indicated that 58.3% of all mycobacteria were detected within 14 days, 37.5% were detected within 21 days, and 4.2% were detected within 28 days [30]. It seems that a final report can be issued after 4 weeks.

2.3 The Microscopic Observation Drug Susceptibility

The microscopic observation drug susceptibility (MODS) assay was developed by Caviedes in 2000. He found that Mycobacterium tuberculosis form the characteristic cable structure in liquid medium. MODS is a kind of liquid culture [31]. Processed CSF is inoculated into a microtiter plate containing broth media and incubated. Growth is examined by an inverted microscope. In TBM patients, the sensitivity is 65%, which is more sensitive than CSF smear. The detection time is median 6 days, which is faster than commercial liquid/solid culture [16]. MODS also performed well in drug susceptibility testing (DST): isoniazid DST concordance was 95.7% (kappa 0.85); rifampicin DST concordance was 96.8% (kappa 0.91) [32]. MODS was recommended by the World Health Organization in 2011.

3 Nucleic Acid Amplification Tests

The conventional tests including microscopy and culture are often limited in the diagnosis of TBM since TBM is a paucibacillary form of tuberculosis. Nucleic acid amplification tests (NAAT) can detect fewer than ten organisms that can be used to identify *M. tuberculosis* in clinical specimens or cultures [33]. The first NAAT for use on CSF specimens was developed since 1990 [34].
3.1 The Polymerase Chain Reaction

The polymerase chain reaction (PCR) is the most common methodology, but alternatives are heterogeneous including real-time PCR, isothermal, strain displacement, or transcription-mediated amplification and ligase chain reaction [35]. NAATs are categorized as commercial or in-house. Most (>90%) laboratories used commercial kits such as the Amplicor *M. tuberculosis* tests (Roche Molecular Systems, Branchburg, NJ, USA) and the Amplified *M. tuberculosis* Direct Test (MTD; Gen-Probe Inc., San Diego, CA, USA). The literature on NAATs has been extensively reviewed. The significant heterogeneity in sensitivity and specificity of in-house PCRs led to no useful comparative information could be obtained [17, 35]. Commercial assays were found to be insensitive at detecting Mtb in CSF samples (sensitivity 56% and specificity 98%) [17]. The specificity of NAATs was high applied to body fluids, for example, for TB meningitis, but sensitivity was poor, indicating that these tests cannot be used reliably to rule out TBM. NAATs improve diagnostic certainty but do not replace microscopy and culture.

3.2 The Xpert MTB/RIF Assay

The Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA) simultaneously detects the presence of *Mycobacterium tuberculosis* and its susceptibility to the rifampin in less than 2 h [36]. The assay is entirely automated, requiring only two manual steps. In 2010, the WHO endorsed the use of Xpert MTB/RIF for use on sputum specimens. Xpert MTB/RIF has been extensively evaluated for *Mycobacterium tuberculosis* detection in sputum specimens and performs well on smear-positive samples (sensitivity 98% compared with 68% in smear-negative samples; specificity 98%) [37]. Although the assay is not recommended by the World Health Organization for the diagnosis of TBM, several studies have evaluated the use of Xpert MTB/RIF for the diagnosis of TBM. The significant heterogeneity has also been found in sensitivity of Xpert MTB/RIF from 27% to 86%. An assay from India reported the sensitivity of Xpert MTB/RIF is 86% [18]. Three studies indicated that the sensitivity of Xpert MTB/RIF was about 60%, which is higher than that of smear microscopy [20, 38, 39]. The report from Tortoli in 2012 indicated the sensitivity is only 27% [19]. Further studies still are required as the studies that have been performed have small subject numbers. The other ability of Xpert MTB/RIF is to detect drug resistance. Xpert MTB/RIF is the only WHO-recommended rapid diagnostic test for detection of TB and rifampicin resistance. However the specificity is lower than the conventional DST. All detected rifampicin-resistant isolates should ideally be confirmed with conventional DST to detect false-positive results [40].
4 Interferon-Gamma Activity and Interferon-Gamma Release Assays (IGRAs)

Interferon-gamma release assays (IGRAs) are whole-blood tests that detect immune responses to a panel of *M. tuberculosis* antigens, which include the measurement of interferon-gamma release in whole blood (QuantiFERON-TB® Gold In Tube [(QFT-IT); Cellestis Limited Chadstone, Vic., Australia] and peripheral blood mononuclear cells (T-SPOT.TB; Oxford Immunotec, Abingdon, UK). On infection of *M. tuberculosis*, macrophages recognize the mycobacteria by toll-like receptor (TLR) followed by phagocytosis and control of mycobacteria. In addition, macrophages also secrete IL-12 to induce IFN-γ production by T cell, which, in turn, increases the phagocytosis and oxidative burst [41]. IGRAs as a high specificity diagnostic tool in TBM received preliminary much attention. However, IGRAs are not recommended for diagnosis of active TB disease. In a meta-analysis, the sensitivity estimates among HIV-infected persons were 76% (95% CI, 45–92%) for T-SPOT and 60% (95% CI, 34–82%) for QFT-GIT [42]. There was no evidence that IGRA was more sensitive than the tuberculin skin test for active tuberculosis diagnosis [42, 43]. The use of IGRAs directly on CSF specimens has been evaluated for the diagnosis of TBM, based on the premise that mononuclear cells localized to infected sites produce more interferon than peripheral blood mononuclear cells PBMC [21]. However, the sensitivity is variable [44, 45]. CSF IGRAs require large volumes of CSF. It is a barrier to perform in practice.

5 Adenosine Deaminase

Adenosine deaminase (ADA) is an enzyme required for the conversion of adenosine to inosine and is found in many tissues, particularly in T lymphocytes from the lymphoid tissue [46]. ADA exists as two isoenzymes: ADA1 and ADA2. It appears that the ADA2 isoenzyme originates mainly from monocytes and macrophages. In tuberculous pleural effusions, most of the ADA activity consists of ADA2 [47]. High ADA levels in tuberculosis appear to be related to the subset of activated T lymphocytes in response to tuberculous antigens. The use of ADA in CSF diagnosis of tuberculosis started from 20 years ago. Many research provided the value of ADA in the TBM diagnosis, but the results are conflicted. According to a meta-analysis from China in 2010, the sensitivity of ADA in the diagnosis of TBM was 0.79 (95% CI 0.75–0.83) and specificity 0.91 (95% CI 0.89–0.93) [22]. A recent study evaluated the performance of ADA tests in 506 patients with microbiologically confirmed TBM. The sensitivity of the ADA was 29.9% [14]. There is a lack of standardization in the ADA cutoff value
for diagnosing TBM. Standardized cutoffs of ADA values for the diagnosis of TBM have not been established, and the values used in the various studies ranged from 5.0 to 15 U/l [2]. Accordingly the different cutoff, the sensitivity, and the specificity are different. ADA values from 1 to 4 U/l (sensitivity >93% and specificity <80%) helped to exclude TBM; values between 4 and 8 U/l were insufficient to confirm or exclude the diagnosis of TBM (P = 0.07), and values >8 U/l (sensitivity <59% and specificity >96%) improved the diagnosis of TBM (P < 0.001). Based on the ROC curve, the ideal cutoff was 5.3 U/l (84% sensitivity and specificity) [48].

6 Cerebral Imaging

Imaging is essential in diagnosis and showing complications of CNS TB and has the advantages of being noninvasive and quick to perform and report, providing the potential for an improved prognosis. MR is superior to CT because it allows earlier detection of the disease, a more exact definition of the spread, and a more detailed representation of complex inflammatory processes [49]. Przybojewski and colleagues identified four features with 100% specificity for TBM: basal enhancement, hydrocephalus, tuberculoma, and infarction in the supratentorial brain parenchyma and brain stem [50]. Four individual criteria had a specificity of 100%, but the sensitivities of these criteria ranged from 15% to 53% only. The above conclusion derived from pediatric case study. In an adult case study, the results showed that of the five major CT features supporting a diagnosis of TBM (hydrocephalus, infarcts, tuberculoma(s), basal meningeal enhancement, and the presence of precontrast basal hyperdensities), hydrocephalus and meningeal enhancement were the most commonly found consensus features in TBM but that the other features were rare [51]. However basal meningeal enhancement is less often detected in adults than in children with TBM. Only 8–34% of cases had this feature and 45% hydrocephalus [1, 52]. So, if CT features are absent, the TBM cannot be ruled out.

Tubercular hydrocephalus is usually communicating. It occurs because thick gelatinous exudate develops around the basal cisterns, the Sylvian fissure, and the brainstem causing obstruction to CSF flow [53]. Noncommunicating or obstructive hydrocephalus can occur because narrowing of the aqueduct and third ventricle by a small tuberculoma causes consequent hydrocephalus [54].

Cerebral infarction occurs in 15–57% of tuberculous meningitis patients, mainly during stage 3 of the illness [55]. Most infarcts involve the thalamus, basal ganglia, and internal capsule regions [56]. Vasculitis and vasospasm are the causes of cerebral infarction in tuberculous meningitis infections [57]. Cerebral infarction is
associated with leptomeningeal enhancement in TBM. The exudate at the basal region surrounds the arteries, leading to arterial narrowing and subsequently stroke. The intense inflammation also causes vasculitis and vasospasm in the nearby vessels [58].

Tuberculomas are among the most common intracranial mass lesions and the most common manifestation of parenchymal TB. They usually occur in the absence of TBM but may occur with meningitis. Tuberculomas may be single or multiple and can be seen anywhere in the brain parenchyma. The number of identified lesions per patient may range from 1 to 12 (or more), with the size varying from 1 mm to 8 cm [59]. Tuberculomas show typical granulomatous reaction. Histopathology is characterized by the presence of epithelioid granuloma with Langhans giant cells. In response to the infection, the activated macrophages, cytokine interferon (IFN), and T cell activity produce a type IV reaction. This reaction combined with ischemia results in central caseation necrosis in the tuberculous granuloma [60]. Imaging findings depend on the stage of tuberculoma, whether it is noncaseating or caseating with solid or liquid center [61]. At the early stage of the tuberculomas, caseating has not yet formed. Tuberculoma usually appears hyperintense on T2W and slightly hypointense on T1W images, which show homogenous enhancement on postcontrast T1W images. A solid caseating tuberculoma appears relatively iso- to hypointense on both T1W and T2W images with an iso- to hyperintense rim on T2W images. It shows rim enhancement on postcontrast T1W images. When the solid center of the caseating lesion liquefies, the center appears hyperintense with a hypointense rim on T2W images. The postcontrast T1W images show rim enhancement [62].

Miliary brain tuberculosis is usually associated with TBM. They typically occur in immunocompromised patients. The infection is characterized by a large amount of M. tuberculosis. Miliary tubercles range from 1–5mm in size and have a mean 2mm which are either not visible on conventional SE MRI images or are seen as tiny foci of hyperintensity on T2W acquisitions. The postcontrast T1W images show numerous, round, small, homogeneous, enhancing lesions [63].

## 7 Scoring System

Given lack of a gold standard, clinicians will have to continue to use their clinical judgment based on clinical examination, inflammatory cerebrospinal fluid (CSF) examinations, imaging studies, and scoring systems, to make the diagnosis and initiate prompt treatment [64]. In 2002, Thwaites GE compared the clinical and laboratory characteristics of tuberous and purulent meningitis and proposed the Thwaites scoring system [65]. In 2005, Thwaites GE modified
possible tuberculous meningitis diagnostic criteria [7]. According to Thwaites criteria, definite, probable, or possible TBM is classified based on the clinical findings, CSF criteria, and the evidence of tuberculosis elsewhere. The results in 2005 from Sunbul suggested that the sensitivity of the Thwaites standard is 95.6%, with a specificity of 70.8% [66]. The results from Shanghai Huashan Hospital showed that the sensitivity is 98.2% and specificity 82.9% [67]. Thwaites’ score is simple, cost-effectiveness, more effective and rapid diagnostic tests. These are needed in the primary care setting where imaging facilities are lacking.

In 2010, a uniform research case definition—the Lancet consensus scoring system (LCSS) for TBM—was developed to improve standardization of diagnosis [68]. LCSS also classifies cases as definite, probable, or possible. Classification is based on a composite score of clinical findings, CSF criteria, cerebral imaging criteria, and the evidence of tuberculosis elsewhere. Cerebral imaging criteria are recommended in LCSS. The LCSS is more detailed and resource intensive. The study demonstrated that the widely used Thwaites’ score compares well with the more detailed and resource intensive Lancet consensus score [64].

8 Conclusions

The best way to improve survival of TBM is by rapid accurate diagnosis and prompt initiation of therapy. There have been encouraging developments in the diagnosis of TBM. However CSF contains low organism numbers, which limit current diagnostic modalities. Because a gold standard is still lacking, clinicians will have to continue make judgment based on clinical examination, inflammatory CSF examinations, imaging studies, and scoring systems. It seems logical that clinicians need to understand the characteristics of the diagnosis, so as to make a comprehensive judgment of the disease.

References


