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LETTERS

T-cell-based assays on cerebrospinal fluid and PBMCs for rapid diagnosis of TB meningitis in non-HIV patients

To the Editors:

Recently, two studies have shown that an ELISPOT assay using mononuclear cells (MCs) compartmentalised in the cerebrospinal fluid (CSF) is an accurate and rapid rule-out or rule-in test for tuberculosis meningitis (TBM) in conjunction with other rapid tests [1, 2]. However, these two studies had limitations: the majority (94%) of enrolled patients were infected with HIV in one study [2], and the CSF-MC ELISPOT assay was performed on a relatively small number of patients in the other [1]. This study builds on our earlier publication [1] and evaluates the clinical utility for the diagnosis of TBM of simultaneous testing with the peripheral blood mononuclear cell (PBMC) and CSF-MC ELISPOT assays in a larger number of non-HIV-infected patients, by using the standardised diagnostic criteria for TBM [3].

Adult patients (aged $\geqslant$16 yrs) with suspected TBM admitted to Asan Medical Center, a 2,700-bed tertiary hospital in Seoul, Republic of Korea, were enrolled prospectively from April 2008 to October 2010. The results of the ELISPOT assays were concealed from the attending physicians to avoid bias because the results of the ELISPOT assays might have affected the attending physicians' decisions on empirical anti-tuberculosis therapy.

Patients with suspected TBM were categorised as definite TBM, probable TBM, possible TBM, not TBM or indeterminate meningitis, according to a recently proposed uniform case definition, with some modifications (tables s1 and s2) [3]. We excluded patients with possible TBM or indeterminate meningitis from the final analysis.

Peripheral venous blood (~8 mL) and CSF (~4 mL) were obtained from participants, and PBMCs and CSF-MCs were immediately separated (within 30 min). The PBMCs and CSF-MCs were isolated by Ficoll-Hypaque density gradients and simple centrifugation, respectively. The collected cells were suspended and the ELISPOT assays (T-SPOT.*TB*; Oxford Immunotec, Oxford, UK) were performed as described elsewhere [1, 4]. The threshold for a positive response was $\geqslant$6 spot-forming cells per well after subtraction of the negative control well, according to the manufacturer's recommendation. A response was classified as indeterminate if the number of spots in the positive-control well was <20 or the number of spots in the negative-control well was >10 [1, 4].

107 subjects with suspected TBM who agreed to simultaneous sampling for PBMC and CSF-MC ELISPOT assays were prospectively enrolled in the study. Of these, two HIV-infected patients and two patients with indeterminate meningitis were excluded. Of the remaining 103, 46 (45%) were classified as having TBM (17 definite TBM, eight probable TBM, and 21 possible TBM), and 57 (55%) as not TBM. Excluding the 21 subjects with possible TBM, 82 subjects were included in the final analysis (31 of these patients had been included in a previous report [1]).

The results of the various diagnostic tests used to assess samples from the 82 patients with suspected TBM are shown in table 1. Eight (10%) and seven (9%) of the 82 subjects gave indeterminate ELISPOT results in the PBMC and CSF-MC ELISPOT assays, respectively. ELISPOT responses to early secretory antigenic target (ESAT)-6 and 10-kDa culture filtrate protein (CFP-10) are shown in figure s1. When we used a cut-off of $\geqslant$6 spots based on the manufacturer's recommendation, the sensitivity and specificity, respectively, of the ELISPOT assays for diagnosing TBM were as follows: PBMC ELISPOT, 88% (95% CI 69–97%) and 58% (95% CI 44–71%); and CSF-MC ELISPOT, 72% (95% CI 51–88%) and 79% (95% CI 66–89%). In addition, when we used a cut-off of $\geqslant$91 spots at the expense of sensitivity, the sensitivity and specificity of the CSF-MC ELISPOT assay were 40% (95% CI 21–61%) and 88% (95% CI 76–95%). We also sought to improve sensitivity and specificity by combining various tests. When a criterion of a PBMC ELISPOT $\geqslant$6 spots or adenosine deaminase (ADA) levels $\geqslant$5.7 IU·L^{-1} was used for diagnosing TBM, we obtained a sensitivity of 100% (95% CI 86–100%). When a criterion of CSF-MC/PBMC ELISPOT ratios $\geqslant$1.0 was used, we obtained a specificity of 98% (95% CI 89–100%).

In the present study, although the diagnostic sensitivity (88%) of the PBMC ELISPOT assay for TBM was similar to or slightly higher than that (75–91%) of previous studies (table s3), it was still not high enough to use as a rule-out test for TBM. Furthermore, the low specificity of PBMC ELISPOT in the current (58%) and previous studies (57–75%) also indicate the limited value of this assay to distinguish between patients with latent infection and those with active disease (table s3). In contrast to the PBMC ELISPOT assay, the CSF-MC ELISPOT assay may increase the sensitivity and specificity of diagnosis of active tuberculosis, because *M. tuberculosis*-specific T-cells are recruited to the sites of the active infection [5–7]. However, the current and previous studies showed that the sensitivity and specificity of CSF-MC ELISPOT with the manufacturer's recommend cut-off value ($\geqslant$6 spots per 2.5×10^5 cells or $\geqslant$24 spots per 10^6 cells) were not high enough to use as a rapid rule-out or rule-in test for diagnosing TBM (table s3). PATEL *et al*. [2] reported that the CSF-MC ELISPOT assay, using a higher cut-off value rather than manufacturer's recommended cut-off value, especially in conjunction with other rapid tests, was an accurate rapid rule-in

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TABLE 1 Diagnostic performance of various tests in 82 patients with suspected tuberculosis meningitis (TBM)

	Sensitivity % (n/N[#] 95% CI)	Specificity % (n/N[#] 95% CI)	PPV % (95% CI)	NPV % (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
Definite[¶] and probable TBM (n=25) compared with not TBM (n=57)[+]						
TST results ⩾10 mm[§]	23 (5/22, 8–45)	89 (39/44, 75–96)	50 (19–81)	70 (56–81)	2.00 (0.65–6.19)	0.87 (0.68–1.12)
PBMC ELISPOT ⩾6 spots	88 (22/25[§§], 69–97)	58 (33/57[§§], 44–71)	58 (41–74)	92 (78–98)	2.69 (1.76–4.13)	0.18 (0.06–0.52)
CSF-MC ELISPOT ⩾6 spots[f]	72 (18/25[§§], 51–88)	79 (45/57[§§], 66–89)	75 (53–90)	88 (76–96)	6.37 (2.90–14.00)	0.28 (0.14–0.57)
CSF-MC ELISPOT ⩾9 spots[##]	72 (18/25[§§], 51–88)	82 (47/57[§§], 70–91)	82 (70–91)	89 (77–96)	9.56 (3.63–25.19)	0.27 (0.14–0.54)
CSF-MC ELISPOT ⩾91 spots[¶¶]	40 (10/25[§§], 21–61)	88 (50/57[§§], 76–95)	91 (59–100)	78 (66–87)	21.25 (2.28–156.64)	0.60 (0.42–0.84)
CSF ADA level ⩾5.7 IU·L^{-1}	88 (22/25, 69–97)	70 (40/57, 57–82)	56 (40–72)	93 (81–99)	2.95 (1.93–4.51)	0.17 (0.06–0.50)
CSF/serum glucose ratio ⩽0.41	72 (18/25, 51–88)	72 (41/57, 58–83)	53 (35–70)	85 (72–94)	2.56 (1.58–4.15)	0.39 (0.20–0.75)
PBMC ELISPOT ⩾6 spots + CSF ADA ⩾5.7 IU·L^{-1}	100 (24/24, 86–100)	53 (24/45, 38–68)	53 (38–68)	100 (86–100)	2.14 (1.57–2.93)	0.00
CSF-MC/PBMC ELISPOT ratio ⩾1.0[++]	54 (13/24, 33–74)	98 (49/50, 89–100)	93 (66–100)	82 (70–90)	27.08 (3.76–195.16)	0.47 (0.30–0.72)
Definite TBM[¶] (n=17) compared with not TBM (n=57)[+]						
TST results ⩾10 mm[§]	36 (5/14, 13–65)	89 (39/44, 75–96)	50 (19–81)	81 (67–91)	3.14 (1.06–9.29)	0.73 (0.48–1.09)
PBMC ELISPOT ⩾6 spots	94 (14/17[§§], 71–100)	58 (33/57[§§], 44–71)	50 (32–68)	97 (85–100)	2.88 (1.90–4.38)	0.09 (0.01–0.59)
CSF-MC ELISPOT ⩾6 spots[f]	82 (14/17[§§], 57–96)	79 (45/57[§§], 66–89)	70 (46–88)	96 (85–99)	7.44 (3.43–16.13)	0.14 (0.04–0.52)
CSF-MC ELISPOT ⩾9 spots[##]	82 (14/25[§§], 57–96)	82 (47/57[§§], 70–91)	78 (52–94)	96 (86–100)	11.16 (4.28–29.10)	0.14 (0.04–0.50)
CSF-MC ELISPOT ⩾91 spots[¶¶]	59 (10/17[§§], 33–82)	88 (50/57[§§], 76–95)	91 (59–100)	89 (78–96)	31.87 (4.41–230.28)	0.38 (0.20–0.72)
CSF ADA level ⩾5.7 IU·L^{-1}	82 (14/17, 57–96)	70 (40/57, 57–82)	45 (27–64)	93 (81–99)	2.76 (1.75–4.35)	0.25 (0.09–0.71)
CSF/serum glucose ratio ⩽0.41	76 (13/17, 50–93)	72 (41/57, 58–83)	45 (26–64)	91 (79–98)	2.72 (1.67–4.46)	0.33 (0.14–0.78)
PBMC ELISPOT ⩾6 spots + CSF ADA ⩾5.7 IU·L^{-1}	100 (16/16, 79–100)	53 (24/45, 38–68)	43 (27–61)	100 (86–100)	2.14 (1.57–2.93)	0.00
CSF-MC/PBMC ELISPOT ratio ⩾1.0[++]	63 (10/16, 35–85)	98 (49/50, 89–100)	91 (59–100)	89 (78–96)	31.25 (4.3–225.67)	0.38 (0.20–0.72)

PPV: positive predictive value; NPV: negative predictive value; TST: tuberculin skin test; PBMC: peripheral blood mononuclear cell; CSF-MC: cerebrospinal fluid-mononuclear cell; ADA: adenosine deaminase. [#]: determined by dividing the number of patients giving positive or negative results by the number of patients tested. [¶]: out of 17 patients with definite TBM, the acid-fast bacilli stain, PCR for *Mycobacterium tuberculosis* complex and *M. tuberculosis* culture were positive in three (18%), 12 (71%) and 16 (94%), respectively. [+]: alternative diagnoses in the not TBM group (n=57) were viral meningitis (n=38), acute bacterial meningitis (n=12), cryptococcal meningitis (n=3), central nervous system aspergillosis (n=1), central nervous system lupus (n=1), aseptic meningitis associated with Kikuchi's disease (n=1), and scrub typhus meningitis (n=1). [§]: a positive criterion for TST as ⩾10 mm was selected according to national guidelines. [f]: manufacturer-recommended cut-off value for the PBMC ELISPOT assay. [##]: receiver operating curve (ROC)-derived optimal cut-off value by Youden's index. [¶¶]: ROC-derived optimal cut-off value selecting for high specificity at the expense of sensitivity. [++]: CSF-MC/PBMC ELISPOT was expressed as zero irrespective of the PBMC ELISPOT result if the CSF-MC ELISPOT result was negative (*i.e.* ⩽5 spots). There were no cases in which the CSF-MC ELISPOT result (numerator) was positive and the PBMC ELISPOT result (denominator) was 0. [§§]: denominator includes samples giving indeterminate results in PBMC or CSF-MC ELISPOT assays.

test for TBM in a tuberculosis- and HIV-endemic setting [2]. We found that CSF-MC/PBMC ELISPOT ratio ⩾1.0, as well as CSF-MC ELISPOT assay with high cut-off value (⩾91 spots) was a useful rule-in test in an indeterminate tuberculosis-/low HIV-burden setting.

Based on these findings, we propose a stepwise diagnostic approach for diagnosing TBM using these combined tests. The combination of PBMC ELISPOT <6 spots with CSF ADA level <5.7 IU·L^{-1} can rule out TBM, and anti-tubeculosis treatment can be discontinued. In our study, of the 82 patients, 26 (32%) met this criterion, all of whom were revealed not to have TBM. In the remaining 56 patients with suspected TBM, CSF-MC/PBMC ELISPOT ratio ⩾1.0 indicates the high possibility of TBM and necessitates maintaining anti-tuberculosis treatment despite its potential toxicity. Of these 56 patients, CSF-MC ELISPOT/PBMC ELISPOT ratio was determined in 48 patients, 14 of whom met the criterion of the CSF-MC/PBMC ELISPOT ratio ⩾1.0. Of these 14 patients, 13 were revealed to have TBM and one was revealed not to have TBM. Therefore, we were able to accurately classify about half the patients with suspected TBM with this approach. However, further prospective studies are needed to validate the practical use of this diagnostic scheme and more accurate diagnostic tests or strategies able to classify the remaining half of patients with suspected TBM need to be developed. In addition, some studies have reported that interferon (IFN)-γ concentration measured in unstimulated body fluid supernatant, such as pleural fluid, pericardial fluid and ascitic fluid, was useful for diagnosing active tuberculosis [8–10]. Therefore, the comparison between unstimulated IFN-γ concentration in CSF and IFN-γ release assays using CSF-MC in patients with suspected TBM will be valuable.

In conclusion, our findings indicate that ELISPOT assays of PBMCs and CSF-MCs are useful adjuncts to current tests for diagnosing TBM. The PBMC ELISPOT assay combined with CSF ADA is a useful rapid rule-out test and the CSF-MC/PBMC ELISPOT ratio is an accurate rule-in test.

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On linezolid efficacy and tolerability

To the Editors:

To further comment on the safety, tolerability and efficacy profile of linezolid in treating ''difficult'' tuberculosis (TB) cases, following the recent study by VILLAR *et al*. [1], we here report on the experience of the E. Morelli Hospital in Sondalo, Italy, a reference centre for difficult-to-treat TB cases, *e.g.* those affected by multidrug-resistant (MDR)- and extensively drug-resistant (XDR)-TB, located in northern Italy [2–3].

As reported elsewhere [3], linezolid has been prescribed ''off label'' in Sondalo, Italy since 2005 to treat patients for whom at least four active drugs cannot be ensured, according to World Health Organization recommendations [4].

Administration of linezolid, within regimens designed to balance efficacy and tolerability, needs to be guided by clear scientific evidence focused on the ideal dosage (per kg body weight per day) and duration [1, 5–9].

The aim of this letter is to describe our recent experience of linezolid tolerability and efficacy between 2009 and 2010.

Methods and definitions are consistent with those used in previous studies by our group [1, 6].

MDR- and XDR-TB have been defined, respectively, as *in vitro* resistance to at least isoniazid and rifampicin (the two most potent first-line drugs for TB treatment) and resistance to isoniazid and rifampicin plus any fluoroquinolone and at least one of the injectable drugs amikacin, capreomycin or kanamycin.

The main results of this study are summarised in tables 1–3.

TABLE 1 Epidemiological characteristics of 12 patients with multidrug-resistant/extremely drug-resistant (XDR) tuberculosis (TB) treated with linezolid in Sondalo, Italy

XDR-TB	4/12 (33)
Resistance to streptomycin	10/12 (83)
Resistance to ethambutol	9/12 (75)
Resistance to pyrazinamide	9/12 (75)
Resistance to fluoroquinolones	7/12 (58)
Resistance to amikacin	3/12 (25)
Resistance to kanamycin	6/11 (54)
Resistance to capreomycin	3/11 (27)
Previous exposure to anti-TB therapy >30 days	9/12 (75)
Median (IQR) number of times treated with anti-TB drugs >1 month	2 (0.5–8)

Data are presented as n/N (%), unless otherwise stated. IQR: interquartile range.