TABLE I
Characteristics of the studied groups

	TBM n (%)	OIM n (%)	NIND n (%)
Cerebrospinal fluid	19	64	73
Age (mean ± standard deviation)	47.9 ± 17.6	49.52 ± 20.26	48.9 ± 20.01
Gender			
Female	7 (37)	27 (40)	50 (69)
Male	12 (63)	37 (60)	23 (31)
Bacteriologic diagnosis			
TB smear negative/culture positive	18 (95)	-	-
TB smear negative/culture negative	1 (5)	-	-
Non TB etiological agent found			
Cryptococcus sp.	-	21 (32.8)	-
Streptococcus pneumoniae	-	16 (25)	-
Neisseria meningitidis	-	6 (9.3)	-
Streptococcus sp.	-	4 (6)	-
Staphylococcus	-	3 (4)	-
Serratia sp.	-	2 (3)	-
Klebsiela sp.	-	2 (3)	-
Escherichia coli	-	1 (1.5)	-
Morganella spp	-	1 (1.5)	-
Corynebacterium spp	-	1 (1.5)	-
Other (viral/not identified)	-	6 (9)/1 (1.5)	-
Biochemical findings in CSF			
Glucose (≥ 40 mg/dL)	7 (36.8)	28 (41.8)	72 (98.6)
Leukocytes (≥ 5/mL)	19 (100)	54 (84.4)	10 (13.7)
Proteins			
PTR_1 (≤ 30 mg/dL)	2 (10.5)	13 (19.4)	68 (93.1)
PTR_2 (30 < PTR > 300 mg/dL)	12 (63.1)	26 (38.8)	5 (6.8)
PTR_3 (≥ 300 mg/dL)	5 (26.3)	25 (37.3)	0
HIV sorology			
Positive	8 (42)	16 (25)	0
Negative	11 (58)	48 (75)	73 (100)

CSF: cerebrospinal fluid; HIV: human immunodeficiency virus; NIND: non-infectious neurological disorders (multiple sclerosis and headache); OIM: other infectious meningoencephalitis; PTR: CSF protein concentration; PTR_1: normal protein concentration; $PTR_{2, 3}$: abnormal protein concentration; TB: tuberculosis; TBM: tuberculosis meningitis. Other abnormal results: glucose ≥ 40 mg/dL and leukocytes ≥ 5/mL.

TABLE II

Enzyme linked immunosorbent assay-IgG and IgA positive frequency in cerebrospinal fluid from tuberculosis meningitis (TBM) and other infectious meningoencephalitis (OIM) according with human immunodeficiency virus (HIV) serology with four antigens and commercial Lionex kit

	Positive/total n (%)			
	TBM		OIM	
Antigens	HIV$^+$	HIV$^-$	HIV$^+$	HIV$^-$
IgG				
MPT-64	2/8 (25)	2/11 (18.2)	0/16 (0)	2/48 (4.1)
MT-10.3	1/8 (12.5)	1/11 (9.1)	0/15 (0)	2/47 (4.2)
16 kDa	4/8 (50)	2/11 (18.2)	1/16 (6.2)	3/45 (6.6)
38 kDa	4/8 (50)	1/11 (9.1)	1/16 (6.2)	1/45 (2.2)
MPT-64/MT-10.3	2/8 (25)	3/11 (27.3)	0/16 (0)	2/48 (4.1)
MPT-64/38 kDa	4/8 (50)	2/11 (18.2)	1/16 (6.2)	3/48 (6.2)
MPT-64/16 kDa	4/8 (50)	2/11 (18.2)	1/16 (6.2)	5/48 (10.4)
All	4/8 (50)	3/11 (27.3)	1/16 (6.2)	7/48 (14.6)
Lionex kit	5/8 (62.5)	4/11 (36.3)	0/16 (0)	3/46 (6.5)
IgA				
MPT-64	6/8 (75)	6/11 (54.5)	0/16 (0)	3/43 (6.9)
MT-10.3	3/8 (37.5)	1/11 (9.1)	1/16 (6.2)	3/42 (7.1)
16 kDa	4/6 (66.6)	0/10 (0)	1/15 (6.6)	5/48 (10.4)
38 kDa	2/7 (28.6)	0/10 (0)	0/15 (0)	1/48 (2.1)
MPT-64/MT-10.3	6/8 (75)	6/11 (54.5)	1/16 (6.2)	5/44 (11.3)
MPT-64/38 kDa	6/8 (75)	6/11 (54.5)	0/16 (0)	4/43 (9.3)
MPT-64/16 kDa	6/8 (75)	6/11 (54.5)	1/16 (6.2)	8/45 (17.8)
All	6/8 (75)	6/11 (54.5)	2/16 (12.5)	9/45 (20)
Lionex kit	3/8 (37.5)	0/11 (0)	0/16 (0)	5/46 (10.8)

results obtained using cut-off based in receiver operator characteristic curves.

APPENDIXES

ELISA (in-house assay) - The assay was performed in polystyrene ELISA plates (Nunc Maxisorp certified, flat bottom, Denmark) separately coated with 50 µL of each antigen in 15 mM carbonate-bicarbonate buffer (pH 9.6) at a predetermined concentration of 1 µg/mL for all antigens and blocked with phosphate buffered saline with 0.01% Tween-20 (PBS-T) containing 5% bovine serum albumin (BSA). The method, design and analysis have been described previously by Kaisermann et al. (2005), with minor modifications. The plates were incubated with a 1:40 dilution of CSF as determined from previous standardised experiments. Horseradish peroxidise-linked goat anti-human immunoglobulin G (IgG) and immunoglobulin (IgA) conjugates (Pierce, Germany) were used at 1:40000 for 16 kDa and 38 kDa and 1:20000 for MPT-64 and MT10.3 antigens in PBS-T containing 1% BSA. The colour was developed by addition of 50 µL of 3,3`,5,5'-tetramethylbenzidine (TMB) solution (Zymed, California, USA) and stopped with 2.5N sulphuric acid solution. In each set of experiments, positive (pooled TB) and negative (pooled control) CSF specimens were used in four serial dilutions as references. The positive and negative pools consisted of a mixture of 50 µL of 10 samples of TBM-CSF and 30 µL of 15 samples of NIND-CSF, respectively. The prepared pools were distributed in small tubes in aliquots sufficient for two sets of experiments. This was done to avoid degradation of the proteins due to successive thawing.

Lionex TB ELISA assay - Kits were kindly provided by Lionex Diagnostics & Therapeutics GmbH, Braunschweig, Germany (www.lionex.de). The kits were used according to the manufacturer's instructions. Briefly, the test samples and controls (standards ready for use provided by the manufacturer) were diluted to 1:200 in the dilution solution. Then, 100 mL of the clinical specimens were added in respective wells of the microplates for detection of IgG, IgM and IgA. After incubation for 45 min at 37°C, the plates were washed three times with 300 µL of washing solution and then 100 µL of conjugate solution was added, except in wells corresponding to the blank. After incubation at 37°C for 30 min and washing as previously described, 100 µL of TMB substrate solution was added into all wells, followed by incubation at 37°C for 10 min in the dark. The reaction was stopped with 100 µL of stop solution. The reading was performed as described by Kaisermann et al. (2005). Both tests were performed without operator knowledge of the group classification of each CSF sample.

Data analysis - All specimens were tested in duplicate. The analysis of the variation coefficient between the duplicates was < 15% on different plates and days. All CSF samples included in the NIND group may be considered as latently infected. ROC curves were constructed with the TBM and OIM groups for each of the antigens. A cut-off point was chosen to optimize specificity value; results were expressed as optical density (OD) x 1000. OD in the range of 10% above to 10% below the cut-off value was taken to identify the grey zone. For the OD values of abnormal distribution, a non-parametric test with Mann-Whitney (independent samples) and Kruskal-Wallis was applied to compare group averages. Bivariate odds ratios and 95% confidence intervals were calculated for demographic and clinical characteristics associated with a positive humoral response in TBM. For most statistical analyses, SPSS for Windows, v.13.0 (SPSS Inc, Chicago, IL, USA) was adopted and MedCalc v. 11.2 (Mariakerke, Belgium) was used to calculate PPV and NPV values.