Comparative Utility of Cytokine Levels and Quantitative RD-1-Specific T Cell Responses for Rapid Immunodiagnosis of Tuberculous Meningitis[▽]

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The rapid diagnosis of tuberculous meningitis (TBM) is problematic. We found in 150 patients with suspected TBM that, similar to RD-1-specific quantitative cerebrospinal fluid (CSF) T-cell responses, unstimulated CSF gamma interferon (IFN- γ) levels when used together with other rapid confirmatory tests (Gram stain and cryptococcal latex agglutination test) may allow the accurate and rapid diagnosis of TBM in a setting in which tuberculosis (TB) and HIV are endemic. In resource-poor settings, a clinical prediction rule (CPR) may be useful to clinicians, and thus the IFN- γ assay may potentially need to be used only when the clinical score is below a prespecified threshold. These preliminary findings will need to be confirmed in further studies.

Tuberculosis (TB) remains one of the commonest global killer infectious diseases and is a burgeoning problem in Africa, where HIV coinfection is common. However, the diagnosis is difficult to confirm and therapy is often empirical based on exclusion of other locally prevalent causes. Available rapid diagnostic tools perform poorly. Smear microscopy has a yield of less than 5% in a programmatic setting (1, 15, 16), liparabinomannan (LAM) antigen detects only about one-third of cases (17), and PCR has a sensitivity of ~55% (14). More recently, we (16) and others (12) have shown that the overnight quantitative RD-1 enzyme-linked immunosorbent spot assay (ELISPOT) T cell assay (T-SPOT-TB) is an excellent rapid rule-in test for tuberculous meningitis (TBM) when combined with other simple and widely available rapid tests (cryptococcal latex agglutination test [CLAT] and bacterial Gram stain). However, the overnight ELISPOT is labor-intensive and yields results only after 16 to 24 h. In contrast, unstimulated gamma interferon (IFN- γ) level determination performed directly on the biological fluid without stimulation of cells, and in the context of TB, has been shown to be a useful and accurate diagnostic marker in pleural fluid (6, 9), ascitic fluid (19), and pericardial fluid (3). Furthermore, another Th1 biomarker, IP-10, has also been shown to be of diagnostic value when using unstimulated pleural fluid in patients with active TB (13). However, only one preliminary study from Spain in a small number of patients has investigated the diagnostic utility of

unstimulated IFN- γ for TBM (10), and none have evaluated IP-10. Given the available data from other body compartments, we hypothesized that unstimulated Th1 cytokines (IFN- γ and IP-10) using unprocessed cerebrospinal fluid (CSF) would perform as well as an overnight RD-1 antigen-stimulated ELISPOT in a setting of high TB and HIV prevalence. We compared their performance to a clinical prediction rule (CPR) derived from clinical and laboratory parameters.

Performance outcomes of the overnight RD-1-specific quantitative T cell assay (T-SPOT-TB), using CSF mononuclear cells from 150 patients with TBM, were recently published (16). Here, using the same cohort, we report on the comparative performance of unstimulated and unprocessed CSF IFN- γ and IP-10. Details of recruitment and ethical approval are outlined in the previous publication (16). After giving informed written consent, patients had a computerized tomogram (CT) scan to exclude contraindications to a lumbar puncture (LP) and had blood and CSF samples taken. Tests to exclude other causes of meningitis were performed as previously described (16). Patients were categorized as (i) definite TBM (CSF *Mycobacterium tuberculosis* culture or positive PCR for *M. tuberculosis*) (21), (ii) probable TBM (clinical features of meningitis; an LP consistent with an aseptic bacterial meningitis, i.e., a CSF glucose level of <2.2 mmol/liter, a CSF/blood glucose ratio of <0.5, a protein level of $\geq$ 0.5 g/liter, lymphocytosis not due to other causes, and one of the following: chest X ray [CXR] with active pulmonary TB, CT scan consistent with TBM [hydrocephalus or basal enhancement], and response to antituberculous therapy), and (iii) non-TBM (an alternate definite cause for meningitis identified with response to appropriate therapy) (20). The definite-TBM and non-TBM groups were used for performance outcome calcu-

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FIG. 1. Summary flow chart of patient categorization and investigations performed at recruitment. FBC, full blood count; U&E, urea and electrolytes; LFT, liver function test.

lations. Overnight T-SPOT-TB ELISPOT using CSF mononuclear cells was performed as previously described (16). Unstimulated IP-10 (Hycult Biotech) and IFN- γ (Cellestis Ltd., Victoria, Australia) level assays using unprocessed CSF were

performed in duplicate. Statistical analysis included patients with all three (ELISPOT, IP-10, and IFN- γ level) results. This was performed as previously outlined (16), and univariate and multivariate analyses were used to derive factors predictive for

TABLE 1. Comparison of the clinical and laboratory parameters in the definite-TB meningitis (culture or PCR positive; $n = 37$) and non-TB meningitis ($n = 48$) groups

Characteristic or parameter ^b	Definite TBM	Non-TBM	<i>P</i> value
Patient characteristic			
No. of patients	37	48	
Mean age, yr (SD)	33.9 (9.6)	32.9 (9.73)	0.5
Age: <36/≥36 yr, ^a no. (%)	22/15 (59.5/40.5)	32/16 (66.7/33.3)	0.1
Sex ratio: male/female, no. (%)	17/20 (45.9/54.1)	14/34 (29.2/70.8)	0.1
Ethnic group: BA/M/E/I, no. (%)	36/1/0/0 (97.3/2.7/0/0)	47/0/0/1 (97.9/0/0/2.1)	0.7
HIV status: P/N/unknown, no. (%)	33/3/1 (89.2/8.1/2.7)	41/6/1 (85.4/12.5/2.1)	0.8
Previous TB: yes/no/unknown, no. (%)	7/26/4 (18.9/70.3/10.8)	21/27/0 (43.8/56.2/0)	0.008
TB contact (within 2 yr): yes/no/unknown, no. (%)	9/24/4 (24.3/64.9/10.8)	13/35/0 (27.1/72.9/0)	0.07
Duration of illness (days): <6/≥6 days, ^a no. (%)	6/29 (17.1/82.9)	9/39 (18.8/81.3)	0.9
Steroid treatment: yes/no, no. (%)	12/25 (32.9/67.6)	7/41 (14.6/85.4)	0.05
CLAT: yes/no, no. (%)	4/33 (10.8/89.2)	25/23 (52.1/47.9)	<0.001
CD4 cells/ μ l, no. (IQR)	84 (53–173)	154 (52–253)	0.15
Hydrocephalus (CT/MRI): yes/no, no. (%)	16/13 (55.2/44.8)	8/11 (42.1/57.9)	0.4
CSF parameter, median (IQR)			
Lymphocytes (cells/ μ l)	98 (16–242)	33 (10–84)	0.02
Neutrophils (cells/ μ l)	60 (20–134)	8 (1–67)	0.004
Protein (g/liter)	1.8 (1.2–2.7)	1.0 (0.8–1.7)	<0.001
CSF glucose (mmol/liter)	1.0 (1.0–1.6)	2.0 (1.5–2.6)	<0.001
CSF/serum glucose ratio	0.2 (0.2–0.3)	0.38 (0.2–0.5)	<0.001
Lymphocytes/total ratio	0.6 (0.2–0.9)	0.81 (0.22–1.0)	0.21

^a We chose a 36-year and 6-day cutoff as this was a significant discriminator between acute septic and aseptic meningitis (22).

^b Abbreviations: BA, black African; M, mixed race; E, European; I, Indian; P, positive; N, negative; MRI, magnetic resonance imaging.

TBM and then applied to derive a clinical score, which was used to determine a receiving operating characteristic (ROC)-derived cut point to dichotomize the cohort into those with TBM and the non-TBM group. In practice, clinical symptoms and laboratory tests are used by clinicians in the pretest phase to surmise whether a patient is likely to have a diagnosis of TBM. These parameters were used to derive the clinical score against which test performance was compared.

Of the 150 patients with suspected TBM, investigation yielded 138 patients (38 with definite TBM, 54 with probable TBM, and 48 non-TBM patients [33 cases of cryptococcal or acute bacterial meningitis and 15 other meningitides]) with evaluable results. Exclusions are shown in the study plan in Fig. 1. Among the cohort, 87% were HIV positive with a median CD4 count of 132 (interquartile range [IQR], 53 to 233) (Table 1). Other significant intergroup differences are shown in Table 1. There was no association between CD4 count or the use of steroids and unstimulated IFN- γ levels in the definite-TBM group ($P = 0.4$ and 0.7 , respectively).

In the definite group, 73% were on a fixed-dose combination of first-line anti-TB treatment with a median duration of treatment of 3 days (IQR, 2 to 4). The non-TBM group had treatment appropriate to the diagnosis considered, mainly amphotericin B for cryptococcal meningitis, with a median duration of 2 days (IQR, 2 to 4). Routine smear microscopy for acid-fast bacilli had a 0% diagnostic yield. The diagnoses in the non-TBM group included cryptococcal meningitis ($n = 28$), bacterial meningitis ($n = 5$), viral meningitis ($n = 10$), neoplastic meningitis ($n = 2$), mucormycosis ($n = 1$), venous sinus thrombosis with CSF change ($n = 1$), and neurosyphilis ($n = 1$).

The median IFN- γ level was significantly higher in the definite-TBM group (0.9 IU/ml [range, 0.51 to 1.92]) than in the non-TBM group (0.2 IU/ml [range, 0.12 to 0.56], $P = <0.001$). Outcome data are presented in Table 2 at an optimal cut point defined by the Youden index (18) and a clinically useful rule-in cut point (cut point, ≥ 0.244 IU/ml). These data were derived when comparing the definite- and non-TBM groups as a whole (Table 2), definite-TBM group with CLAT and bacterial meningitides on the basis of CLAT and Gram staining (Table 2), and with cryptococcal and acute bacterial meningitis excluded on a similar basis (Table 2). The sensitivity and specificity in the latter group were 92% (95% confidence interval [CI], 78 to 98) and 100% (95% CI, 78 to 100), respectively. The relevant dot plots and comparative ROC curves for each group are shown in Fig. 2A to F. The sensitivity and specificity (95% CI) of ELISPOT at a cut point of ≥ 46 spot-forming units were 82% (65 to 92) and 100% (78 to 100), respectively (16). Compared to unstimulated IFN- γ levels, this was not significantly different ($P = 0.9$). The median values of IP-10 in the definite-TBM and non-TBM groups were 4,364 (range, 3,941 to 4,942) and 40,44 (2,059 to 4,712), respectively ($P = 0.2$) (Fig. 2C and D). Comparative ROC curves of all 3 assays (RD-1 ELISPOT, unstimulated IFN- γ , and IP-10) are shown in Fig. 2.

The multivariate analysis confirmed 4 factors that were predictive for TBM (Table 3). These factors were used to generate a CPR to which we applied ROC curve analysis generating performance outcome data (summarized in Table 4). A cut point of ≥ 6 was clinically most useful as a rule-in test for the diagnosis of TBM. When unstimulated IFN- γ (level, ≥ 0.224 IU/ml) was applied only to those ruled out by the CPR, then

TABLE 2. Performance outcomes of unstimulated CSF IFN- γ levels (95% CI) at different cut points in the definite-TBM and non-TBM groups^d

Group compared with definite TBM (<i>n</i> = 37) ^c	Cut point (IU/ml)	% (CI)			
		Sensitivity	Specificity	PPV ^e	NPV ^f
Unselected non-TBM	≥0.6 ^a	73 (56–86)	76 (77–88)	71 (54–85)	79 (64–89)
	≥0.244 ^b	92 (78–98)	58 (43–72)	63 (49–76)	90 (74–98)
Non-TBM, Gram stain or CLAT positive	≥0.6 ^a	73 (56–86)	67 (48–82)	71 (54–85)	69 (50–84)
	≥0.244 ^b	92 (78–98)	39 (23–58)	81 (49–76)	67 (55–78)
Non-TBM, Gram stain or CLAT negative	≥0.6 ^a	73 (58–86)	100 (78–100)	100 (87–100)	81 (67–90)
	≥0.244 ^b	92 (78–98)	100 (78–100)	100 (90–100)	94 (84–99)

^a Statistically derived optimal cut point determined by Youden's index (maximal sensitivity and specificity given by the inflection point of the ROC curve [18]).
^b Cut point chosen from ROC curve to maximize clinical utility, i.e., maximal specificity to enable use as a rule-in test when combined with other rapid diagnostic tests, i.e., CLAT and Gram stain.
^c Unselected non-TBM, $n = 48$ and area under the curve, 0.78 (95% CI, 0.65–0.87); non-TBM, Gram stain or CLAT positive (i.e., concurrently positive by alternative rapid tests), $n = 33$ and area under the curve, 0.71 (95% CI, 0.58–0.84); non-TBM, Gram stain or CLAT negative (i.e., all causes of meningitis other than cryptococcal and acute bacterial meningitides), $n = 15$ and area under the curve, 0.93 (95% CI, 0.85–1.0).
^d The first comparison was between the definite-TB meningitis ($n = 37$) group and the unselected non-TB meningitis ($n = 48$) group. To evaluate whether other concomitantly used rapid tests could enhance the specificity of the IFN- γ assay, the data were also analyzed when the non-TBM groups were divided into those who had a positive Gram stain or CLAT and those non-TBM patients who had a negative Gram stain or CLAT.
^e PPV, positive predictive value.
^f NPV, negative predictive value.

FIG. 2. Unstimulated IFN- γ levels and the corresponding ROC curve (A and B) when comparing the definite-TBM, probable-TBM, and non-TBM groups stratified by whether other rapid tests (Gram stain and CLAT) were concurrently negative (meningitides other than cryptococcal or bacterial meningitis) or positive (cryptococcal plus bacterial meningitis). Similar comparisons are shown for unstimulated IP-10 levels (C and D) and the RD-1 ELISPOT (E and F). SFC, spot-forming cells; AUC, area under the curve.

the rule-in value did not substantially change and was significantly higher than the CPR alone ($P < 0.0001$). Applying this cut point to the probable group identified 38/51 (74.5%) patients as having TBM (above the 0.244-IU/ml cut point). Furthermore, the sensitivity when combining the definite ($n = 37$) and probable ($n = 51$) groups was 81.8% (72/88).

This study shows that determination of CSF unstimulated IFN- γ levels performed as well as the overnight ELISPOT in a high-HIV-prevalence setting. However, the discriminatory value of unstimulated IFN- γ is conditional upon combination with two other rapid, cheap, and widely available assays, i.e.,

CLAT and Gram stain. In the probable group, unstimulated IFN- γ levels defined 74.5% of cases as TBM. Thus, the true sensitivity of the test lies between 74% and 92%. Unstimulated IFN- γ , in the context of TBM, has previously been diagnostically evaluated only in a single preliminary study which enrolled 12 Spanish patients with culture-positive TBM (10). In this study, Juan and colleagues found that unstimulated IFN- γ had a sensitivity of 70% (95% CI, 50% to 90%) and a specificity of 95% (95% CI, 90% to 98%) with use of a radioimmunoassay (10). In contrast, we confirmed the accuracy of unstimulated IFN- γ using a standardized enzyme-linked

TABLE 3. Univariate and multivariate analysis to identify factors predictive for the diagnosis of TBM

Laboratory characteristic	Univariate analysis		Multivariate analysis			
	OR ^a (95% CI)	P value	OR (95% CI)	P value	β coefficient	Score
Lymphocyte count, ≥ 200 (cells/ μ l)	6.0 (1.7–20.3)	0.004	6.3 (1.2–32.9)	0.03	2.0	2
Neutrophil count, ≥ 36 (cells/ μ l)	4.5 (1.8–11.2)	<0.001				
Protein level, ≥ 2.5 g/liter	5.3 (1.8–18.1)	0.008				
CSF glucose, ≤ 1 mmol/liter	11.6 (3.5–38.9)	<0.001				
Ratio of CSF/serum glucose, ≤ 0.2	14.2 (3.7–54.0)	<0.001	7.7 (1.6–36.1)	0.01	2.0	2
CD4 count, ≤ 200 (cells/ μ l)	2.4 (0.9–6.3)	0.08	6.2 (1.6–23.7)	0.007	1.8	2
CLAT test (negative)	9.0 (2.8–29.2)	<0.001	11.1 (2.7–46.5)	0.001	2.4	2
Previous TB (no)	3.3 (1.2–9.1)	0.02				

^a OR, odds ratio.

immunosorbent assay (ELISA) in a larger definitive study and in a high-HIV-prevalence setting, which has the highest burden of TBM. Larger studies in different settings are now required to confirm our findings, which lend themselves to the development of a user-friendly point-of-care assay for the rapid diagnosis of TBM. Reassuringly, and unlike the ELISPOT (2, 5), the assay performs well in CSF in HIV-infected persons and is not confounded by CD4 count in this subgroup. This is crucial given the diagnostic conundrum posed by patients with suspected TBM and HIV, where the CSF can be acellular (8, 11, 15) or with a neutrophil predominance (8), and the clinical presentation is often atypical. Another key advantage is that it is independent of CSF lymphocyte count, which is often reduced in HIV-infected persons (4, 7, 11).

Our preliminary findings indicate that in resource-poor settings the CPR may be useful for clinicians; however, this requires confirmation in larger studies. If confirmed, the IFN- γ assay need be used only when the clinical score is below a prespecified threshold. IP-10, hitherto unevaluated in the CSF, showed no discriminatory value in those suspected with TBM.

Our study has several limitations. Larger numbers would have narrowed our confidence intervals. The CPR and IFN- γ levels (used together with CLAT and a Gram stain)

presented here apply only to high-HIV- and -TB-prevalence settings.

In conclusion, the unstimulated IFN- γ levels in unprocessed CSF may be a useful confirmatory test for TBM when combined with other rapid and inexpensive tests such as Gram stain and CLAT. Our preliminary findings now need confirmation in larger studies in different settings.

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TABLE 4. Clinical prediction rule compared to unstimulated IFN- γ , RD-1-specific ELISPOT, and IP-10 results in the definite-TBM ($n = 37$) and non-TBM (CLAT- and Gram-negative; $n = 15$) groups^d

Value	% (CI)				
	Sens	Spec	PPV	NPV	Agreement
Clinical prediction rule ≥ 4	87 (71–95)	71 (56–83)	70 (54–82)	87 (73–96)	78 (67–86)
Clinical prediction rule ≥ 6	46 (30–63) ^a	97 (68–100)	94 (73–100)	41 (25–59)	60 (45–73)
IFN- $\gamma \geq 0.244$ IU/ml	92 (78–98)	100 (78–100)	100 (90–100)	83 (59–96)	94 (84–99)
Clinical prediction rule < 6 but IFN- $\gamma \geq 0.244$ IU/ml	92 (78–98) ^{a,b}	93 (68–100)	97 (85–100)	82 (57–96)	92 (82–98)
ELISPOT ≥ 46 SFU	82 (65–92)	100 (78–100)	100 (88–100)	68 (45–86)	87 (74–94)
Clinical prediction rule < 6 but ELISPOT ≥ 46 SFU	89 (75–97) ^b	93 (68–100)	97 (85–100)	78 (52–94)	90 (79–97)
IP-10 $\geq 4,214$ pg/ml ^c	62 (45–78)	60 (32–84)	79 (60–92)	39 (20–61)	62 (47–75)

^a Comparison of the sensitivity between the clinical prediction rule alone (cut point ≥ 6) to a combination of IFN- γ levels (≥ 0.244 IU/ml) and the clinical prediction rule (< 6) ($P = < 0.001$).

^b Comparison of the negative predictive value between the clinical prediction rule (≥ 6) alone to a combination of IFN- γ levels (≥ 0.244 IU/ml) and the clinical prediction rule (< 6) ($P = 0.005$).

^c $P = 0.9$.

^d Abbreviations: Sens, sensitivity; Spec, specificity; PPV, positive predictive value; NPV, negative predictive value; SFU, spot-forming cells.

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REFERENCES

1. Bhigjee, A. I., et al. 2007. Diagnosis of tuberculous meningitis: clinical and laboratory parameters. *Int. J. Infect. Dis.* **11**:348–354.
2. Bocchino, M., B. Bellofiore, A. Matarese, D. Galati, and A. Sanduzzi. 2009. IFN-gamma release assays in tuberculosis management in selected high-risk populations. *Expert Rev. Mol. Diagn.* **9**:165–177.
3. Burgess, L. J., H. Reuter, M. E. Carstens, J. J. Taljaard, and A. F. Doubell. 2002. The use of adenosine deaminase and interferon-gamma as diagnostic tools for tuberculous pericarditis. *Chest* **122**:900–905.
4. Cecchini, D., et al. 2009. Tuberculous meningitis in HIV-infected and non-infected patients: comparison of cerebrospinal fluid findings. *Int. J. Tuberc. Lung Dis.* **13**:269–271.
5. Chapman, A. L., et al. 2002. Rapid detection of active and latent tuberculosis infection in HIV-positive individuals by enumeration of *Mycobacterium tuberculosis*-specific T cells. *AIDS* **16**:2285–2293.
6. Dheda, K., et al. 2009. Utility of quantitative T-cell responses versus unstimulated interferon- γ for the diagnosis of pleural tuberculosis. *Eur. Respir. J.* **34**:1118–1126.
7. Dube, M. P., P. D. Holtom, and R. A. Larsen. 1992. Tuberculous meningitis in patients with and without human immunodeficiency virus infection. *Am. J. Med.* **93**:520–524.
8. Garcia-Monco, J. C. 1999. Central nervous system tuberculosis. *Neurol. Clin.* **17**:737–759.
9. Jiang, J., H. Z. Shi, Q. L. Liang, S. M. Qin, and X. J. Qin. 2007. Diagnostic value of interferon-gamma in tuberculous pleurisy: a metaanalysis. *Chest* **131**:1133–1141.
10. Juan, R. S., et al. 2006. Interferon gamma quantification in cerebrospinal fluid compared with PCR for the diagnosis of tuberculous meningitis. *J. Neurol.* **253**:1323–1330.
11. Karstede, A. S., S. Valtachanova, R. Barriere, and H. H. Crewe-Brown. 1998. Tuberculous meningitis in South African urban adults. *Q. J. Med.* **91**:743–747.
12. Kim, S. H., et al. 2010. Rapid diagnosis of tuberculous meningitis by T cell-based assays on peripheral blood and cerebrospinal fluid mononuclear cells. *Clin. Infect. Dis.* **50**:1349–1358.
13. Lighter, J., M. Rigaud, M. Huie, C. H. Peng, and H. Pollack. 2009. Chemokine IP-10: an adjunct marker for latent tuberculosis infection in children. *Int. J. Tuberc. Lung Dis.* **13**:731–736.
14. Pai, M., et al. 2003. Diagnostic accuracy of nucleic acid amplification tests for tuberculous meningitis: a systematic review and meta-analysis. *Lancet Infect. Dis.* **3**:633–643.
15. Patel, V. B., et al. 2004. Multidrug-resistant tuberculous meningitis in KwaZulu-Natal, South Africa. *Clin. Infect. Dis.* **38**:851–856.
16. Patel, V. B., et al. 2010. Cerebrospinal T-cell responses aid in the diagnosis of tuberculous meningitis in a human immunodeficiency virus- and tuberculosis-endemic population. *Am. J. Respir. Crit. Care Med.* **182**:569–577.
17. Patel, V. B., et al. 2010. Comparison of a clinical prediction rule and a LAM antigen-detection assay for the rapid diagnosis of TBM in a high HIV prevalence setting. *PLoS One* **5**:e15664.
18. Schisterman, E. F., N. J. Perkins, A. Liu, and H. Bondell. 2005. Optimal cut-point and its corresponding Youden index to discriminate individuals using pooled blood samples. *Epidemiology* **16**:73–81.
19. Sharma, S. K., et al. 2006. Diagnostic accuracy of ascitic fluid IFN-gamma and adenosine deaminase assays in the diagnosis of tuberculous ascites. *J. Interferon Cytokine Res.* **26**:484–488.
20. Thwaites, G., et al. 2000. Tuberculous meningitis. *J. Neurol. Neurosurg. Psychiatry* **68**:289–299.
21. Thwaites, G., et al. 2009. British Infection Society guidelines for the diagnosis and treatment of tuberculosis of the central nervous system in adults and children. *J. Infect.* **59**:167–187.
22. Thwaites, G. E., et al. 2002. Diagnosis of adult tuberculous meningitis by use of clinical and laboratory features. *Lancet* **360**:1287–1292.