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*Original Article*

# Positive interferon-gamma release assay results are correlated with paradoxical reaction in tuberculous meningitis

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**Abstract**: Background: Evidence suggests that T cell-based interferon-γ release assays (IGRAs) may be useful for the diagnosis of tuberculosis. The present study aims to investigate the clinical significance of blood T-SPOT.TB assay, a commercially available IGRAs, in tuberculous meningitis (TBM) patients. Methods: Records of consecutive TBM and non-TBM meningitis patients admitted from July 2011 to March 2016 at a tertiary hospital were retrospectively reviewed. Clinical and magnetic resonance (MR) findings, as well as T-SPOT.TB results were comprehensively assessed. Results: A total of 61 TBM patients and 85 non-TBM meningitis patients were enrolled. The sensitivity and specificity of T-SPOT.TB for TBM patients were 62.3% and 72.9%, respectively. Positive T-SPOT.TB results were related to diagnostic category (P=0.015), TB outside of the central nervous system (CNS) (P=0.002), active TB outside of CNS (P=0.009), hydrocephalus (P=0.039) and basal exudates (P=0.031) on MR, and paradoxical reaction to anti-tuberculosis drugs (P=0.032). The logistic regression analysis showed that TB outside of CNS was the only independent predictor for positive T-SPOT.TB (P=0.013). Conclusion: These results collectively indicate that blood T-SPOT.TB should be supplementary for the diagnosis of TBM, rather than serving as a single test to diagnose or exclude the disease. However, positive T-SPOT.TB results may reflect typical clinical and MR features of TBM. Moreover, positive results are associated with paradoxical reaction to treatment, which is related to immune status to tuberculosis infection. Thus, T-SPOT.TB results provide potentially useful information for the consideration of immune-modulating therapy.

**Keywords:** Interferon-gamma release assay, tuberculous meningitis, paradoxical reaction, T-SPOT.TB

## Introduction

Tuberculosis is considered as one of the leading causes of death due to infectious diseases worldwide [1]. Although tuberculous meningitis (TBM) represents only approximately 1% of all tuberculosis cases, it kills or disables about half of the people affected [2]. Treatment delay has been long recognized as the strongest risk factor for poor prognosis. However, early diagnosis of TBM is notoriously difficult because clinical features in the early stage are non-specific and laboratory tests are insensitive [3]. Definitive diagnosis of TBM mainly relies on the confirmation of *M. tuberculosis* inside the central nervous system (CNS). Unfortunately, sensitivities for CSF Ziehl-Neelsen staining and nucleic acid amplification techniques in TBM are both less than 60% [2]. In clinical practice, the majority of clinically diagnosed TBM cases were classified as probable or possible, particularly for HIV-negative patients.

Compared with the tuberculin skin test (TST) in diagnosing TBM, T cell-based interferon-γ (IFN-γ) release assays (IGRAs), which measure the release of IFN-γ after in vitro stimulation with *M. tuberculosis*-specific antigens, such as early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), have the ability to reduce false positive results and show higher sensitivity and specificity. The sensitivity of

**Table 1.** Baseline clinical characteristics in suspected meningitis

| Characteristics | TBM (n=61) | Non-TBM (n=85) | *P* Value |
|---|---|---|---|
| Age, mean years ± SE | 39.34±1.886 | 42.02±1.931 | 0.337# |
| Male sex, n (%) | 38 (62.3) | 57 (67.1) | 0.552 |
| With tuberculosis outside of CNS, n (%) | 29 (47.5) | 13 (15.3) | <0.001 |
| Underlying condition or illness, n (%) | | | |
| No underlying illness | 27 (44.3) | 34 (40.0) | 0.607 |
| Diabetes mellitus | 9 (14.8) | 12 (14.1) | 0.960 |
| Metabolic syndrome and related diseases* | 11 (18.0) | 24 (28.2) | 0.859 |
| Autoimmune diseases | 7 (11.5) | 6 (7.1) | 0.109 |
| Chronic hepatitis B virus infection | 9 (14.8) | 13 (15.3) | 0.332 |
| Pregnancy and related conditions** | 3 (4.9) | 1 (1.2) | 0.128## |
| Dysfunction of major organs | 2 (3.3) | 3 (3.5) | 1.000## |
| Prior trauma and/or surgery | 3 (4.9) | 1 (1.2) | 0.113## |
| Receiving immunosuppressive treatment | 5 (8.2) | 7 (8.2) | 0.959 |
| Immunosuppressed condition*** | 9 (14.8) | 13 (15.3) | 0.911 |

*Including hypertension, abnormal glucose tolerance, overweight and obesity, lipid metabolism disorder, hepatic adipose infiltration, and arteriosclerotic artery disease, et al. **Including application of assisted reproductive technology and pathological abortion. ***Defined as patients with underlying diseases such as malignancy, liver cirrhosis, chronic renal failure, pregnancy or patients receiving immunosuppressive treatment. #Student's t test. ##Fisher's exact test.

**Table 2.** Results of blood T-SPOT.TB in different subgroups of patients with meningitis

| Characteristics | Positive, n (%) | Negative, n |
|---|---|---|
| TBM | 38 (62.3) | 23 |
| Diagnostic category | | |
| Definite and probable | 31 (72.1) | 12 |
| Possible TBM | 7 (38.9) | 11 |
| Evidence for TB infection other than CNS | | |
| With | 24 (82.8) | 5 |
| Without | 14 (43.75) | 18 |
| Non-TBM | 23 (27.1) | 62 |
| Evidence for TB infection other than CNS | | |
| With | 8 (61.5) | 5 |
| Without | 15 (20.8) | 57 |

IGRAs was highest in active pulmonary TB, with specificity depending on the burden of latent TB infection. Researches also suggest that IGRAs are particularly useful for the diagnosis of extrapulmonary TB, but the sensitivity is relatively lower, with a range of 79.8-89% [4]. There was limited information for IGRA-based TBM diagnosis, especially in high-burden countries. As a commercially available IGRA, T-SPOT.TB assay detects IFN-γ induced by ESAT-6 and CFP-10. Thus, the present study attempted to investigate the clinical significance of T-SPOT.TB assay in TBM.

## Materials and methods

### *Patients*

Patients admitted to the Third Affiliated Hospital of Sun Yat-sen University consecutively during July 2011 and March 2016 with meningitis, including TBM, viral meningitis, cryptococcal meningitis and purulent meningitis, and underwent T-SPOT.TB assays, were enrolled in this study. According to the standardized clinical case definition published in 2010 [5], patients were diagnosed with (1) "definite" TBM if acid-fast bacilli were found in CSF or mycobacterial polymerase chain reaction was positive in CSF; (2) "probable" TBM if they had a total diagnostic score higher than 12, including 2 points from CSF or cerebral imaging criteria; and (3) "possible" TBM if they presented with a total diagnostic score of ranging from 6 to 11 points. Viral meningitis was diagnosed according to patients' medical history, viral detection, magnetic resonance imaging (MRI), and response to antivirus therapy, while cryptococcal and purulent meningitis were diagnosed based on the presence of pathogens in the CSF. The local Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University approved the

**Table 3.** Diagnostic performance of the blood T-SPOT.TB in tuberculous meningitis

| | Sensitivity* % (95% CI) | Specificity** % (95% CI) | Positive predictive value % (95% CI) | Negative predictive value % (95% CI) | Positive likelihood ratio (95% CI) | Negative likelihood ratio (95% CI) |
|---|---|---|---|---|---|---|
| All cases | 62.3 (48.9-74.1) | 72.9 (62.0-81.7) | 62.3 (48.9-74.1) | 72.9 (62.0-81.7) | 2.30 (1.54-3.43) | 0.517 (0.371-0.719) |
| Diagnostic category | | | | | | |
| Definite and probable TBM | 72.1 (56.1-84.2) | 72.9 (62.0-81.7) | 57.4 (43.3-70.5) | 83.8 (73.0-91.0) | 2.66 (1.79-3.96) | 0.382 (0.235-0.624) |
| Possible TBM | 38.9 (18.3-63.8) | 72.9 (62.0-81.7) | 23.3 (10.6-42.7) | 84.9 (74.2-91.9) | 1.44 (0.731-2.82) | 0.838 (0.575-1.22) |
| Evidence for TB infection other than CNS | | | | | | |
| Without TB outside of CNS | 43.7 (26.8-62.1) | 79.2 (67.7-87.5) | 48.3 (29.9-67.1) | 76.0 (64.5-84.8) | 2.10 (1.15-3.82) | 0.710 (0.520-0.971) |
| With TB outside of CNS | 82.8 (63.5-93.5) | 38.5 (15.1-67.7) | 75.0 (56.2-87.9) | 50.0 (20.1-79.8) | 1.34 (0.848-2.13) | 0.448 (0.159-1.27) |

*Number of patients with a positive result/number of patients tested. **Number of patients with a negative result/number of patients tested.

procedures of the study. All patients provided written informed consent.

*Clinical, laboratory and MRI assessments*

The age, gender, medical history, and clinical presentation of each patient were retrieved from the patient's medical records. Disturbance of consciousness was defined by a Glasgow Coma Scale rating below 8. Cranial nerve palsy, movement impairment, and the presence of seizures were also recorded. Severity of TBM was graded into three stages, including stage 1: fully conscious and without specific symptoms; stage 2: lethargy or cranial nerve palsies; and stage 3: stupor, severe illness, gross paralysis or paresis [6]. HIV antibody tests, chest radiographs, and other necessary examinations for extra-CNS tuberculosis were carried out. Opening pressure, cells, protein, glucose, and chloride were examined in CSF, which was also subjected to pathogenic culture.

Brain MRI was performed using a 1.5-Tesla MR scanner (GE Health care, Milwaukee, USA). T1, T2, fluid attenuated inversion recovery (FLAIR), diffusion weighted imaging (DWI), T1 contrast and magnetic resonance angiography (MRA) images were obtained in the first week of hospitalization. Evidence of infarcts, hydrocephalus, basal exudates, tuberculomas and vasculitis was recorded.

*T-SPOT.TB*

The T-SPOT.TB assay was performed upon enrollment and interpreted according to the manufacturer's insert guidelines [7, 8]. Briefly, peripheral blood mononuclear cells (PBMC) were separated from peripheral venous blood, and $2.5\times10^5$ PBMC were plated per well in wells precoated with anti-human IFN-γ antibody. The PBMC were cultured at 37°C for 18 h. The number of spot-forming cells (SFCs) in each well was counted automatically. Laboratory staffs were blind to the clinical characteristics of the patients.

*Treatments and prognosis*

TBM patients received routine antituberculosis treatments and dexamethasone. A paradoxical reaction was defined when observing one of the following symptoms in a patient whose clinical symptoms initially improved with antituberculosis treatment: the worsening of a preexisting insults, the appearance of new insults on MR, and/or transient worsening of CSF parameters [9]. Paradoxical reactions on MR included the appearance of new tuberculomas, hydrocephalus, infarction, vasculitis, optochiasmatic and spinal arachnoiditis and the expansion of an existing insult. Paradoxical reactions during the first three months of treatment were documented. Disability status was recorded at time of enrollment and 6 months post-admission. The follow-up outcome was evaluated with the modified Barthel index (MBI), and patients were classified into two categories including (1) poor outcome, i.e., MBI score lower than 50 or death; and (2) good outcome, i.e., MBI higher than 50.

*Statistical analysis*

Statistical analysis was performed using SPSS 19.0 under the Windows environment (SPSS Inc., Chicago, IL, USA). The diagnostic value of T-SPOT.TB assay was expressed in terms of sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio. Ninety-five percent confidence intervals (CIs) were calculated using the Wilson score method. The relationship between T-SPOT.TB results and various clinical and MRI findings was assessed. Qualitative data were analyzed with the $X^2$ tests or Fisher's exact test, and quantitative data were analyzed using two-tailed Mann-Whitney U-tests or student's t test. In addition, logistic regression analysis was applied to identify the independent predictive factors of positive T-SPOT.TB.

## Results

*Patient characteristics*

A total of 61 patients with TBM and 85 patients with other meningitis were reviewed in the present study. All patients were HIV-negative. In the patient group with TBM, there were 2 patients with "definite" TBM, 41 patients with "probable" TBM, and 18 patients with "possible" TBM. In the non-TBM meningitis patient group, there were 38 patients with cryptococcal meningitis, 34 patients with viral meningitis, and 13 patients with purulent meningitis. Their clinical characteristics at time of admission are summarized by patient group in **Table 1**.

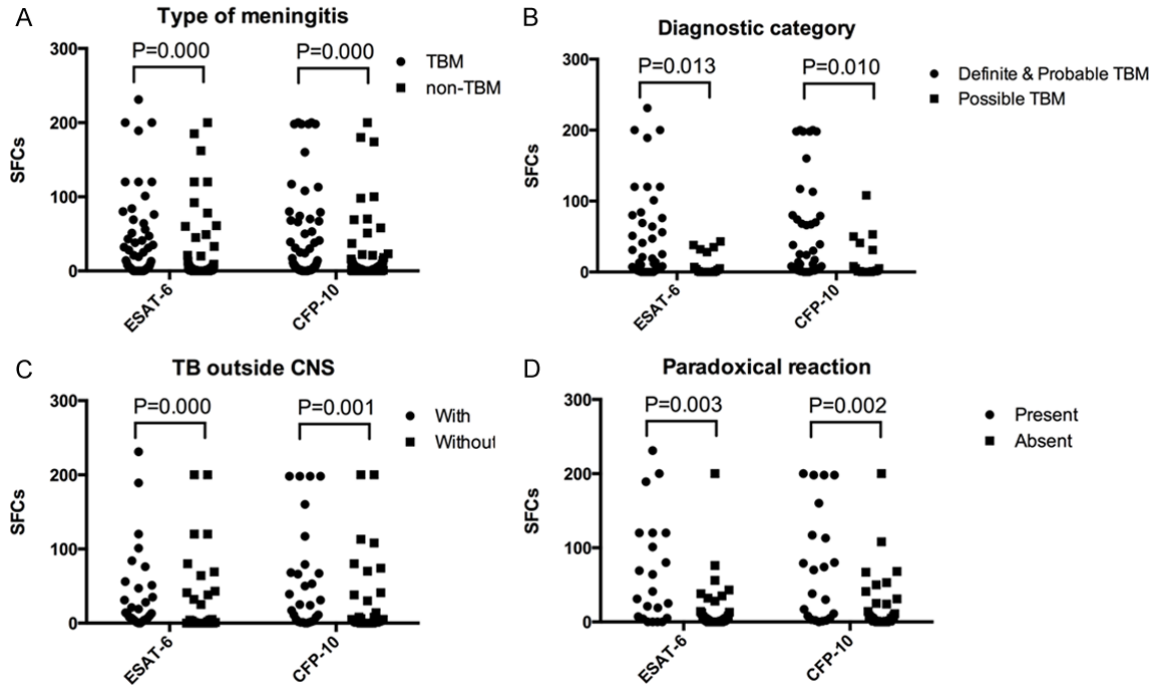


**Figure 1.** Scatter plots displaying counts of spot-forming cells (SFCs) in peripheral blood mononuclear cells using the T-SPOT.TB according to the following criteria: A. Type of meningitis; B. Diagnostic category of tuberculous meningitis (TBM); C. With or without tuberculosis (TB) outside of the central nervous system (CNS); and D. Presence or absence of paradoxical reaction. Differences between patient groups were assessed using Mann-Whitney U-tests.

*Diagnostic value of the blood T-SPOT.TB for TBM*

Thirty-eight TBM patients (out of 61, 62.3%) were T-SPOT.TB positive, while 23 non-TBM patients (out of 85, 27.1%) were positive (P=0.000) (**Table 2**). As shown in **Table 3**, the sensitivity and specificity of T-SPOT for diagnosing TBM were 62.3% (95% CI; 48.9-74.1%) and 72.9% (95% CI; 62.0-81.7%), respectively. The sensitivity of the blood T-SPOT.TB for diagnosing TBM differed between patient groups; patients with possible TBM and definite/probable TBM were identified with sensitivities of 38.9% and 72.1%, respectively. The sensitivity of T-SPOT.TB in patients with TB infection outside CNS was higher than in patients without TB outside CNS (82.8% and 43.7%, respectively), though the specificity was lower (38.5% and 79.2%, respectively).

As displayed in **Figure 1A-C**, patients with TBM showed higher numbers of SFCs for ESAT-6 and CFP-10 than non-TBM meningitis patients (both P=0.000). The numbers of SFCs for ESAT-6 and CFP-10 were compared in TBM patients with different diagnostic categories and presence of tuberculosis outside of CNS. Patients with definite and probable TBM had significantly higher SFCs numbers for ESAT-6 (P=0.013) and CFP-10 (P=0.010) than patients with possible TBM. In addition, TBM patients with TB outside of CNS exhibited significantly higher SFCs numbers for ESAT-6 and CFP-10 (P=0.000 and P=0.001, respectively).

*Factors associated with positive T-SPOT.TB results in TBM*

Correlation tests between T-SPOT.TB results and clinical and MR manifestations in TBM patients are displayed in **Table 4**. Positive T-SPOT.TB results were associated with TB outside of CNS (P=0.002), active TB outside of CNS (P=0.009), diagnostic category (P=0.015), and hydrocephalus (P=0.039) and basal exudates (P=0.031) on MR. As shown in logistic regression analysis, TB outside of CNS was the only independent predictor for positive T-SPOT.TB results (P=0.013) (**Table 5**).

*Correlations between positive T-SPOT.TB and prognosis*

Fifty-seven patients finished the first six months of treatment, and paradoxical reaction to anti-

**Table 4.** Factors associated with positive result of T-SPOT.TB in tuberculous meningitis

| Feature, n (%) | Positive result (n=38) | Negative result (n=23) | *P* Value |
|---|---|---|---|
| Clinical presentation | | | |
| Age | | | 0.765 |
| ≥60 years | 4 (10.5) | 3 (13.0) | |
| <60 years | 34 (89.5) | 20 (87.0) | |
| TB outside of CNS | 24 (63.1) | 5 (21.7) | 0.002 |
| Active TB | 15 (39.5) | 2 (8.7) | 0.009 |
| Disturbance of consciousness | 7 (18.4) | 5 (21.7) | 0.752 |
| Cranial nerve injury | 13 (34.2) | 7 (30.4) | 0.761 |
| Focal weakness | 11 (28.9) | 7 (30.4) | 0.902 |
| Epilepsy | 10 (26.3) | 3 (13.0) | 0.220 |
| Diagnostic category | | | 0.015 |
| Definite and Probable TBM | 31 (81.6) | 12 (52.2) | |
| Possible TBM | 7 (18.4) | 11 (47.8) | |
| Stage of disease | | | 0.394 |
| Stage I | 12 (31.6) | 11 (47.8) | |
| Stage II | 11 (28.9) | 4 (17.4) | |
| Stage III | 15 (39.5) | 8 (34.8) | |
| MR abnormality | | | |
| Hydrocephalus | 12 (31.6) | 2 (8.7) | 0.039 |
| Basal exudates | 25 (65.8) | 9 (39.1) | 0.031 |
| Infarction | 14 (36.8) | 5 (21.7) | 0.217 |
| MRA abnormality | 16 (42.1) | 7 (30.4) | 0.384 |
| Tuberculomas | 11 (28.9) | 4 (17.4) | 0.310 |
| Paradoxical reaction to anti-tuberculosis drugs* | 17 (50.0) | 5 (21.7) | 0.032 |
| Poor prognosis* | 6 (17.6) | 4 (17.4) | 0.980 |

*Total N=57, including 34 T-SPOT.TB positive cases and 23 T-SPOT.TB negative cases.

tuberculosis drugs occurred in 22 patients. Positive T-SPOT.TB results were associated with paradoxical reaction (P=0.032) (**Table 4**). SFCs numbers for ESAT-6 and CFP-10 were also higher in patients who developed paradoxical reaction (P=0.003 and P=0.002, respectively) (**Figure 1D**). The prognosis was good in 47 patients, while poor in 10 patients. However, T-SPOT.TB results were not significantly associated with the prognosis (**Table 4**).

## Discussion

Still until now, the diagnosis of TBM in the clinical practice is made by clinical manifestation. The majority of patients are diagnosed with probable or possible TBM, especially in HIV negative patients, and a fraction of patients are diagnosed with definite TBM (e.g., 5.9% in our local center) [10]. Using blood T-SPOT.TB to diagnose TBM is very convenient, since T-SPOT.TB tests can be completed within 24 hours, and is not affected by previous BCG vaccination. The diagnostic value of T-SPOT.TB has been demonstrated to be greater than TST, even in HIV patients [8, 11, 12]. The present study comprehensively assessed the performance of blood T-SPOT.TB assay for TBM under real clinical condition, in which the majority of the TBM cases could not be etiologically diagnosed. Although results showed that the sensitivity and specificity of T-SPOT.TB were both inadequate, positive result of T-SPOT.TB could provide additional information in the diagnosis and management of TBM.

Since the results of blood T-SPOT.TB could not be used to differentiate latent tuberculosis infection (LTBI) and active TB, the specificity of the T-SPOT.TB test for active TB depends on the prevalence of LTBI. A nationwide TB survey in 2000 identified China as the second-highest burden country for tuberculosis, with an LTBI rate of nearly 44.5% [13]. In Korea, another high-burden country, the specificity of blood T-SPOT.TB for TBM was 57-63% [14, 15]. In line with these findings, our data indicated that the specificity of T-SPOT.TB for TBM was approximately 72.9% (95% CI, 62.0-81.7%). IFN-γ releasing largely depended on the antigens in circulating blood, fewer antigens could be released into circulation in the circumstance of extrapulmonary tuberculosis (EPTB). Diagnostic sensitivity varied with site of infection in EPTB and was lower than in PTB [16]. As revealed by the meta-analyses conducted by Fan et al., the pooled sensitivity of T-SPOT.TB for EPTB was 90% (95% CI, 86-93%) [17]. Because

**Table 5.** Multivariate logistic regression analysis of factors related to positive T-SPOT.TB results in tuberculous meningitis

| Factor | β | SE | $X^2$ Walds | P | OR | 95% CI |
|---|---|---|---|---|---|---|
| TB outside of CNS | 1.677 | 0.683 | 6.026 | 0.014 | 5.350 | 1.402-20.413 |
| Diagnostic category | -0.382 | 0.725 | 0.277 | 0.599 | 0.683 | 0.165-2.828 |
| Hydrocephalus | 0.648 | 0.992 | 0.427 | 0.513 | 1.912 | 0.274-13.370 |
| Basal exudates | 1.007 | 0.700 | 2.067 | 0.150 | 2.737 | 0.694-10.798 |
| Paradoxical reaction to anti-tuberculosis drugs | 0.416 | 0.804 | 0.268 | 0.605 | 1.516 | 0.313-7.337 |

the blood brain barrier further influences the transfer of antigens, sensitivity in TBM could be even lower. The sensitivity of blood T-SPOT.TB in TBM was estimated at approximately 62.5-79% in previous studies [4, 15, 18]. According to our results, the overall sensitivity was 62.3% (95% CI, 48.9-74.1%). Our data further indicated the diagnostic performance of T-SPOT.TB differed according to the diagnostic category, i.e., 72.1% for probable/definite TBM, and 38.9% for possible TBM. As indicated, the sensitivity and specificity for TBM were both inadequate for application in a high-burden situation. The results of IGRAs depend on antigenic load, host responsiveness to these antigens, and host-pathogen interactions, the SFCs in PBMC are higher in high antigen-load situations such as definite or probable TBM, as well as in TBM cases with TB outside of CNS.

As revealed by univariate analyses, positive results of T-SPOT.TB were associated with presentation of tuberculosis outside of CNS. This finding is consistent with the principle that T-SPOT.TB detects PBMCs reactive to TB-related antigens in the peripheral blood. In addition, as revealed by the logistic regression analysis, TB outside of CNS was the only predictive factor for positive T-SPOT.TB results, indicating that peripheral blood T-SPOT.TB could be considered as alternative evidence for tuberculosis outside of CNS, which should be considered as supportive evidence for the diagnosis of TBM. The result should be analyzed in combination with the clinical manifestations and other diagnostic methods, such as CSF, cranial computed tomography/MRI, and et al. However, positive T-SPOT.TB results were also associated with hydrocephalous and basal exudates on MR, which are typical manifestations of TBM. Additionally, T-SPOT.TB results were associated with diagnostic classification, which is made upon the basis of clinical presentation. Indeed, to the authors' knowledge, no similar information has not been reported for TBM. These results indicated that results of T-SPOT.TB could also serve as an indicator of disease state. TBM patients with positive T-SPOT.TB would be expected to have more typical presentations.

Though positive T-SPOT.TB was not related to prognosis directly, our results indicated a correlation with paradoxical treatment reaction to anti-tuberculosis drugs. As already known, the manifestation of tuberculosis infection largely depends on the intensity of the immune response to *Mycobacterium tuberculosis* [19]. Mycobacterial cell wall antigens are present in the affected brain tissues. After effective anti-tuberculosis treatment, massive amounts of mycobacterial antigens are released and trigger an exaggerated inflammatory reaction, which results in CSF and MR worsening and clinical deterioration. This phenomenon is the so-called paradoxical reaction, which usually does not require changes in anti-tuberculosis therapy and would be alleviated by continuing treatment. It is crucial to distinguish paradoxical reaction from diagnostic error, treatment failure, drug hypersensitivity, concomitant infections and so on. Misinterpreting this benign treatment reaction could result in diagnostic confusion and treatment discontinuation, which may lead to an unfavorable prognosis. When paradoxical reaction in the form of acute exacerbations becames life threatening and disabling, treatment with immuno-modulatory drugs is required [9]. T-SPOT.TB positive patients, especially those with higher SFCs counts, may have an intense reaction to *Mycobacterium tuberculosis* and tend to develop paradoxical reaction. Thus, proper immunomodulatory therapy in these patients should be carefully considered as a treatment option.

## Conclusions

In summary, the present study demonstrated that T-SPOT.TB should be supplementary for the diagnosis of TBM, rather than being applied as a single test to diagnose or exclude the disease. The results of T-SPOT.TB reflect the clinical features of the disease, as well as immune status to tuberculosis infection in TBM patients, thus providing useful information regarding the utility of immune-modulating therapy.

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## Disclosure of conflict of interest

None.

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