Letter to the Editor

Diagnostic Value of Cerebrospinal Fluid T-SPOT.TB for Tuberculousis Meningitis in China

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The aim of this study was to evaluate the diagnostic value of the cerebrospinal fluid (CSF) T-SPOT.TB test for the diagnosis of TB meningitis (TBM). A retrospective analysis of 96 patients with manifested meningitis was conducted; T-SPOT.TB test was performed for diagnosing TBM to determine the diagnostic sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). A receiver operating characteristic (ROC) curve was also drawn to assess the diagnostic accuracy. The sensitivity, specificity, PPV, and NPV of CSF T-SPOT.TB test were 97.8%, 78.0%, 80.3%, and 97.5%, respectively, for 52 patients (54.2%) of the 96 enrolled patients. The area under the curve (AUC) was 0.910, and the sensitivities of CSF T-SPOT.TB for patients with stages I, II, and III of TBM were 96.7%, 97.2%, and 98.9%, respectively. CSF T-SPOT.TB test is a rapid and accurate diagnostic method with higher sensitivity and specificity for diagnosing TBM.

Key words: T-SPOT.TB; Tuberculous meningitis; Cerebrospinal fluid; Diagnosis

Tuberculosis (TB) is still a global public health problem, with an estimated 10.4 million new TB cases worldwide and 918,000 TB cases in China in 2015[1]. The incidence of extrapulmonary TB is about 25%-30%[2]. The diagnosis of extrapulmonary TB is generally more difficult than that of TB. Tuberculous meningitis (TBM) is a type of extrapulmonary TB accounting for approximately 1% of all TB cases[3]. Despite this low proportion of TBM among TB cases, TBM remains the most lethal form of TB, with a fatality rate of up to 50% and 20%-30% of surviving patients having permanent sequelae of the central nervous system[4]. Therefore, rapid and effective diagnosis and early treatment of TBM are particularly critical factors to determine the outcome. However, the clinical manifestations of TBM lack specificity, due to which it is notoriously difficult to diagnose TBM promptly. The detection of acid-fast bacilli (AFB) in cerebrospinal fluid (CSF) smear can directly be used to diagnose TBM, although the detection rate is low[5]. Furthermore, despite a higher sensitivity of the Lowenstein-Jensen (L-J) culture medium, it cannot be used to guide the clinical diagnosis and timely treatment as this test requires a long time to provide a result.

The T-SPOT.TB test that was developed gradually in recent years is a diagnostic method for TB and is based on the detection of IFN-gamma-secreting T cells by specific antigen stimulation; it is primarily used for the diagnosis of TB infection. A previous study has reported a sensitivity and specificity of 84.95% and 85.12%, respectively, for the T-SPOT.TB test in diagnosing active pulmonary TB[6]. Unfortunately, this test has been rarely applied in the diagnosis of TBM. The aim of our study was to assess the diagnostic value of CSF T-SPOT.TB (Oxford Immunotec Ltd., Abingdon, UK) in diagnosing TBM in the Chinese population.

In this retrospective study, a total of 96 HIV-negative patients with suspected meningitis were enrolled from Beijing Chest Hospital during 2010-2013. Informed consent was obtained from all subjects, and the study protocol was approved by the ethics committee of Beijing Chest Hospital. The patients were categorized into two groups based on the diagnostic criteria for TBM, one was TBM group and another was non-TBM group, according to 'a consensus case definition for tuberculous

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meningitis,' as follows: definite, probable, possible, or not TBM, respectively. (1) Definite TBM was diagnosed based on microbiological identification or evidence from commercial nucleic acid amplification tests of central nervous system *Mycobacterium* tuberculosis infection. (2) Probable TBM: when imaging is available a diagnostic score of 12 or above is required, and when imaging is not available, a diagnostic score of 10 or above is required. (3) Possible TBM: when imaging is available a diagnostic score of 6-11 is required, and when imaging is not available, a score of 6-9 is required. The definite, probable, and possible TBM cases belonged to the TBM group. In addition, we classified stages I, II, and III of TBM according to the clinical signs of patients presenting with TBM, which can be easily assessed for severity based on modifications of the Medical Research Council staging system[8]. About 10 mL of CSF was collected for performing T-SPOT.TB testing, adenosine deaminase (ADA) testing, bacteriological testing, and so on; these tests were conducted within 4 h after sampling.

Data analysis was conducted by SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). The characteristics of all subjects were analyzed descriptively; the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were assessed between CSF-ADA testing and CSF T-SPOT.TB testing among patients with TBM and patients with different stages of TBM, respectively. The ROC curve was drawn to assess the diagnostic accuracy, and the Youden's Index (YI) was used to select the optimum cut points on the ROC curve and calculate the areas under the curve (AUCs).

A total of 96 HIV-negative patients were evaluated during the 3-year study period. The proportion of patients with TBM and that of non-TBM cases were 54.2% (52/96) and 45.8% (44/96), respectively. Of the TBM patients, 12 patients were confirmed as having TBM by bacteriological, or polymerase chain reaction (PCR) tests. Of the 52 patients with TBM, 12, 26, and 14 patients had definite, probable, and possible TBM, respectively. The median age was 32 years (range: 23.00-44.25 years), 61.5% (32/52) of females was higher compared to male, and patients with stages I, II, and III of TBM accounted for 23.1% (12/52), 57.7% (30/52), and 19.2% (10/52), respectively.

The comparison between the T-SPOT.TB test and the ADA test for the diagnostic accuracy is illustrated in Table 1 and Figure 1. The sensitivity (97.8%) and specificity (78.0%) of the T-SPOT.TB test were higher than those of the ADA test (63.0% and 74.0%, respectively); meanwhile, the AUC for the T-SPOT.TB test was 0.910, whereas it was only for 0.689 for the ADA test.

We further compared the T-SPOT.TB and ADA tests for different stages of TBM, and the results showed that the sensitivity and specificity of the T-SPOT.TB test for stage I (96.7% and 77.8%), stage II (97.2% and 77.9%), and stage III (98.9% and 78.1%) were, respectively, significantly higher than those for the ADA test. In addition, the sensitivity and specificity of the T-SPOT.TB test for stage III of TBM were higher than those for stages I and II, as shown in Table 2.

Currently, the diagnosis of TBM is a global issue and has become increasingly important. The conventional methods of culturing on solid and liquid media are the gold standard for diagnosis, though most of the cases show negative results, which usually causes difficulty in visualization of AFB in direct smears or in cultures of CSF for TBM. Thakur et al. found that smear positivity for AFB was 7.9%,

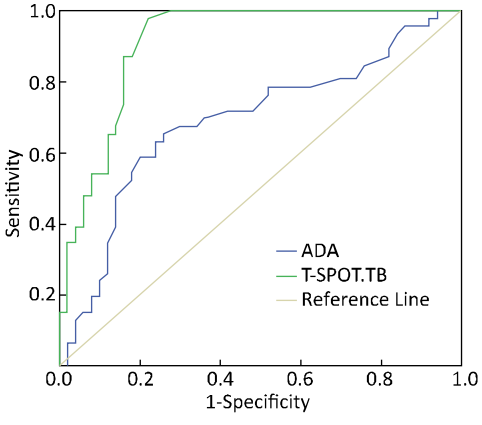

Figure 1. ROC curve analysis of T-SPOT.TB and ADA tests for diagnosing TBM.

Table 1. ROC Curve Results of T-SPOT.TB and ADA Tests for Diagnosing TBM

Test	Sensitivity (%)	Specificity (%)	AUC	Cut-off	YI	PPV	NPV
T-SPOT.TB	97.8 (87.0-99.9)	78.0 (63.7-88.0)	0.910 (0.850-0.970)	10.000	0.758	80.3 (67.2-89.3)	97.5 (85.3-99.9)
ADA	63.0 (47.5-76.4)	74.0 (59.4-85.0)	0.689 (0.580-0.798)	3.75	0.392	69.0 (52.8-81.9)	68.5 (54.3-80.1)

Table 2. Comparison of T-SPOT.TB and ADA Tests for Different Stages of TBM

Stage	Test	Sensitivity	Specificity
I	T-SPOT.TB	96.7 (88.1-99.8)	77.8 (65.9-91.9)
	ADA	61.6 (48.3-75.5)	73.5 (59.4-82.5)
II	T-SPOT.TB	97.2 (88.1-99.8)	77.9 (66.0-86.3)
	ADA	61.8 (54.8-73.9)	73.4 (60.2-83.6)
III	T-SPOT.TB	98.9 (90.2-99.9)	78.1 (64.2-88.0)
	ADA	65.4 (55.2-76.9)	74.2 (65.3-85.0)

while the automated Bactec MGIT 960 system displayed a higher rate of recovery of MTB (27.4%) than that by L-J media (10.9%)[9]. In our study, AFB were not found in any patient, but 12 patients (23.1%) had a positive result of culture or PCR of CSF. Thus, the low sensitivity of bacteriological examination of CSF cannot fulfill the diagnostic criteria of TBM.

In our study, we showed that the sensitivity of the CSF T-SPOT.TB test was 97.8%, which was significantly higher than those reported from South Korea (59%)[10] and by a meta-analysis (77%)[11], indicating that the T-SPOT.TB test was useful in diagnosing TBM. In recent years the CSF-ADA test was reported to have considerable value in the diagnosis of TBM, with a sensitivity of 92% and a specificity of 90.47%[12], whereas, in the present study, the sensitivity (63.0%) and specificity (74.0%) of the ADA test were lower. We also found the ADA test had 69.0% PPV and 68.5% NPV, which were lower than those for the CSF T-SPOT.TB test (80.3% and 97.5%, respectively). We also used ROC curves to compare the T-SPOT.TB test and the ADA test, which showed that the AUCs for the two diagnostic methods were 0.910 and 0.689, respectively. Thus, our data suggest that the T-SPOT.TB test is a rapid and accurate diagnostic method for TBM in China, and the diagnostic value of ADA test for TBM was unfavorable.

In addition, in the current study, we compared the diagnostic performance of the T-SPOT.TB and ADA tests in patients with different stages of TBM, and the results showed that the sensitivities of the T-SPOT.TB test for stages I, II, and III of TBM were significantly higher than those of the ADA test, and the corresponding specificities of the T-SPOT.TB test were also slightly higher. Furthermore, the sensitivity and specificity of the T-SPOT.TB test were the highest for stage III of TBM, whereas no similar studies on T-SPOT.TB test for different stages of TBM have been reported yet. Our study indicated that the T-SPOT.TB test may currently be the most effective method for diagnosing TBM in China.

Several limitations exist in our study. First, the number of enrolled cases was small, due to which we could not further analyze the definite, probable, and possible TBM cases. A large sample size is needed to determine the diagnostic value of CSF T-SPOT.TB for TBM in the future. The second limitation is the bias in patient selection. All the patients in our study were recruited from one hospital near the northeast of China, which cannot represent the status of entire China. Despite these limitations, our study still has several strengths. To the best of our knowledge, this is the first study to investigate the diagnostic value of CSF T-SPOT.TB test for different stages of TBM. Our data confirmed that the CSF T-SPOT.TB test showed a favorable diagnostic performance in patients with different stages of TBM, and we also found that the CSF T-SPOT.TB test had higher sensitivity and specificity for diagnosing TBM. These results can probably be used as a reference to apply CSF T-SPOT.TB as a routine laboratory test for the diagnosis of TBM.

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