The microbiological diagnosis of tuberculous meningitis: results of Haydarpasa-I study

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Abstract

We aimed to provide data on the diagnosis of tuberculous meningitis (TBM) in this largest case series ever reported. The Haydarpasa-1 study involved patients with microbiologically confirmed TBM in Albania, Croatia, Denmark, Egypt, France, Hungary, Iraq, Italy, Macedonia, Romania, Serbia, Slovenia, Syria and Turkey between 2000 and 2012. A positive culture, PCR or Ehrlich–Ziehl–Neelsen staining (EZNs) from the cerebrospinal fluid (CSF) was mandatory for inclusion of meningitis patients. A total of 506 TBM patients were included. The sensitivities of the tests were as follows: interferon-γ release assay (Quantiferon TB gold in tube) 90.2%, automated culture systems (ACS) 81.8%, Löwenstein Jensen medium (L-J) 72.7%, adenosine deaminase (ADA) 29.9% and EZNs 27.3%. CSF-ACS was superior to CSF L-J culture and CSF-PCR ($p < 0.05$ for both). Accordingly, CSF L-J culture was superior to CSF-PCR ($p < 0.05$). Combination of L-J and ACS was superior to using these tests alone ($p < 0.05$). There were poor and inverse agreements between EZNs and L-J culture ($\kappa = -0.189$); ACS and L-J culture ($\kappa = -0.172$) ($p < 0.05$ for both). Fair and inverse agreement was detected for CSF-ADA and CSF-PCR ($\kappa = -0.299$, $p < 0.05$). Diagnostic accuracy of TBM was increased when both ACS and L-J cultures were used together. Non-culture tests contributed to TBM diagnosis to a degree. However, due to the delays in the diagnosis with any of the cultures, combined use of non-culture tests appears to contribute early diagnosis. Hence, the diagnostic approach to TBM should be individualized according to the technical capacities of medical institutions particularly in those with poor resources.

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Introduction

Tuberculosis remains a major global health problem and is second to human immunodeficiency virus infection as an infectious cause of death [1]. In 2011, the global tuberculosis prevalence was 13 million, and the incidence was 8.7 million while mortality due to tuberculosis was 1.4 million [2]. Tuberculous meningitis (TBM), one of the extrapulmonary tuberculous diseases, occurs in <1% of all cases [3], and it is the most severe form of tuberculosis [4]. TBM is seen in all age groups but recent data from Germany indicated that individuals aged 15 years and over accounted for 88% of all patients [2]. The mortality rate for TBM ranges between 20% and 69% worldwide with up to half of survivors experiencing irreversible sequelae (e.g. paraplegia, blindness, motor, cognitive deficits) [4,5].

Prognosis of the disease is largely inter-related to early diagnosis leading to initiation of proper treatment [2]. Cerebrospinal fluid (CSF) examination is the mainstay in the diagnosis of TBM. Definitive diagnosis depends on detection of tuberculous bacilli in the CSF either by smear examination or by culture [1,3]. However, it comprises many challenges because quick, reliable and affordable diagnostic tests are not always available. The sensitivity of CSF smear microscopy is low (10–60%) and depends on the capacity of laboratories and technicians' experience. Added to that, the sensitivity of CSF culture is as low as 25% and availability of results after 2–6 weeks of incubation causes delays in making the proper diagnosis and initiating treatment [3]. Hence, the diagnostic algorithm of TBM should be re-evaluated with combinations of older and newer diagnostic modalities. For this reason, in this multinational cohort we investigated the laboratory implications of the largest microbiologically confirmed TBM case series ever reported and the main aim for this study is to provide data for the optimization of diagnostic approaches.

Methods

Study design and patient selection

This retrospective, multicentre and multinational cohort Haydarpasa-1 Study involved patients hospitalized for TBM between 2000 and 2012. An MS Windows®-based computer database was designed and data were collected from 43

participating centres in 14 countries (Albania, Croatia, Denmark, Egypt, France, Hungary, Iraq, Italy, Macedonia, Romania, Serbia, Slovenia, Syria and Turkey). The participating centres of the Haydarpasa-1 Study are shown in Fig. 1. The Institutional Review Board of Istanbul Fatih Sultan Mehmet Training and Research Hospital approved the study protocol. Inclusion criteria were age over 14 years and clinical evidence of meningitis (fever, nuchal rigidity and CSF abnormalities) and microbiological confirmation of TBM. At least one of positive CSF culture, PCR analysis and Ehrlich–Ziehl–Neelsen staining (EZNs) was mandatory for the inclusion of the patient into the study [4]. The diagnosis of TBM was made by the clinicians at the participating centres. In addition to CSF and routine laboratory analyses, a neurological scale of Glasgow coma score (GCS), which aims to score the conscious state of the patient, was also recorded by the clinicians for each patient on admittance. A GCS of ≥13 was accepted as mild, 9–12 as moderate, and ≤8 as poor [6]. This paper evaluated only the diagnostic issues related to the TBM. The clinical parameters, therapeutic issues and outcome analysis will be published elsewhere.

Laboratory tests

The CSF samples were centrifuged at 3000 ***g*** for 15 min, and two drops of the deposit were stained by the EZNs method. The remaining CSF samples were cultured on conventional Löwenstein Jensen (L-J) medium and in liquid mycobacterial growth indicator tubes of the automated culture system (ACS) (BACTEC® MGIT® 960, BACTEC®9000 MB, Becton Dickinson Diagnostic Systems, Sparks, MD, USA and BacT/Alert® MB, bioMérieux Diagnostics, Durham, NC, USA) for 6 weeks for the isolation of the *Mycobacterium tuberculosis*. In the molecular diagnosis of TBM conventional PCR, PCR-hybridization (Cobas® Amplicor, Grenzach-Whylen, Roche, Germany) and real-time PCR (ProbeTec®, Becton Dickinson, Oxford, UK; GeneProof®, GeneProof, Brno, Czech Republic;

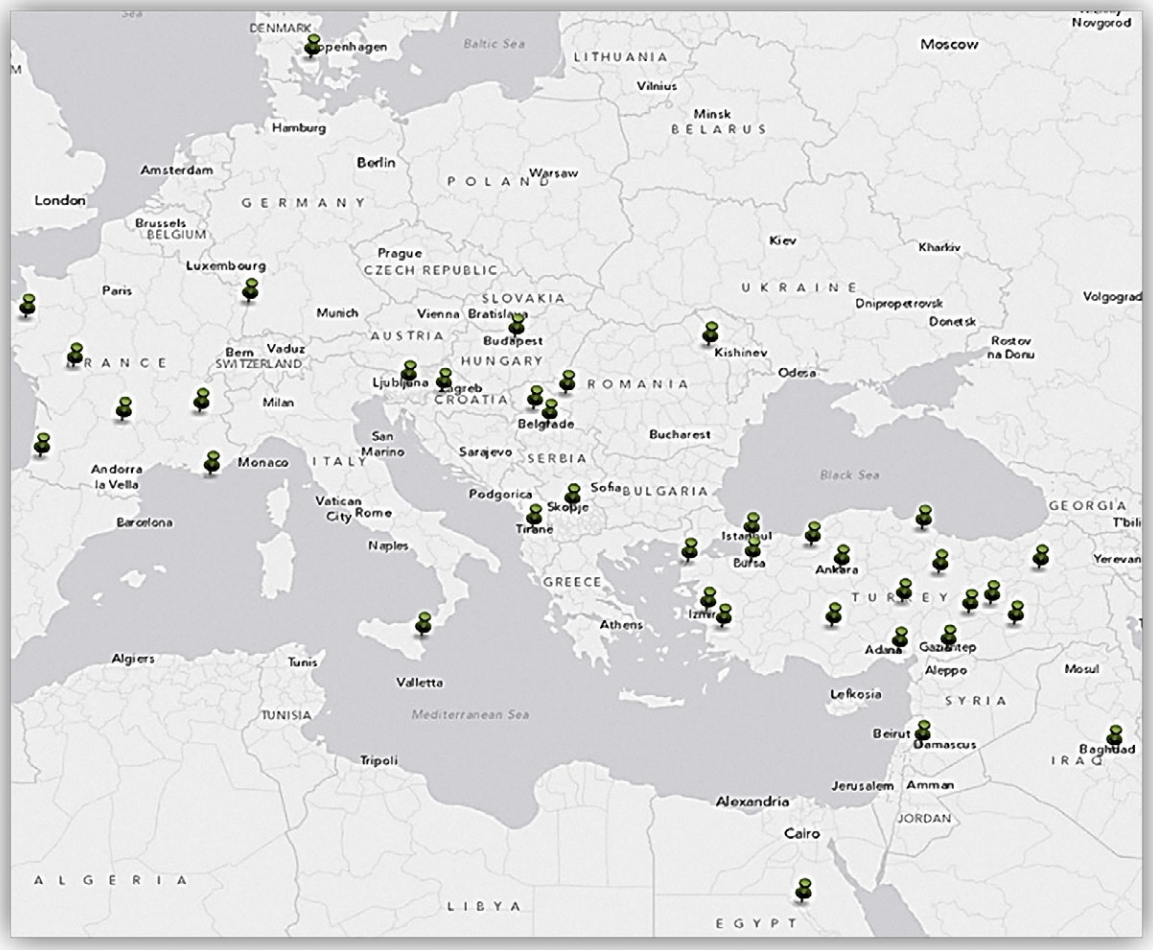

FIG. 1. The cities where participating centres are located.

GenExpert®, Cepheid, Sunnyvale, CA, USA) tests have been used in the participating centres. All of these molecular tests were performed in accordance with the manufacturer's instructions. Different PCR test kits were employed, but their results were analysed as one block in this study.

Although three different types of interferon-γ (IFN-γ) release assay (IGRA) (QuantiFERON®-TB Test, QuantiFERON®-TB Gold Test and QuantiFERON®-TB Gold Test In-Tube, all products marketed by Cellestis Ltd., Carnegie, Vic., Australia) were used, due to consistent specificity of >99% in low-risk individuals and a sensitivity as high as 92% in individuals with active disease, the results of QuantiFERON®-TB Gold In-Tube test were included solely in our study. This *in vitro* diagnostic test used a peptide cocktail simulating esat-6, cfp-10 and tb 7.7(p4) antigens associated with *M. tuberculosis* infection to stimulate cells for IFN-γ in heparinized whole blood drawn directly into specialized blood collection tubes (Quantiferon-TB Gold In-Tube Package Insert, Cellestis, 2006) (http://www.Fda.Gov/downloads/advisorycommittees/committeesmeetingmaterials/medicaldevices/medicaldevicesadvisorycommittee/microbiologydevicespanel/ucm260551.pdf, retrieved October 2013). The results were calculated and interpreted according to the manufacturer's instructions. CSF adenosine deaminase (ADA) activity was evaluated with different marketed test kits by the Giusti method, and kinetic determination and spectrophotometric method in the participating centres [7]. All estimations were performed according to the manufacturer's guidelines and CSF ADA activity was quantified as Unit/liter. Various cut-off values were taken for diagnosing TBM and final results were presented as positive or negative by the participants.

Statistics

The data analysis was performed with SPSS in the Windows® V.16.0 program and with GraphPad Prism® in the Windows® V.5 program. Descriptive statistics were presented as frequencies, percentages for categorical variables and as mean ± SD (range) for continuous variables. In comparing the groups, the Chi-square and Fisher exact tests were used. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and likelihood ratio of the diagnostic tests were calculated by GraphPad Prism®. An inter-rater reliability analysis using the κ statistic was performed to determine consistency between the tests. κ-value 0.00–0.20 was interpreted as poor, 0.21–0.40 as fair, 0.41–0.60 as moderate, 0.61–0.80 as substantial and 0.81–1.00 as perfect agreement [8]. All tests were two-tailed and in comparing the sensitivity of the data, $p < 0.05$ was considered significant.

Results

Main demographics and laboratory characteristics of TBM cases

In this study, 506 TBM cases (240 females, 47.4%) with microbiological confirmation for *M. tuberculosis* were included and patients infected with *Mycobacterium bovis* or non-tuberculous mycobacteria were excluded from the study. The mean age of the patients was 39.69 ± 18.42 (14–89) years. The mean white blood cell count was 9.5 ± 4.3 (1–27.3) × 10^3/mm^3 (n = 470), the mean erythrocyte sedimentation rate was 43.7 ± 26.4 (2–140) mm/h (n = 367). CSF analyses were as follows: mean leucocyte count 320.5 ± 492.1 (0–4450)/mm^3, mean percentage of mononuclear cells 67.7 ± 26.9 (0–100), mean protein level 307.1 ± 425.1 (21–3500) mg/dL, mean CSF/blood glucose ratio 0.28 ± 0.15 (0–92.8), formation of spider web coagulum 24.6% (50/203) and xanthochromia 51.7% (171/331).

CSF culture results

Overall, 412 (81.4%) patients were culture positive. In 319 of 439 (72.6%) patients L-J culture yielded the pathogen and in 157 out of 192 (81.8%) patients ACS yielded the pathogen from the CSF cultures. In 66 (13%) cases, the microorganism was isolated in both L-J and ACS. Neither L-J nor ACS was performed in 29 (5.7%) cases. When automated systems were investigated in detail, the bacterium was isolated with MGIT-960 in 144 patients, BACTEC-9000 MB in five patients and MB/BacT Alert in eight patients. In two cases the microorganism was recovered in both manual MGIT and L-J. In two cases the microorganism was recovered from Middlebrook 7H12 together with MGIT960.

Diagnostic tests for TBM other than CSF cultures

The uses of other diagnostic tests were as follows: CSF-PCR (n = 206), IGRA (n = 41), CSF-EZNs (n = 469), and CSF-ADA (n = 137).

(a) Sensitivities of the diagnostic tests in the diagnosis of TBM: IGRA (QuantiFERON®-TB Gold Test In-Tube) (90.2%) was the most sensitive method followed by CSF-ACS (81.8%) in this study. When L-J was combined with IGRA with or without EZN, the cumulative sensitivity was 100%. In addition, concordant use of IGRA and EZN with or without PCR had 100% sensitivity. The sensitivities of the tests are presented in Table 1.

(b) The contribution of non-culture tests to diagnosis in case of culture negativity: When L-J and ACS were individually combined with other diagnostic tests, the contributions

TABLE 1. The sensitivities of the microbiological diagnostic tests in microbiologically confirmed tuberculosis meningitis (*n* = 506)

	Total	Positive	Sensitivity (%)
Use of diagnostic tests			
IGRA	41	37	90.2
CSF-ACS	192	157	81.8
CSF L-J culture	439	319	72.7
CSF-PCR	206	118	57.3
CSF-ADA	137	41	29.9
CSF EZNs	469	128	27.3
Concordant use of diagnostic tests			
L-J, ACS, IGRA, and EZNs,	2	2	NA
L-J, ACS, and IGRA	2	2	NA
ACS, EZNs, and IGRA	2	2	NA
ACS and IGRA	2	2	NA
EZNs, PCR, and IGRA	34	34	100
ACS, L-J, and EZNs	152	146	96.1
ACS and PCR	88	84	95.5
ACS and L-J	154	146	94.8
ACS, L-J, and PCR	70	66	94.3
L-J, EZNs, and IGRA	34	34	100
ACS and EZNs	188	163	86.7
L-J and PCR	166	138	83.1
L-J and IGRA	41	41	100
EZNs and IGRA	41	41	100
EZNs and PCR	192	119	62

CSF, cerebrospinal fluid; L-J, Löwenstein–Jensen medium; ACS, automated culture system; EZNs, Ehrlich–Ziehl–Neelsen staining; IGRA, interferon-γ release assay; ADA, adenosine deaminase; NA, not applicable.

of other tests to TBM diagnosis in the L-J arm was seemingly high (12.8% for EZN, 24.6% for PCR and 10.9% for ADA). But the combined use of ACS and L-J did not benefit much from the non-culture tests (0.7% for EZN, 2.8% for PCR and 14.2% for ADA). These data are presented in Table 2.

(c) The comparisons of diagnostic test results according to L-J culture results: CSF-EZN tended to be more positive when L-J culture was negative (p <0.0001). We could not establish any other association for other tests, which are presented in Table 3.

(d) The comparisons of diagnostic test results according to ACS culture results: CSF-PCR tended to be negative when ACS culture was negative (p <0.0001). We could not establish any other association for other tests presented in Table 4.

(e) The efficacies of diagnostic tests in predicting culture positivity: The most sensitive test was IGRA indicating culture positivity for L-J and ACS (73% and 82%). However, PCR was the most likely test indicating ACS positivity. The efficacies of non-culture tests in predicting culture positivity are presented in Table 5.

(f) The efficacies of microbiological tests according to GCS: Both EZNs and PCR were significantly more positive when the GCS was >13 (p 0.027 and p 0.006, respectively). The relationships between the severity of disease and the efficacy of microbiological diagnosis are presented in Table 6.

(g) The agreements between the diagnostic tests: There were poor and inverse agreements between EZNs staining and L-J culture (κ = −0.189; p <0.0001); ACS culture and L-J culture (κ = −0.172; p 0.021); and IGRA and L-J culture (κ = −0.112; p 0.05). Accordingly, fair and inverse agreement was detected for CSF-ADA and CSF-PCR (κ = −0.299; p 0.003). The agreements between the diagnostic tests are presented in Table 7.

Comparison of diagnostic tests for TBM

When the diagnostic tests were compared with each other, the results were as follows: CSF-ACS culture (*n* = 157/192, 81.8%) was superior to CSF L-J culture (*n* = 319/439, 72.7%) (χ^2: 5.98, p 0.015, OR (95% CI): 0.59 (0.39–0.90)). Accordingly, CSF-ACS culture (*n* = 157/192, 81.8%) was better than CSF-PCR test (*n* = 118/206, 57.3%) (χ^2: 27.91, p <0.0001, OR (95% CI): 3.35 (2.11–5.29)), and CSF L-J culture (*n* = 319/439, 72.7%) was superior to CSF-PCR test (*n* = 118/206, 57.3%) (χ^2: 15.19, p <0.0001, OR (95% CI): 1.98 (1.40–2.81)). On the other hand, the comparisons of IGRA with microbiological diagnostic tests were as follows: CSF L-J culture (*n* = 319/439, 72.7%) was found to be worse than IGRA test (*n* = 37/41, 90.2%) (p 0.014, OR (95% CI): 3.48 (1.21–9.97)). Similarly, CSF-ACS culture (*n* = 157/192, 81.8%) was inferior to IGRA test (*n* = 37/41, 90.2%) (p 0.007, OR (95% CI): 3.83 (1.31–11.18)). Accordingly, the IGRA test (*n* = 37/41, 90.2%) was significantly more effective than the CSF-PCR test (*n* = 118/206, 57.3%) in indicating TBM (p <0.0001, OR (95% CI): 6.89 (2.37–20.07)).

TABLE 2. The contribution of other tests to tuberculous meningitis diagnosis in case of culture negativity

	L-J, ACS and other test		L-J and other test		ACS and other test	
Other tests	*n*/*N*	%	*n*/*N*	%	*n*/*N*	%
EZNs	1/152	0.7	53/414	12.8	9/188	4.7
PCR	2/70	2.8	41/166	24.6	2/88	2.3
IGRA	ND	ND	3/3	100.0	ND	ND
ADA	2/14	14.2	11/101	10.9	2/38	5.3

n/*N*, number of tests positive/number tested; ND, not determined; L-J, Löwenstein–Jensen medium; ACS, automated culture system; EZNs, Ehrlich–Ziehl–Neelsen staining; IGRA, interferon-γ release assay; ADA, adenosine deaminase.

TABLE 3. Comparison of diagnostic test results according to Löwenstein–Jensen medium culture positivity

Test methods	Results	CSF L-J culture[a] Positive (*n* = 319)	Negative (*n* = 120)	p value	OR (95% CI)
CSF-PCR	Pos	47 (48.5)	41 (59.4)	0.207	0.64 (0.34–1.12)
	Neg	50 (51.5)	28 (40.6)		
IGRA	Pos	27 (87.1)	3 (100)	1.0	0.87 (0.04–20.0)
	Neg	4 (12.9)	0 (0.0)		
CSF-ADA	Pos	12 (18.2)	11 (31.4)	0.143	0.49 (0.19–1.25)
	Neg	54 (81.8)	24 (68.6)		
CSF-EZNs	Pos	53 (17.7)	53 (46.5)	<0.0001	0.25 (0.15–0.4)
	Neg	247 (82.3)	61 (53.5)		

[a]Data expressed as *n* (%); Pos, positive; Neg, negative; OR, Odds ratio; CI, confidence interval.
CSF, cerebrospinal fluid; L-J, Löwenstein–Jensen medium; EZNs, Ehrlich–Ziehl–Neelsen staining; IGRA, interferon-γ release assay; ADA, adenosine deaminase.

TABLE 4. Comparison of diagnostic test results according to automatic automated culture system positivity

Test methods	Results	Automatic automated culture system culture media[a] Positive (*n* = 157)	Negative (*n* = 35)	p value	OR (95% CI)
CSF-PCR	Pos	39 (47.6)	2 (5.7)	<0.0001	14.97 (3.37–66.53)
	Neg	43 (42.4)	33 (94.3)		
IGRA	Pos	2 (100)	0 (0)ND	NA	NA
	Neg	0 (0.0)	0 (0)ND		
CSF-ADA	Pos	18 (50)	2 (100)	0.143	0.49 (0.19–1.25)
	Neg	18 (50)	0 (0)		
CSF-EZNs	Pos	40 (25.9)	9 (26.5)	0.952	0.98 (0.42–2.26)
	Neg	114 (74.1)	25 (73.5)		

[a]Data expressed as *n* (%); OR, Odds ratio; CI, confidence interval; Pos, positive; Neg, negative; NA, not applicable, ND, not determined.
CSF, cerebrospinal fluid; L-J, Löwenstein–Jensen medium; ACS, automated culture system; EZNs, Ehrlich–Ziehl–Neelsen staining; IGRA, interferon- γ release assay; ADA, adenosine deaminase.

TABLE 5. The efficacy of diagnostic test methods in terms of culture positivity

	CSF-PCR (95% CI)	IGRA (95% CI)	CSF-ADA (95% CI)	CSF-EZNs (95% CI)
According to L-J				
Sensitivity	0.48 (0.38–0.59)	0.73 (0.68–0.77)	0.18 (0.1–0.30)	0.17 (0.14–0.22)
Specificity	0.41 (0.29–0.53)	0.10 (0.03–0.23)	0.69 (0.51–0.83)	0.54 (0.44–0.63)
PPV	0.53 (0.42–0.64)	0.90 (0.86–0.93)	0.52 (0.31–0.73)	0.50 (0.40–0.6)
NPV	0.35 (0.25–0.47)	0.03 (0.01–0.08)	0.31 (0.21–0.42)	0.20 (0.16–0.25)
Likelihood ratio	0.81	0.81	0.58	0.38
According to ACS				
Sensitivity	0.48 (0.36–0.59)	0.82 (0.76–0.87)	0.50 (0.33–0.67)	0.26 (0.19–0.34)
Specificity	0.94 (0.81–0.99)	0 (0–0.84)	0 (0–0.84)	0.74 (0.56–0.87)
PPV	0.95 (0.83–0.99)	0.98 (0.96–0.99)	0.90 (0.68–0.99)	0.82 (0.68–0.91)
NPV	0.43 (0.32–0.55)	0 (0–0.10)	0 (0–0.19)	0.18 (0.12–0.25)
Likelihood ratio	8.32	0.82	0.50	0.98

CSF, cerebrospinal fluid; CI, confidence interval; IGRA, interferon-γ release assay; ADA, adenosine deaminase; EZNs, Ehrlich–Ziehl–Neelsen staining; L-J, Löwenstein–Jensen medium; ACS, automated culture system; PPV, positive predictive value; NPV, negative predictive value.

TABLE 6. The efficacy of microbiological diagnostic tests according to Glasgow Coma Score of tuberculous meningitis patients

	Mild GCS (*n* = 188)	Moderate GCS (*n* = 139)	Severe GCS (*n* = 61)	p value
CSF-PCR				
Positive	53 (65.4)	24 (39.3)	9 (45)	0.006
Negative	28 (34.69	37 (60.7)	11 (55)	
CSF-ACS				
Positive	62 (76.5)	39 (78)	25 (86.2)	0.54
Negative	19 (23.5)	11 (22)	4 (13.8)	
CSF L-J culture				
Positive	114 (76.5)	101 (81.5)	39 (76.5)	0.57
Negative	35 (23.5)	23 (18.5)	12 (23.5)	
CSF-EZNs				
Positive	51 (30.4)	24 (17.4)	12 (21.4)	**0.027**
Negative	117 (69.6)	114 (82.6)	44 (78.6)	

Data expressed as *n* (%).
CSF, cerebrospinal fluid; ACS, automated culture system; L-J, Löwenstein–Jensen medium; EZNs, Ehrlich–Ziehl–Neelsen staining.

TABLE 7. The inter-relations between the microbiological diagnostic tests in tuberculosis meningitis patients

	CSF L-J culture	CSF-ACS culture	CSF-PCR	IGRA	CSF-ADA Pos:41; Neg:96
CSF-EZNs Pos:128; Neg:341	κ = −0.189 **p <0.0001** (*n* = 414)	κ = −0.002 p 0.952 (*n* = 188)	κ = 0.10 p 0.056 (*n* = 192)	κ = −0.196 **p 0.031** (*n* = 41)	κ = 0.05 p 0.586 (*n* = 108)
CSF-L-J culture Pos:319; Neg:120		κ = −0.172 **p 0.021** (*n* = 154)	κ = −0.11 p 0.163 (*n* = 166)	κ = −0.112 p 0.05 (*n* = 34)	κ = −0.10 p 0.131 (*n* = 101)
CSF-ACS culture Pos:157; Neg:35			κ = 0.03 p 0.50 (*n* = 88)	κ = NA p NA (*n* = 2)	κ = −0.105 p 0.168 (*n* = 38)
CSF-PCR Pos:118; Neg:88				κ = −0.091 p 0.554 (*n* = 41)	κ = −0.299 **p 0.003** (*n* = 68)
IGRA Pos:37; Neg:4					κ = 0.106 p 0.36 (*n* = 15)

Data expressed as κ and p values. The numbers in parenthesis represent frequency. Pos, positive; Neg, negative, NA, not applicable.
CSF, cerebrospinal fluid; L-J, Löwenstein–Jensen medium; ACS, automated culture system; EZNs, Ehrlich–Ziehl–Neelsen staining; IGRA, interferon-γ release assay; ADA, adenosine deaminase.
Bold indicates statistically significant (p <0.05).

Combination of CSF L-J and ACS cultures (*n* = 146/154, 94.8%) was superior to CSF L-J culture (*n* = 319/439, 72.7%) alone (χ^2: 33.02, p <0.0001, OR (95% CI): 6.87 (3.27–14.42)). Accordingly, concordant use of CSF L-J and ACS cultures (*n* = 146/154, 94.8%) was superior to CSF-ACS culture (*n* = 157/192, 81.8%) alone (χ^2: 13.34, p 0.0003, OR (95% CI): 4.07 (1.83–9.06)).

Discussion

Tuberculous meningitis is associated with high mortality and neurological sequelae if untreated [9]. Early diagnosis is the critical step for the start of treatment of the disease. The diagnosis of CNS tuberculosis is still a complex issue because of the poor sensitivity and frequently delayed results of conventional tests, and lack of standardization or applicability problems of newer techniques. The detection of acid-fast bacilli in the CSF by EZNs or isolation of the microorganism by culture has long been accepted as the gold standard for the diagnosis of TBM [4]. Many authors reported finding acid-fast bacilli in <20% of TBM patients [10] and culture positivity rates were reported to range around 25–75% [4].

The ACS have been shown to be more efficient than L-J culture in the diagnosis of TBM [11]. Our findings confirm that CSF-ACS was superior to CSF L-J culture. Nevertheless, the agreement between these two culture types was poor. Concordant use of L-J and ACS provided significant benefit over using L-J or ACS alone. Hence, the diagnostic approach in CNS tuberculosis should be similar to that of other mycobacterial infections in which the use of solid medium along with a liquid medium is believed to maximize recovery [12]. Unfortunately, only 13% of the treating clinicians in this study preferred this approach while in 5.7% of the cases no culture of any kind was performed. In addition, CSF-ACS and L-J cultures were found to be significantly better than molecular methods (81.8%, 72.7% and 57.3%, respectively). The efficacy of molecular methods was previously reported to fall behind culture methods [13–16]. This seemed to be the case in TBM, too. Hence, we consider ACS as the gold standard in the confirmation of diagnosis of TBM and if only one type of culture is possible in the hospital, ACS should be preferred.

Amplification of mycobacterial DNA by the use of molecular assays, such as nucleic acid amplification techniques, PCR (including real-time and nested PCR) in particular, potentially allows rapid diagnosis of TBM. Molecular tests can also pick up dead bacteria and may further contribute to diagnosis. Hence, this feature may warrant molecular tests to be used in combination with other diagnostic tests. However, the sensitivity of PCR lies between EZNs and culture according to our data. In a meta-analysis on the role of nucleic acid amplification techniques in TBM, the pooled specificity was 98% but the sensitivity was 56% [17]. In our study, molecular tests detected 57.3% of the patients. Besides, a positive PCR result was not likely to indicate L-J culture positivity and no agreement was detected between molecular test methods and L-J or ACS cultures. Consequently, molecular methods cannot replace culture methods as the gold standard of diagnosis and should be used in combination with other diagnostic tools to diagnose TBM. On the other hand, since they are less affected by the use of antibiotics, in comparison to cultures and EZNs, molecular methods may provide additional advantages in identifying the disease [18,19].

The foremost diagnostic modality in many countries of high tuberculosis endemicity with poor resources is EZNs because of its low cost [20]. According to our data, EZNs provided positive results in one-quarter of the patients and it had the lowest sensitivity in this study. In addition, EZNs was not likely to suggest L-J or ACS culture positivity, and the agreement between L-J culture and EZNs was poor and inverse. Hence, a

surprising number of specimens were EZNs positive and culture negative, a finding that is in contrast to those known for the pulmonary form of the disease [1]. The probable reason for this situation may be the low bacterial count in CSF samples compared with sputum. The low bacterial count may have occurred by the immune reaction of host or by use of various drugs. However, there was not an inverse agreement between EZNs and ACS as in L-J. This is probably because of the high sensitivity of the ACS compared with L-J medium. Interestingly, EZNs or molecular testing was significantly more positive when GCS was mild. This is probably due to mild inflammation in less severe forms of TBM, which may contribute to higher yields for EZNs and PCR.

Assays based on the detection of IFN-γ from lymphocytes after the administration of *M. tuberculosis* antigens have been introduced into clinical practice in recent years [21]. In some case series IFN-γ assays were found be useful in the diagnosis of TBM [22,23]. On the other hand, there are reports indicating the failure of these assays in the diagnosis of TBM patients [24]. In a study from Japan, 50% of patients with culture-positive TBM yielded negative IGRA results [25]. In our study, IGRA (QuantiFERON®-TB Gold Test In-Tube) was positive in 73% of L-J and 82% of ACS culture-positive patients and in 92% of the microbiologically confirmed cases. Moreover, we found that although the IGRA test was superior to CSF L-J and ACS cultures, it was not likely to predict culture positivity as in molecular tests, ADA and EZNs. On the other hand, this situation may provide an advantage particularly in favour of IGRA because this most sensitive test tended to be positive in culture-negative patients. Accordingly, the agreement between IGRA and L-J culture positivity was poor and inverse. The probable reason for this situation may be that the strong immune reaction, in which IFN-γ is also a part, may contribute to eradication of the bacterium from CSF leading to culture negativity. Molecular tests provided positive results in slightly more than half of the patients, although they were inferior to IGRA. But we could not disclose agreement between IGRA and CSF-PCR in this study. Hence, these non-culture tests appear not to be confirmatory tests, but rather supplementary tests. Hence, these two tests have different kinetics in diagnosis and they should be used separately.

Adenosine deaminase is most commonly present in human lymphoid tissue and in active T lymphocytes. Hence, it increases largely by the induction of T-cell-mediated immune responses. A meta-analysis reported that the sensitivity and specificity of ADA in TBM were 79% and 91%, respectively [7]. But, publication bias was questioned by a recent report to result in the overestimation of diagnostic accuracy in that meta-analysis [4]. According to our data, although its specificity and sensitivity for L-J culture positivity were 69% and as low as 18%, respectively, ADA was not very likely to indicate the isolation of the pathogen both in L-J culture and by ACS. In addition, other CNS disorders may produce positive ADA results [26,27] and this may limit its use. However, we detected a poor and inverse agreement between ADA and PCR. The probable reason may be that the activated T lymphocytes may contribute to lower efficacy of PCR in ADA-positive samples.

The strength of this study is that it is by far the largest microbiologically confirmed TBM case series. Although its retrospective design is a limitation, it is nearly impossible to provide such a large prospective cohort sample. Another limitation was that there were numerous molecular tests used in the participating centres. We combined all of them as one block for statistical comparisons and were supposed to neglect differences between their sensitivities. In conclusion, in the diagnosis of TBM, ACS has the highest sensitivity and should be the reference standard followed by L-J culture. Diagnostic accuracy is increased when both tests are used together. In addition, tests like EZNs, IGRA, ADA and PCR only contributed slightly to the TBM diagnosis. However, as the major problem in diagnosis of TBM appears to be the long time period for the recovery of the microbe, up to 2–6 weeks [10], combined use of EZNs, PCR, ADA and IGRA may circumvent the delays and appear to help early diagnosis. Hence, our data indicate that diagnosis of TBM should be made by combination of diagnostic tests in two steps. In the first step, non-culture tests like EZNs, PCR, ADA and IGRA should be performed in combination according to their availability in the institution for the rapid diagnostic clues. In the second coexistent step, both ACS and L-J cultures should be performed together for the confirmation of TBM. If only one type of culture is feasible in the hospital, that should be ACS in the diagnosis of TBM.

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Transparency Declaration

We have no competing interests to declare. The study has not been presented in any meeting previously.

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