MAJOR ARTICLE

Rapid Diagnosis of Tuberculous Meningitis by T Cell–Based Assays on Peripheral Blood and Cerebrospinal Fluid Mononuclear Cells

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Background. The role of the new *Myocbacterium tuberculosis*–specific enzyme-linked immunosorbent spot (ELISPOT) assay for diagnosis of tuberculous meningitis (TBM) has not yet been fully assessed. Here, we conducted a prospective, blinded, observational study to evaluate the diagnostic accuracy of this assay, compared with the conventional tests, for diagnosing TBM.

Methods. All adult patients with suspected TBM were enrolled at a tertiary care hospital (Seoul, South Korea) during a 12-month period. ELISPOT assays were performed on peripheral mononuclear cells and mononuclear cells from cerebrospinal fluid (CSF).

Results. Eighty-nine patients with suspected TBM were enrolled. Of these, 31 (35%) were classified as having TBM (10 confirmed, 6 highly probable, and 15 probable cases), and 55 (62%) were classified as not having active tuberculosis. The remaining 3 (3%) with possible TBM were excluded from the final analysis. The sensitivities and specificities, respectively, of the tested methods for diagnosing TBM were as follows: CSF adenosine deaminase level >5.8 U/L, 89% (95% confidence interval [CI], 69%–98%) and 73% (95% CI, 58%–84%); peripheral mononuclear cells ELISPOT, 71% (95% CI, 51%–86%) and 57% (95% CI, 42%–70%); and CSF mononuclear cells ELISPOT assay, 59% (95% CI, 36%–79%) and 89% (95% CI, 72%–98%). The combined sensitivity of an adenosine deaminase level >5.8 U/L or a positive peripheral mononuclear cells ELISPOT assay result was 94% (95% CI, 79%–99%), conferring a negative likelihood ratio of 0.14 (95% CI, 0.03–0.55) when both test results were negative.

Conclusion. ELISPOT assays using peripheral mononuclear cells and CSF mononuclear cells are useful adjuncts to the current tests for diagnosing TBM, particularly when used in combination with the assessment of adenosine deaminase level in CSF.

Although tuberculous meningitis (TBM) is a serious global health problem, clinicians are not always able to correctly initiate therapy on the basis signs and symptoms, the results of routine analyses of cerebrospinal fluid (CSF), and radiological findings [1]. Delays in the initiation of therapy, which are often attributable to the use of slow or relatively insensitive conventional diagnostic tests, have been associated with high mortality rates in patients with TBM [2]. Therefore, we urgently need a faster, more sensitive, and specific test for the diagnosis of TBM in clinical practice.

Recently, a new generation of diagnostic tuberculosis (TB) assays, which use *Mycobacterium tuberculosis*–specific antigens encoded by genes located in the region of difference 1 (RD1), have shown promising results in the diagnosis of latent TB infection and active pulmonary or extrapulmonary TB [3–6]. Less is known, however, about the usefulness of these assays for TBM diagnosis in daily clinical practice. Furthermore, there is some question regarding which clinical samples should be used for such tests, because mononuclear cells that are compartmentalized at the sites of infection (eg, cells in pleural fluid [7, 8], bronchoalveolar lavage fluid [9–11], and CSF [12–15]) have higher interferon-γ response rates, compared with peripheral mononuclear cells (PBMCs). To address these questions, we

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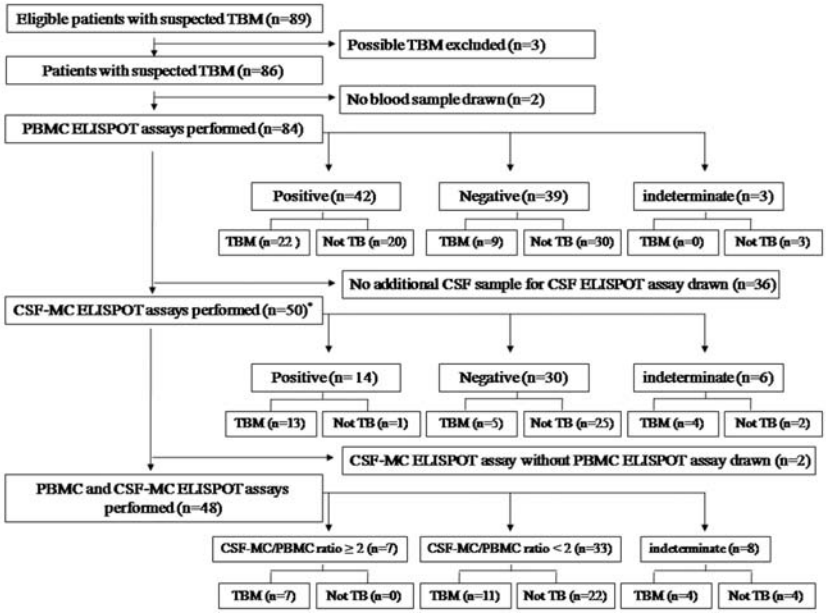

Figure 1. Study flow diagram. CSF-MC, cerebrospinal fluid mononuclear cells; PBMC, peripheral mononuclear cells; TB, tuberculosis; TBM, tuberculous meningitis. *CSF samples for CSF-MC enzyme-linked immunosorbent spot (ELISPOT) assays were obtained from 2 patients whose blood samples were not obtained.

conducted a prospective, blinded, observational study to evaluate the diagnostic accuracy of circulating and compartmentalized mononuclear cell–based enzyme-linked immunosorbent spot (ELISPOT) assays for the diagnosis of active TB in patients with suspected TBM.

METHODS

Study population. Adult patients with suspected TBM who were admitted to Asan Medical Center, a 2700-bed tertiary hospital in Seoul, South Korea, were prospectively enrolled from April 2008 through March 2009. If the attending physicians (J.K.K. and S.A.L.) considered TBM to be part of the differential diagnosis and the individual was 16 years of age or older, we invited him/her to participate in this study and to provide informed consent. Tuberculin skin test was performed, as described elsewhere [4]. Microbiological and pathological specimens for diagnosis of TBM were processed using standard techniques and procedures, as described elsewhere [4]. Decisions regarding anti-TB therapy were made by the attending physicians (ie, J.K.K. and S.A.L) on the basis of each patient's initial clinical features, blood test results, image findings, and CSF profiles. The results of the ELISPOT assays were concealed from the attending physicians, to avoid a bias because the results of the ELISPOT assay may have affected the attending physicians' decisions on empirical anti-TB therapy [12, 16]. The study protocol was approved by the Institutional Review Board of our hospital.

Clinical category of TBM. All cases were independently classified by 2 of the study investigators (O.-H.C. and S.-H.K.) who were blinded to the ELISPOT results. Classification was based on clinical, histopathological, radiological, and microbiological information collected over at least 3 months of follow-up care. The clinical categorization of patients with suspected TBM was performed as described elsewhere [12, 14, 17, 18]. Briefly, patients were classified as having "confirmed TBM" if clinical specimens were found to be positive for *M. tuberculosis* in culture or by *M. tuberculosis* polymerase chain reaction (PCR) assay. Patients were classified as having "highly probable TBM" if there were CSF findings of lymphocytic pleocytosis, increased protein levels, and sterile cultures, plus $\geqslant 2$ of the following supporting criteria. Patients were classified as having "probable TBM" if there were CSF findings of lymphocytic pleocytosis, raised protein levels, and sterile cultures, plus 1 of the following supporting criteria. The supporting criteria included (1) computed tomography/magnetic resonance images revealing hydrocephalus, granulomas, or basal exudates; (2) evidence of extraneural TB; and (3) appropriate responses to anti-TB chemotherapy. Patients were classified as having "possible TBM" if they did not fulfill the above criteria, but a diagnosis of active TB could not be excluded. Patients were classified as "not TB" when some other diagnosis had been made, or when there was clinical improvement in the absence of anti-TB therapy within 3 months after hospital admission, because untreated TBM would be expected to cause death within that period [12, 17].

ELISPOT assays. The ELISPOT assays (T-SPOT.*TB*; Ox-

Table 1. Baseline Clinical Characteristics of 86 Patients with Suspected Tuberculosis (TB) Meningitis

Characteristic	TB meningitis[a] (n = 31)	Inactive TB (n = 55)
Age, mean years ± SD	45.4 ± 14.8	44.0 ± 19.6
Male sex	15 (48)	38 (69)
Clinical diagnosis		
TB meningitis	31[b] (100)	NA
Viral meningitis	NA	31 (56)
Partially treated bacterial meningitis	NA	14[c] (25)
Fungal meningitis	NA	5[d] (9)
Leptomeningeal seeding of malignant tumor	NA	1 (2)
Other	NA	4[e] (7)
Underlying condition or illness		
HIV infection	0 (0)	2 (4)
Transplantation	0 (0)	1 (2)
Hematologic malignancy	1 (3)	3 (5)
Solid tumor	1 (3)	2 (4)
Liver cirrhosis	0 (0)	3 (5)
Rheumatologic disease	2 (6)	1 (2)
Chronic renal failure	1 (3)	2 (4)
Diabetes	2 (6)	4 (7)
No underlying illness	24 (77)	37 (67)
Immunosuppressive condition[f]	5 (16)	12 (22)
Prior TB treatment	4 (13)	1 (2)

NOTE. Data are no (%) of patients, unless otherwise indicated. HIV, human immunodeficiency virus; NA, not applicable; SD, standard deviation.

[a] TB meningitis includes confirmed (n = 10), highly probable (n = 6), and probable (n = 15) cases of TB. Three cases of possible TB meningitis were excluded in this analysis.

[b] Of 31 patients, 5 (16%) patients had miliary TB at initial presentation.

[c] These 14 cases include cases of partially treated bacterial meningitis (n = 12), neurolisteriosis (n = 1), and neurobrucellosis (n = 1).

[d] These 5 cases include cases of cryptococcal meningitis (n = 5).

[e] These 4 cases include cases of central nervous system toxoplasmosis (n = 1), nocardiosis (n = 1), central nervous system lupus (n = 1), and pituitary apoplexy (n = 1).

[f] Immunosuppressive condition is defined as patients with underlying diseases, such as HIV infection, malignancy, liver cirrhosis, and chronic renal failure, and/or patients who were receiving immunosuppressive treatment.

ford Immunotec) were performed as described elsewhere [12]. Briefly, peripheral venous blood (~8 mL) was obtained from participants, and PBMCs were immediately (within 30 min) separated and collected. Concurrent with venous sampling, ~4 mL samples of CSF (median volume of CSF, 4.0 mL; interquartile range, 3–7 mL) were obtained from patients who agreed to additional CSF sampling, and cerebrospinal fluid mononuclear cells (CSF-MCs) were immediately (within 30 min) separated and collected. The collected cells were suspended in AIM-V media (GIBCO) at concentrations of 2.5×10^6 cells/mL for PBMCs and 2.5×10^6 cells/mL for CSF-MCs. The prepared PBMCs and CSF-MCs were distributed (2.5×10^5 cells/well) to plates that had been precoated with anti–human interferon-g antibody, and the samples were cultured for 18 h. The resulting spots were counted using an automated microscope (ELiSpot 04 HR; Autoimmune Diagnostika GmbH). We used criterion for indeterminate outcomes as described elsewhere [12]. In brief, a response was classified as indeterminate if the number of spots for the positive control well was <20 or the number of spots for the negative control well was >10.

Statistical analyses. Statistical analyses were performed using SPSS for Windows, version 12.0 (SPSS), and the MedCalc software package, version 11.1 (MedCalc Software). Continuous variables were compared using the Mann-Whitney U test or the Student's t test. All tests of significance were 2-tailed, and $P \leqslant .05$ was considered to be significant. Diagnostic performance was expressed in terms of sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio. For each of the tests used herein for diagnosis of TBM, we assessed the optimal cut-off point by constructing a receiver operating characteristic (ROC) curve that plotted the rate of sensitivity against the rate of false-positive results over a range of cut-off values [19]. We

Table 2. Comparison of Various Diagnostic Tests in 86 Patients with Suspected Tuberculosis (TB) Meningitis

Characteristic	TB meningitis[a] (n = 31)	Inactive TB (n = 55)
Tuberculin skin test		
Induration size ⩾5 mm after 48 h	7/31 (23)	7/45 (16)
Induration size ⩾10 mm after 48 h	7/31 (23)	6/45 (13)
ELISPOT assay		
ELISPOT assay on PBMCs	22/31 (71)	20/53[b] (38)
ELISPOT assay on CSF-MCs	13/22[c] (59)	1/28[d] (4)
CSF-MC/PBMC ratio ⩾2	7/22[e] (32)	0/26[f] (0)
Results of diagnostic tests for tuberculosis		
CSF profile		
WBC count, median cells × 10^6/L (IQR)	145 (66–188)	75 (30–355)
Lymphocyte percentage, median % (IQR)	67 (30–81)	53 (20–82)
Neutrophil percentage, median % (IQR)	16 (2–66)	19 (3–62)
Protein level, median mg/L (IQR)	172 (139–241)	85 (44–120)
Glucose level, median g/dL (IQR)	49 (35–73)	61 (50–71)
CSF/serum glucose ratio, median ratio (IQR)	0.39 (0.28–0.54)	0.51 (0.38–0.55)
CSF adenosine deaminase level, median U/L (IQR)	10.6 (7.3–15.4)	3.7 (2.5–9.8)
Positive AFB stain from CSF or other sample	4/31[g] (13)	0/55 (0)
Positive *M. tuberculosis* PCR from CSF or other sample	4/31[g] (13)	0/55 (0)
Positive *M. tuberculosis* culture from CSF or other sample	7/31[g] (23)	0/55 (0)

NOTE. Data are proportion (%) of patients, unless otherwise indicated. CSF, cerebrospinal fluid; CSF-MC, cerebrospinal fluid mononuclear cell; ELISPOT, enzyme-linked immunosorbent spot; IQR, interquartile range; PBMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction; WBC, white blood cell.

[a] TB meningitis includes confirmed (n = 10), highly probable (n = 6), and probable (n = 15) cases of TB. Two cases of possible TB meningitis were excluded from this analysis.

[b] Of the 53 patients for whom the PBMC ELISPOT assay was performed, 3 samples yielded indeterminate ELISPOT results.

[c] Of the 22 patients for whom the CSF ELISPOT assay was performed, 4 samples yielded indeterminate ELISPOT results.

[d] Of the 28 patients for whom the CSF ELISPOT assay was performed, 2 samples yielded indeterminate ELISPOT results.

[e] The CSF-MC ELISPOT assay was performed concurrently with the PBMC ELISPOT assay in 22 of 31 patients. Of these 22 patients, 4 samples yielded indeterminate results on the CSF-MC or PBMC ELISPOT assays.

[f] The CSF-MC ELISPOT assay was performed concurrently with the PBMC ELISPOT assay in 26 of 55 patients. Of these 26 patients, 4 samples yielded indeterminate results on the CSF-MC or PBMC ELISPOT assays.

[g] Positive results of AFB stain, *M. tuberculosis* PCR, and *M. tuberculosis* culture were obtained from CSF specimens in 2, 3, and 5 patients and from other sites in 2, 1, and 2 patients, respectively.

selected an optimal cut-off value as the point on the ROC curve farthest from the diagonal line that maximized the sum of the sensitivity and the specificity [20]. To evaluate the preferred test for prediction of TBM, we compared the areas under the ROC curves [19].

RESULTS

Patient characteristics. Eighty-nine subjects with suspected TBM were prospectively enrolled in the study. Of these, 10 (11%) subjects were classified as having confirmed TBM, 6 (7%) as having highly probable TBM, 15 (17%) as having probable TBM, and 55 (62%) as not TB. The 2 independent study investigators were in complete agreement on these classifications. The investigators disagreed on the classification of 3 patients (3%), who were therefore classified as having possible TBM and were excluded from the final analysis (Figure 1). The baseline clinical characteristics of the 86 patients with TBM and those classified as not TB are shown in Tables 1 and 2.

Diagnostic performances of the PBMC and CSF-MC ELISPOT assays. The PBMC ELISPOT assay was successfully performed on 84 (98%) of 86 enrolled subjects (Figure 1). The samples from 3 (4%) of the 84 patients yielded indeterminate ELISPOT results. Of the 86 patients with suspected TBM, the CSF-MC ELISPOT assay was performed on 50 (58%) subjects who agreed to additional CSF sampling (Figure 1). As shown in Table 3, the patients who consented to participate in the CSF-MC ELISPOT assays were older and had higher white blood cell counts, higher protein levels, lower glucose levels, and higher adenosine deaminase (ADA) levels in their CSF, relative to those who did not consent to the CSF-MC ELISPOT

Table 3. Comparisons of Clinical Characteristics and Cerebrospinal Fluid (CSF) Profiles between Patients Who Did and Did Not Consent to Participate in the CSF Mononuclear Cell (CSF-MC) Enzyme-Linked Immunosorbent Spot (ELISPOT) Assay

Characteristic	CSF-MC ELISPOT assay[a] (n = 50)	No CSF-MC ELISPOT assay (n = 36)	P
Age, mean years ± SD	41.4 ± 17.2	48.9 ± 18.3	.05
Male sex	31 (62)	22 (61)	.93
Clinical diagnosis			
TB meningitis			
Overall	22 (44)	9 (25)	.07
Confirmed	8 (16)	2 (6)	
Highly probable	3 (6)	3 (8)	
probable	11 (22)	4 (11)	
Viral meningitis	14 (28)	17 (47)	.07
Partially treated bacterial meningitis	7 (14)	7 (19)	.50
Fungal meningitis	5 (10)	0 (0)	.07
Leptomeningeal seeding of malignant tumor	1 (2)	0 (0)	>.99
Other	1 (2)	3 (8)	.30
No underlying illness	35 (70)	26 (72)	.82
Immunosuppressive condition[b]	9 (18)	8 (22)	.63
CSF profile			
WBC count, median cells × 10^6/L (IQR)	120 (50–250)	55 (30–340)	.01
Lymphocyte percentage, median % (IQR)	62 (23–81)	52 (21–88)	.93
Neutrophil percentage, median % (IQR)	24 (2–64)	13 (3–49)	.74
Protein level, median mg/L (IQR)	134 (74–231)	60 (37–115)	.004
Glucose level, median g/dL (IQR)	53 (34–68)	64 (57–72)	.01
CSF/serum glucose ratio, median ratio (IQR)	0.40 (0.29–0.55)	0.51 (0.46–0.58)	.01
CSF adenosine deaminase level, median U/L (IQR)	7.5 (3.3–13.9)	3.4 (1.8–6.5)	.002
Positive AFB stain from CSF or other sample	2 (4)	2 (6)	.64
Positive *M. tuberculosis* PCR from CSF or other sample	3 (6)	1 (3)	.65
Positive *M. tuberculosis* culture from CSF or other sample	5 (10)	2 (6)	.70

NOTE. Data are no (%) of patients, unless otherwise indicated. CSF, cerebrospinal fluid; CSF-MC, cerebrospinal fluid-mononuclear cells; IQR, interquartile range; PCR, polymerase chain reaction; TB, tuberculosis; WBC, white blood cell.

[a] Three cases of possible TB meningitis were excluded from this analysis.

[b] Immunosuppressive condition is defined as patients with underlying diseases, such as human immunodeficiency virus infection, malignancy, liver cirrhosis, and chronic renal failure, and/or patients who were receiving immunosuppressive treatment.

assay. The samples from 6 (12%) of the 50 participating patients yielded indeterminate ELISPOT results. The factors associated with the indeterminate results obtained in some of the 50 patients who consented to the CSF-MC ELISPOT assay are shown in Table 4. The volume of CSF obtained and the white blood cell count in the CSF did not appear to influence the likelihood of obtaining an indeterminate result for the CSF-MC ELISPOT assay. The responses to ESAT-6 and CFP-10 in the PBMC and CSF-MC ELISPOT assays are detailed in Figure 2*A*. We performed both PBMC and CSF-MC ELISPOT assays simultaneously in 48 (56%) of the 86 patients with suspected TBM (Figure 1). Among these patients, data from 8 (17%) subjects yielded indeterminate results from either the CSF-MC or the PBMC ELISPOT assay (Figure 1). The ratios of the CSF-MC to PBMC ELISPOT assay results are shown in Figure 2*B*.

On the basis of the ROC curve obtained for the PBMC ELISPOT assay (Figure 3*A*), we determined that the optimal cut-off was ⩾45 spots. However, we herein selected a cut-off for the PBMC ELISPOT assay of ⩾6 spots, on the basis of clinical relevance and the manufacturer's recommendation. When we used this cut-off, the sensitivity and specificity of the PBMC ELISPOT were 71% (95% confidence interval [CI], 51%–86%) and 57% (95% CI, 42%–70%), respectively. On the basis of our ROC curve for the CSF-MC ELISPOT assay (Figure 3*A*), we selected an optimal cut-off of ⩾6 spots. When we used

Table 4. Factors Associated with Indeterminate Results in 50 Patients Who Underwent a Cerebrospinal Fluid Mononuclear Cell Enzyme-Linked Immunosorbent Spot Assay

This table is available in its entirety in the online version of *Clinical Infectious Diseases*

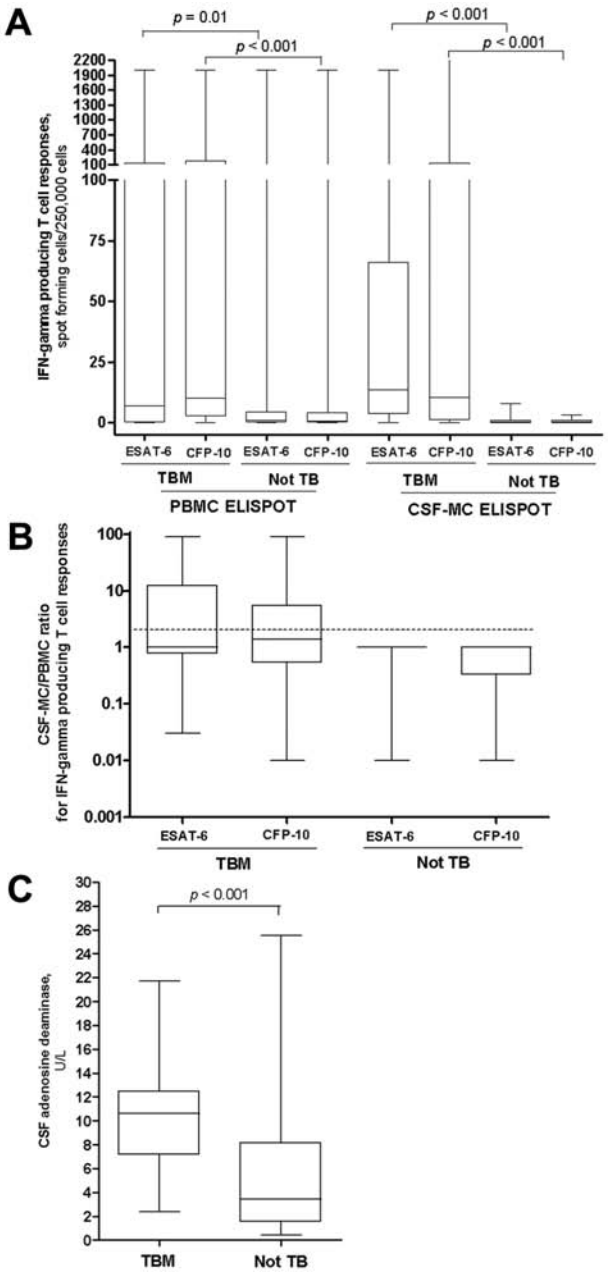

Figure 2. Box-and-whisker plot showing responses to ESAT-6 and CFP-10, according to the peripheral mononuclear cell (PBMC) and cerebrospinal fluid mononuclear cell (CSF-MC) enzyme-linked immunosorbent spot (ELISPOT) assays (*A*), the ratio of CSF-MC to PBMC ELISPOT assay results (*B*), and CSF adenosine deaminase levels (*C*) in patients with suspected tuberculous meningitis (TBM). The *boxes* indicate the lower and upper quartiles, the *central lines* indicate the median, and the ends of the *whiskers* indicate the 95% confidence intervals.

this cut-off, the sensitivity and specificity of the CSF-MC ELISPOT assay were 59% (95% CI, 36%–79%) and 89% (95% CI, 72%–98%), respectively. The diagnostic performances of the ELISPOT assays in the subsets of patients who were tested with the CSF-MC ELISPOT assay ($n = 50$) or with only the PMBC ELISPOT assay ($n = 36$) are shown in Table 5. The diagnostic performances of ELISPOT assays, by TBM diagnostic category, are shown in Table 6.

Comparison of various diagnostic tests for TBM. The results of the various diagnostic tests used to assess the samples from 86 patients with suspected TBM are shown in Table 2. The acid fast bacilli stain, *M. tuberculosis* PCR, and cultures for *M. tuberculosis* were positive in 13%, 13%, and 23% of all subjects, respectively. The CSF ADA levels are detailed in Figure 2*C*. Our ROC curve analysis revealed that the optimal cut-off values for the CSF ADA levels and CSF/serum glucose ratios were 5.8 IU/mL and 0.44, respectively (Figure 3*A*). The diagnostic performances for these cut-off values are shown in Table 5. The sensitivity and specificity of ADA levels in CSF (>5.8 U/L) were 89% (95% CI, 69%–98%) and 73% (95% CI, 58%–84%), respectively.

Figure 3*A* and 3*B* show the relative discriminative accuracies of the various tests, as assessed by the area under the ROC curves for all patients ($n = 86$) and for the subgroup that participated in the CSF-MC ELISPOT assay ($n = 50$). If we assume that sensitivity and specificity are equally important, the CSF-MC ELISPOT assay appears to be the preferred test for the diagnosis of TBM. However, the highest sensitivity was obtained when we combined ADA >5.8 U/L or positive PBMC ELISPOT results (94%; 95% CI, 79%–99%); this combined test conferred a negative likelihood ratio of 0.14 (95% CI, 0.03–0.55) when the results from both tests were negative.

DISCUSSION

Here we assessed the clinical usefulness of the recently developed T cell–based ELISPOT assay in patients with suspected TBM. We found that the PBMC ELISPOT assay in patients with suspected TBM had a 71% sensitivity for the diagnosis of active TB. We previously showed that the sensitivity of the ELISPOT assay for the diagnosis of extrapulmonary TB was 94% [4, 5]. Thus, the sensitivity of the PBMC ELISPOT assay to TBM appears to be slightly lower than that to other types of extrapulmonary TB. It is not yet known whether RD1 peptide-specific T cell responses are influenced by differences in disease status or host condition. Recently, Goletti et al [21] demonstrated that patients with severe pulmonary TB exhibited a lower response to selected RD1 peptides, compared with patients with less severe disease. Other studies have correlated the magnitude of the response to RD1 peptides with the patient's bacterial antigenic load [22–24]. Hence, the relatively low sensitivity of the PBMC ELISPOT assay in patients with TBM may be partially explained by low antigenic loading and the severe manifestation of TBM. However, further studies will be required to determine whether ELISPOT assay performance differs among patients with various clinical manifestations of extrapulmonary TB.

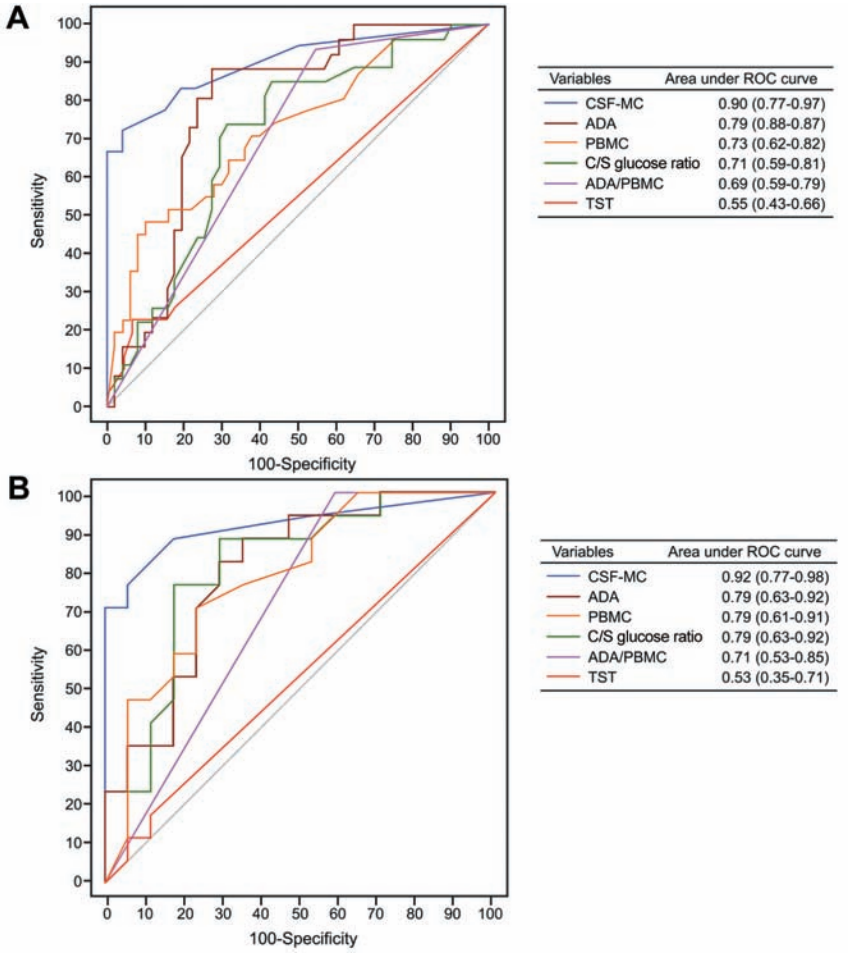

Figure 3. Receiver operating characteristics (ROC) curves for the various tests used to detect tuberculous meningitis in all patients ($n = 86$) (*A*) and in the subgroup that underwent cerebrospinal fluid mononuclear cell (CSF-MC) enzyme-linked immunosorbent spot (ELISPOT) assays ($n = 50$) (*B*). ADA, adenosine deaminase levels in CSF; ADA/PBMC, positive ADA test (>5.8 IU/L) or positive PBMC ELISPOT assay (≥6 spots); CSF-MC, ELISPOT assay of CSF-MCs; C/S glucose ratio, CSF/serum glucose ratio; PBMC, ELISPOT assay of peripheral blood mononuclear cells; TST, tuberculin skin test.

Because *M. tuberculosis*–specific T cells are recruited to the infection site(s) in cases of active TB [7–15, 25], it seems logical that enumeration of effector T cells by ELISPOT assay at the infection site could increase the specificity of active TB diagnosis, compared with that obtained from assaying the blood alone [10, 11]. Consistent with this hypothesis, our results showed that the CSF-MC ELISPOT for TBM had a specificity of 89% (95% CI, 72%–98%). However, we assumed that inflamed meninges may inevitably allow for circulating *M. tuberculosis*–specific lymphocyte migration in patients with inactive TB and latent TB infection. So, the comparison of the results of ELISPOT assays on samples obtained from the site of infection to those of ELISPOT assays performed on the blood can give additional information to distinguish between active TB and inactive TB. We found that a ratio (≥2) of the CSF-MC ELISPOT to the PBMC ELISPOT results could be used to distinguish patients with active TB from patients without TB, and that the use of this ratio conferred high specificity (ie, nearly 100%) in diagnosis. These findings are consistent with those from our previous study [12]. Therefore, the present findings confirm our hypothesis that the use of *M. tuberculosis*–specific ELISPOT assays to test samples from the infection site may provide a more specific diagnosis of active TB, compared with assaying blood samples alone (Table 2 and Figure 2*B*). Notably, fewer than one-half of the patients who received a diagnosis of TBM had increased concentrations of *M. tuberculosis*–specific lymphocytes at the infected sites (CSF-MC ELISPOT/PBMC ELISPOT ratio ≥2), whereas no TBM-free patient had more *M. tuberculosis*–specific lymphocytes in the CSF than in the blood. This is in contrast to previous findings that increased concentrations of *M. tuberculosis*–specific lymphocytes are compartmentalized at the infected sites, including pleural fluid [7, 8], bronchoalveolar lavage fluid [9–11], peritoneal fluid [25], and CSF [13]. We do not know precisely why our results differ from the previous observations. However, a similar phenomenon has been described in patients with TBM

Table 5. Diagnostic Accuracy of Diagnosis of Tuberculosis (TB) Meningitis in 86 Patients with Suspected TB Meningitis

Population, assay	Sensitivity[a]		Specificity[b]		PPV, % (95% CI)	NPV, % (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
	Proportion	Percentage (95% CI)	Proportion	Percentage (95% CI)				
All patients (n = 86)								
Tuberculin skin test result ⩾10 mm	7/31	23 (10–41)	39/45	87 (73–95)	54 (25–81)	62 (49–74)	1.69 (0.63–4.56)	0.90 (0.72–1.12)
PBMC ELISPOT ⩾6 spots	22/31	71 (51–86)	30/53	57 (42–70)	49 (34–64)	77 (61–89)	1.64 (1.12–2.39)	0.51 (0.28–0.93)
CSF-MC ELISPOT ⩾6 spots	13/22	59 (36–79	25/28	89 (72–98)	81 (54–96)	74 (56–87)	5.52 (1.79–16.98)	0.46 (0.27–0.77)
CSF ADA level >5.8 IU/L	23/26	89 (69–98)	37/51	73 (58–84)	62 (45–78)	93 (79–98)	3.22 (2.02–5.14)	0.16 (0.05–0.47)
CSF/serum glucose ratio ⩽0.44	20/27	74 (54–89)	35/51	69 (54–81)	56 (38–72)	83 (69–93)	2.36 (1.49–3.75)	0.38 (0.19–0.73)
PBMC ELISPOT or CSF ADA > 5.8 IU/L	29/31	94 (79–99)	25/55	46 (32–59)	49 (36–63)	93 (76–99)	1.72 (1.32–2.22)	0.14 (0.03–0.55)
CSF-MC ELISPOT assay performed (n = 50)								
Tuberculin skin test result ⩾10 mm	2/22	9 (1–29)	21/23	91 (72–99)	50 (7–67)	51 (35–67)	1.05 (0.16–6.79)	0.99 (0.82–1.19)
PBMC ELISPOT ⩾6 spots	15/22	68 (45–86)	17/26	65 (44–83)	63 (41–81)	71 (49–87)	1.97 (1.08–3.59)	0.48 (0.24–0.95)
CSF-MC ELISPOT ⩾6 spots	13/22	59 (39–79)	25/28	89 (72–98)	81 (54–96)	74 (56–87)	5.52 (1.79–16.98)	0.46 (0.27–0.77)
CSF ADA >5.8 IU/L	19/21	91 (69–99)	16/27	59 (39–78)	63 (44–80)	89 (65–99)	2.22 (1.38–3.57)	0.16 (0.04–0.62)
CSF/serum glucose ratio ⩽0.44	17/22	77 (55–92)	17/27	63 (42–81)	63 (42–81)	77 (55–92)	2.09 (1.21–3.59)	0.36 (0.16–0.82)
PBMC ELISPOT or CSF ADA level >5.8 IU/L	21/22	96 (77–99)	14/28	50 (31–69)	60 (42–76)	93 (68–99)	1.91 (1.30–2.79)	0.09 (0.01–0.64)
Only PBMC ELISPOT assay performed (n = 36)								
Tuberculin skin test results ⩾10 mm	5/9	56 (21–86)	18/22	82 (59–95)	56 (21–86)	82 (59–95)	3.06 (1.06–8.83)	0.54 (0.25–1.16)
PBMC ELISPOT ⩾6 spots	7/9	78 (39–97)	13/27	48 (29–68)	33 (15–57)	87 (59–98)	1.50 (0.91–2.48)	0.46 (0.12–1.67)
CSF ADA >5.8 level IU/L	4/5	80 (28–99)	21/24	88 (68–97)	57 (18–90)	95 (77–99)	6.40 (2.04–20.12)	0.23 (0.04–1.33)
CSF/serum glucose ratio ⩽0.44	3/5	60 (15–95)	18/24	75 (53–90)	33 (7–70)	90 (68–99)	2.40 (0.89–6.49)	0.53 (0.17–1.59)
PBMC ELISPOT or CSF ADA level >5.8 IU/L	8/9	89 (52–99)	11/27	41 (22–61)	33 (16–99)	92 (61–99)	1.50 (1.02–2.21)	0.27 (0.04–1.82)

NOTE. ADA, adenosine deaminase; CI, confidence interval; CSF, cerebrospinal fluid; CSF-MC, cerebrospinal fluid-mononuclear cell; ELISPOT, enzyme-linked immunosorbent spot; NPV, negative predictive value; PBMC, peripheral blood mononuclear cell; PPV, positive predictive value; TB, tuberculosis.

[a] Sensitivity is determined by dividing the no of patients with a positive test result by the no of patients tested.

[b] Specificity is determined by dividing the no of patients with a negative test result by the no of patients tested.

Table 6. Diagnostic Accuracy of Diagnosis of Tuberculous Meningitis in 86 Patients with Suspected Tuberculous Meningitis

This table is available in its entirety in the online version of *Clinical Infectious Diseases*

[12, 14]. This could indicate that the inflamed blood-brain barrier allows for less efficient *M. tuberculosis*–specific lymphocyte migration than do inflamed serosal surfaces [14]. Alternatively, the clinical presentation of TBM may occur earlier in the disease process, prior to significant lymphocyte compartmentalization [14]. Another possible explanation could be that the cell counts in the CSF may decrease over time after sampling and may be falsely low in CSF ELISPOT assays [26], although we did separate the CSF-MC from the CSF within 30 min after obtaining the samples. Therefore, the number of viable cells in CSF can modify the sensitivity of CSF-MC ELISPOT assay.

Given the high cost of false-negative diagnoses of TBM (which is fatal if left untreated), we sought to improve this sensitivity by testing the combined use of various tests. Among the diagnostic tests and combinations of these tests, we found that the ADA/PBMC ELISPOT assay provided the highest sensitivity (94%) but had low specificity (46%). Thus, the use of this strategy for TBM diagnosis could lead to false positives, potentially exposing patients without TBM to unnecessary anti-TB treatments. However, the use of a ratio ($\geqslant$2) of CSF-MC/PBMC ELISPOT as a cut-off provided the highest specificity. Therefore, the step-wise use of both of these combined analyses may provide clinicians with more valuable information. For example, we propose a diagnostic role of ELISPOT assays in patients with suspected TBM (Figure 4). However, further prospective studies will be needed to validate the practical use of this diagnostic strategy.

Our study has a few limitations. First, some may argue that 68% of the patients were classified as having highly probable or probable TBM, mainly on the basis of brain image findings or CSF findings and their clinical responses to anti-TB therapy, in the absence of microbiologic confirmation. However, bacteriological confirmation is not sufficiently sensitive to be used alone for the evaluation of new diagnostic tests, which may be more sensitive than mycobacterial culture [17, 27]. Therefore, as other investigators have done in other studies [4, 5, 12, 14, 17, 18, 25], we herein relied on clinical categories as reference standards for diagnosis, incorporating information from mycobacterial culture, acid fast bacilli stain, and *M. tuberculosis* PCR tests, as well as clinical, radiological, and therapeutic outcome criteria. That is, we applied strictly predefined case definitions to patients who had culture-negative TBM or were non TB, and 2 independent study investigators were asked to classify the patients without knowledge of the ELISPOT assay results. In addition, the attending physicians were blinded to the results of the ELISPOT assays, so the test results would not affect their decisions regarding empirical anti-TB treatment. Furthermore, when subgroup analyses were performed on patients with confirmed TBM or with confirmed and highly probable TBM (Table 6), the sensitivities and specificities of the various diagnostic tests were similar. Second, there may be a selection bias because CSF-MC ELISPOT assays were performed in 50 (58%) of 86 patients, and patients with severe illness or more suspected TBM were included in this subgroup (Table 3). To address this potential issue, we calculated diagnostic performances and generated ROC curves of various tests on the subset of patients that participated in the CSF-MC ELISPOT assay (Table 5 and Figure 3*B*). Our results suggest that selection bias did not substantially affect our study results. Finally, we obtained a relatively high proportion of indeterminate results in our CSF-MC ELISPOT assay. Six (12%) of the 50 patients who agreed to additional CSF sampling had indeterminate results in the CSF-MC ELISPOT assay. Previous studies on the use of peripheral blood samples for an interferon-γ–releasing assay also reported that ~10% of samples yielded indeterminate results in routine clinical practice [28, 29]. However, further studies on this issue should examine technical parameters, such as the optimal CSF volume, optimal time range after CSF sampling, and optimal cut-off values, for CSF-MC ELISPOT assay. In conclusion, our present findings indicate that ELISPOT assays of PBMC and CSF-MC can be useful adjuncts to the current tests for the diagnosis of TBM, particularly when used in combination with ADA level in CSF.

The figure is available in its entirety in the online edition of *Clinical Infectious Diseases*.

Figure 4. Proposed diagnostic role of enzyme-linked immunosorbent spot assays in patients with suspected tuberculous meningitis.

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