Is it possible to differentiate tuberculous and cryptococcal meningitis in HIV-infected patients using only clinical and basic cerebrospinal fluid characteristics?

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Background. Tuberculous and cryptococcal meningitis (TBM and CM) are the most common causes of opportunistic meningitis in HIV-infected patients from resource-limited settings, and the differential diagnosis is challenging.
Objective. To compare clinical and basic cerebrospinal fluid (CSF) characteristics between TBM and CM in HIV-infected patients.
Methods. A retrospective analysis was conducted of clinical, radiological and laboratory records of 108 and 98 HIV-infected patients with culture-proven diagnosis of TBM and CM, respectively. The patients were admitted at a tertiary centre in São Paulo, Brazil. A logistic regression model was used to distinguish TBM from CM and derive a diagnostic index based on the adjusted odds ratio (OR) to differentiate these two diseases.
Results. In multivariate analysis, TBM was independently associated with: CSF with neutrophil predominance (odds ratio (OR) 35.81, 95% confidence interval (CI) 3.80 - 341.30, p=0.002), CSF pleocytosis (OR 9.43, 95% CI 1.30 - 68.70, p=0.027), CSF protein >1.0 g/L (OR 5.13, 95% CI 1.38 - 19.04, p=0.032) and Glasgow Coma Scale <15 (OR 3.10, 95% CI 1.03 - 9.34, p=0.044). Nausea and vomiting (OR 0.27, 95% CI 0.08 - 0.90, p=0.033) were associated with CM. Algorithm-related area under the receiver operating characteristics curve was 0.815 (95% CI 0.758 - 0.873, p<0.0001), but an accurate cut-off was not derived.
Conclusion. Although some clinical and basic CSF characteristics appear useful in the differential diagnosis of TBM and CM in HIV-infected patients, an accurate algorithm was not identified. Optimised access to rapid, sensitive and specific laboratory tests is essential.

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Tuberculous and cryptococcal meningitis (TBM and CM) are the most common causes of opportunistic meningitis in HIV-infected patients.[1-5] TBM and CM share similar clinical and laboratory features, resulting in delays to diagnosis and poorer outcomes, particularly in settings where confirmatory diagnosis is not possible.[6] Clinical algorithms have been proposed to simplify the diagnosis and treatment of these neurological infections,[6-8] but a lack of sensitivity and specificity precludes their implementation in clinical practice.

The objective of this study was to compare clinical and laboratory findings of TBM and CM in HIV-infected patients to propose an algorithm to differentiate these diseases.

Methods

We retrospectively analysed data from the clinical records of 108 and 98 HIV-infected patients with a culture-proven diagnosis of TBM or CM, respectively. The patients were admitted between March 1999 and June 2008 at the Emílio Ribas Institute of Infectious Diseases, São Paulo, Brazil. This hospital is a 250-bed tertiary teaching centre and the main referral institution for HIV-infected patients in São Paulo State. HIV infection was diagnosed by ELISA and confirmed by Western blot. We evaluated demographic, clinical, radiological and laboratory information. The study was approved by the ethical and scientific boards of the institution.

Cerebrospinal fluid (CSF) was obtained by lumbar puncture at hospital admission. Pleocytosis was defined as a leukocyte count >5 cells/μL. The normal range of lumbar CSF glucose is >2.22 mmol/L (40 mg/dL) and CSF protein <0.45 g/L (45 mg/dL). Neutrophil pleocytosis was defined as >50% CSF leukocytes.

The comparison between groups (TBM v. CM) was performed using the Mann-Whitney *U*-test for numeric variables and Yates corrected χ^2 or Fisher's exact test as appropriate for categorical variables. Odds ratio (OR) and 95% confidence interval (CI) were adjusted using a binary logistic regression model (Wald test) and included diagnosis of TBM v. CM as the dependent variable. Covariates and factors associated with TBM (v. CM) in univariate analysis were included to derive a diagnostic index based on clinical, laboratory and radiological findings. A receiver operating characteristics (ROC) curve was derived to calculate the accuracy of the logistic model. An estimation of cut-off for the diagnosis index was made to distinguish between TBM and CM. Statistical significance was defined as p<0.05, and SPSS 20.0 (IBM Corp., USA) software was used for statistical analysis.

Results

Table 1 shows a comparison of the clinical, radiological and laboratory characteristics of HIV-infected patients with TBM and CM. CSF

with three abnormal parameters (pleocytosis, protein elevation and depressed glucose) was observed in 55.6% and 36.1% of patients with TBM and CM, respectively (p=0.002). The in-hospital case-fatality rate was similar between the groups (TBM 29% v. CM 29%, p=0.578).

Microscopy was of limited value. Among TBM patients, Ziehl-Neelsen staining of CSF showed acid-fast bacilli (AFB) in only 5.5% (6/108) of cases. Among cryptococcal patients, India ink staining of CSF showed yeast compatible with *Cryptococcus* spp. in 84% (82/98) of cases. A cryptococcal antigen latex agglutination test of CSF was positive in only 82% (80/98) of cases.

Table 1. Comparison of clinical and laboratory characteristics among 206 HIV-infected patients with tuberculous or cryptococcal meningitis

Characteristics	Tuberculous meningitis, *n* (%) (*n*=108)	Cryptococcal meningitis, *n* (%) (*n*=98)	*p*-value
Demographic data			
Age (years), median (IQR)	36 (30 - 42)	39 (34 - 43)	0.008*
Male sex	78 (72.2)	76 (77.6)	0.520
CD4 (T-cells/mL), median (IQR) (*n*=183)	65 (30 - 122)	36 (17 - 87)	0.003*
CD4 ≥50 cells/mL	60 (61.2)	30 (35.3)	0.001
Prior HAART use	36 (51.0)	46 (46.9)	0.643
Prior HIV diagnosis	63 (61.2)	95 (96.9)	<0.0001*
Clinical data			
Fever	84 (77.8)	53 (54.1)	0.001*
Headache	82 (75.9)	87 (88.8)	0.006*
Meningeal signs	20 (19)	11 (11.8)	0.241
Seizures	12 (11.1)	16 (16.3)	0.309
Nausea and vomiting	41 (38.0)	54 (55.1)	0.011*
GCS <15	68 (63.0)	26 (26.5)	<0.0001*
Extra-CNS disease	52 (48.2)	50 (51.0)	0.572
Images and laboratory data			
Ventricular dilatation on cranial CT	25 (24.8)	9 (10.8)	0.021
Haemoglobin (g/dL), median (IQR)	10.7 (9.4 - 12.0)	11.9 (11.0 - 13.3)	0.004*
CSF characteristics			
Leukocytes (cells/mL), median (IQR)	160 (16 - 333)	6 (2 - 64)	<0.0001*
Lymphocyte predominance	50/89 (56.2)	48/49 (98.2)	<0.0001*
Neutrophil predominance	39/89 (43.8)	1/49 (1.8)	<0.0001*
CSF glucose (mmol/L), median (IQR), *n*=205	1.6 (1.1 - 2.7)	2.1 (1.3 - 2.7)	0.142
CSF blood glucose ratio, median (IQR), *n*=183	0.3 (0.2 - 0.5)	0.3 (0.2 - 0.5)	0.601
CSF protein, median (IQR), *n*=205	0.2 (0.1 - 2.9)	0.1 (0.1 - 1.4)	<0.0001*
CSF pleocytosis†	89 (82.4)	49 (50.0)	<0.0001*
CSF glucose <2.22 mmol/L	68 (63.0)	51 (52.0)	0.157
CSF protein ≥0.45 g/L	97 (90.0)	80 (81.6)	0.185
CSF protein ≥1.0 g/L	77 (71.3)	34 (35.1)	<0.0001*
Normal CSF‡	5 (4.6)	13 (13.4)	0.049*
One CSF abnormal parameter	12 (11.1)	23 (23.7)	0.027*
Two CSF abnormal parameters	31 (28.7)	26 (26.8)	0.641
Three CSF abnormal parameters	60 (55.6)	35 (36.1)	0.002*
Prognosis			
In-hospital case fatality rate	31 (28.7)	28 (28.6)	0.578

CNS = central nervous system; CT = computed tomography; IQR = interquartile range; HAART = highly active antiretroviral therapy.
*Statistically significant.
†Pleocytosis as ≥5 leukocytes/μL.
‡CSF parameters: ≥5 leukocytes/μL, glucose <2.22 mmol/L and protein ≥0.45 g/L.

Tables 2 and 3 show the results of univariate and multivariate modelling to identify variables associated with TBM.

A diagnostic index was derived using a multivariate model as follows: 1.635 × (protein >1.0 g/L) + 1.132 × (Glasgow Coma Scale (GCS) <15) - 1.296 × (nausea and vomiting) + 3.578 × (CSF with polymorphs predominance) + 2.244 × (pleocytosis).

The parameters in parenthesis were coded 1 if present or 0 if absent. We excluded the value of the constant in the calculation of the index. The diagnostic index was a number that varied from -1.296 to 8.589, and negative values favoured a diagnosis of CM, while positive values favoured a diagnosis of TBM. The area under the ROC curve was 0.815 (95% CI 0.758 - 0.873; p<0.0001). At a cut-off of 1.04, sensitivity was 96.0% and specificity 73.1%. Values >1.04 presented a remarkable fall in the level of sensitivity – a cut-off of 5.33 presented a specificity of 100% but a sensitivity of 34.7% – while values <1.04, such as -0.73, presented higher sensitivity (100%) but lower specificity (3.2%). Fig. 1 shows the ROC curve of the logistic model.

Discussion

To the best of our knowledge this is the largest study comparing culture-proven CM and TBM in HIV-infected patients. Although some clinical and CSF characteristics appear useful to the discrimination of these two diseases, a diagnostic index could not be derived because of a lack of sensitivity and specificity, similar to that reported in a previous study,[6] which derived an area under the ROC curve very close to ours in spite of the smaller number of patients in the groups. A highly accurate logistic model did not result in an index with a cut-off sufficiently sensitive and specific to distinguish TBM and CM using clinical and laboratory features.

In the current study, CSF protein >1.0 g/L, GCS <15, absence of nausea and vomiting, neutrophil pleocytosis and CSF pleocytosis were independently associated with TBM diagnosis. There are few studies with a heterogeneous design comparing TBM and CM. Similar to our findings, GCS <15,[6] pleocytosis,[6] and neutrophil pleocytosis[9] have been found related to TBM. Fever,[6,10] neck stiffness,[6] brain CT abnormalities,[10,11]

Table 2. Univariate analysis of associated variables with tuberculous meningitis among 206 HIV-infected patients with tuberculous or cryptococcal meningitis

	Unadjusted		Adjusted*	
Variable	**OR (95% CI)**	***p*-value**	**OR (95% CI)**	***p*-value**
Male gender	0.81 (0.43 - 1.52)	0.520		
Age <40 years	2.22 (1.24 - 2.96)	0.009		
CD4 T-cell count ≥50 cells/μL	2.90 (1.59 - 5.28)	0.001		
Previous diagnosis of HIV	0.05 (0.02 - 0.16)	<0.0001		
GCS <15†	4.50 (2.48 - 8.19)	<0.0001	3.10 (1.03 - 9.34)	0.044
Fever	2.84 (1.55 - 5.19)	0.001		
Headache	0.33 (0.15 - 0.73)	0.006		
Triad of meningism, fever and headache	2.49 (0.96 - 6.46)	0.072		
Nausea and vomiting	0.48 (0.27 - 0.83)	0.011	0.27 (0.08 - 0.90)	0.033
Haemoglobin <10 g/dL	2.92 (1.53 - 5.57)	0.001		
CSF pleocytosis‡	4.68 (2.49 - 8.79)	<0.0001	9.43 (1.30 - 68.70)	0.027
CSF neutrophil predominance	32.9 (5.6 - 195.4)	<0.0001	35.81(3.80 - 341.30)	0.002
CSF protein ≥1.0 g/L	4.60 (2.56 - 8.28)	<0.0001	5.13 (1.38 - 9.04)	0.032
Normal CSF protein, glucose and leukocytes	0.31 (0.11 - 0.88)	0.049		
Abnormal CSF protein, glucose and leukocytes	2.21 (1.27 - 3.88)	0.008		
Ventricular dilatation on cranial CT	2.71 (1.20 - 6.08)	0.021		
Brain atrophy on cranial CT	0.35 (0.15 - 0.78)	0.015		

*Adjusted OR was measured by binary logistic regression model (Wald test) and included baseline fever, headache, T-cell CD4 count, CSF protein, GCS at admission, nausea and vomiting, pleocytosis and CSF neutrophil pleocytosis.
†At admission.
‡Pleocytosis defined as ≥5 leukocytes/μL.

Table 3. Multivariate logistic regression model to identify associated variables with tuberculous meningitis among 206 HIV-infected patients with tuberculous or cryptococcal meningitis

Variable	**β**	**OR (95% CI)**	***p*-value**
CSF protein ≥1.0 g/L	1.635	5.13 (1.38 - 19.04)	0.014
GCS <15*	1.132	3.10 (1.03 - 9.34)	0.044
Nausea and vomiting	–1.296	0.27 (0.08 - 0.90)	0.033
Neutrophil pleocytosis†	3.578	35.81 (3.80 - 341.30)	0.002
CSF pleocytosis‡	2.244	9.43 (1.30 - 68.70)	0.027
Constant	–4.252		

*At admission.
†Neutrophil pleocytosis defined as >50% CSF leukocytes.
‡Pleocytosis defined as leukocytes >5 cells/μL.

and extrameningeal disease[10] have been reported more frequently in TBM in other studies than in our study. In these studies, the variables of headache,[11] vomiting,[11] altered sensorium,[11] high opening pressure,[6] low CSF white blood cell count[6] and advanced immunosupression[9,11] were more frequent in patients with CM. These results indicate that some clinical and laboratory characteristics seem useful in the differential diagnosis of TBM and CM; however, in clinical practice, they do not usually allow for sufficient discrimination between these two diseases.

Culture is the mainstay for diagnosis of TBM and CM, but alternative tests are evolving to provide more rapid and reliable diagnosis. In clinical practice, the India ink microscopy stain is usually available for cryptococcal diagnosis in resource-limited settings. Yet, in referral centres in Africa, India ink has only ~85% sensitivity,[12] similar to the sensitivity in our study. The use of India ink can be particularly problematic for early and/or low-burden infections, with sensitivity only 40% for quantitative cultures <1 000 colony-forming U/mL of CSF.[12] Thus, cryptococcal antigen (CrAg) tests could be necessary in at least 15% of patients with CM and negative India ink microscopy. In people with AIDS who have a negative India ink microscopy, the most common cause of meningitis is *Cryptococcus* in high-burden regions.[12] Recently, the World Health Organization included the CSF latex CrAg agglutination or lateral flow immunochromatographic assay (LFA) as the preferred diagnostic approach for CM.

In our study, the sensitivity of CSF CrAg latex agglutination was only 82%, lower than usually reported for this test (93 – 100%).[13] CSF CrAg LFA (Immuno-Mycologics, USA) has a reported sensitivity of 99.3%, specificity of 99.1%, positive predictive value of 99.5% and negative predictive value of 98.7%.[12] CRAg LFA is a 'dipstick' test that requires only a drop of CSF and is relatively inexpensive, quick and easy to interpret. Unlike traditional latex agglutination, CrAg LFA does not require laboratory infrastructure or cold-chain transport.[13]

Unfortunately, rapid diagnosis of TBM is more complicated. Detection of AFB in patient samples using Ziehl-Neelsen staining is widely employed for diagnosis of TBM. AFB microscopy is, however, insensitive in TBM, with sensitivity rates of <10 - 20%.[14] The sensitivity of AFB microscopy in our study was only 5%. The sensitivity of smear microscopy in TBM can be maximised by examination of large-volume CSF samples (>6 mL) using several CSF specimens collected over a few days and prolonged slide examination (≥30 min).[15] However, these criteria are rarely achieved in practice. Recent modifications to the Ziehl-Neelsen stain have shown optimistic results,[16] but replication in other sites is required. Over several decades, nucleic acid amplification tests (NAATs) of *Mycobacterium tuberculosis* have been evaluated for the diagnosis of TBM. A recent systematic review and meta-analysis of commercial NAATs with a CSF of *M. tuberculosis* culture-positive gold standard found a sensitivity of 64% and a specificity of 98%.[17] Despite suboptimal sensitivity, the rapid turnaround time of NAATs compared with culture enhances their role in the early accurate diagnosis of TBM. However, NAATs

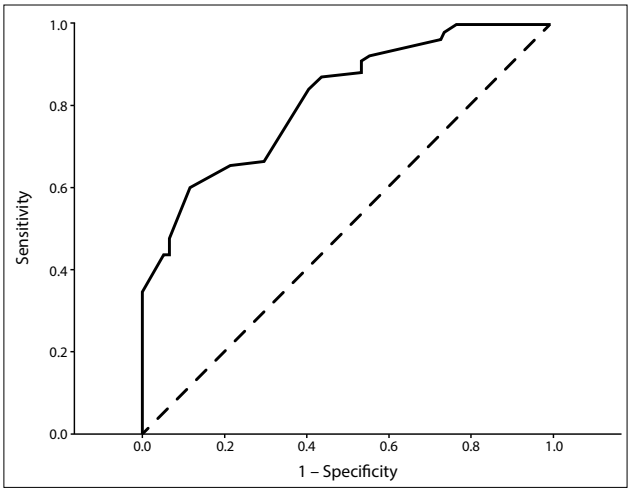

Fig. 1. Logistic regression model ROC curve for HIV-infected patients with TBM or CM. Area under the curve was 0.815 (95% CI 0.758 - 0.873), p<0.0001. At a cut-off of 1.04, sensitivity was 96.0% and specificity 73.1%.

are unavailable for most resource-limited laboratories. Yet, the availability of automated NAATs via the GeneXpert system (Cepheid, USA) is increasing, but testing a large volume of centrifuged CSF remains essential.

In terms of the current status of meningitis diagnosis in HIV-infected patients, we would recommend a cryptococcal antigen assay (ideally LFA) as an initial approach.[18] Following a negative test in an adequate clinical and CSF context, empiric treatment for TBM should strongly be considered. However, it is important to consider bacteria (e.g. *Streptococcus pneumoniae, Neisseria meningitidis, Listeria monocytogenes*) and syphilitic meningitis in the differential diagnosis of meningitis in HIV-infected patients. More-rare aetiologies include other fungal pathogens (i.e. *Histoplasma capsulatum*, *Candida, Coccidioides*) and viruses (i.e. herpes virus, enterovirus).[19,20]

This study has limitations. We did not have CSF opening pressure available for the whole population. We included only culture-proven cases and not culture-negative cases, tested with techniques such as NAAT. Therefore, we did not compare clinical and laboratory differences between culture-positive and culture-negative cases. However, we did include a reasonable number of cases, all of which were culture proven, allowing rigorous case definition.

In conclusion, although some clinical and basic CSF characteristics appear useful in the differential diagnosis of TBM and CM in HIV-infected patients, an accurate algorithm was not identified. In this scenario, optimised access to more rapid, sensitive and specific laboratory tests is essential.

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