Comparison of a Clinical Prediction Rule and a LAM Antigen-Detection Assay for the Rapid Diagnosis of TBM in a High HIV Prevalence Setting

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Abstract

Background/Objective: The diagnosis of tuberculous meningitis (TBM) in resource poor TB endemic environments is challenging. The accuracy of current tools for the rapid diagnosis of TBM is suboptimal. We sought to develop a clinical-prediction rule for the diagnosis of TBM in a high HIV prevalence setting, and to compare performance outcomes to conventional diagnostic modalities and a novel lipoarabinomannan (LAM) antigen detection test (Clearview-TB®) using cerebrospinal fluid (CSF).

Methods: Patients with suspected TBM were classified as definite-TBM (CSF culture or PCR positive), probable-TBM and non-TBM.

Results: Of the 150 patients, 84% were HIV-infected (median [IQR] CD4 count = 132 [54; 241] cells/µl). There were 39, 55 and 54 patients in the definite, probable and non-TBM groups, respectively. The LAM sensitivity and specificity (95%CI) was 31% (17;48) and 94% (85;99), respectively (cut-point $ \geq 0.18$). By contrast, smear-microscopy was 100% specific but detected none of the definite-TBM cases. LAM positivity was associated with HIV co-infection and low CD4 T cell count (CD4<200 vs. >200 cells/µl; $ p = 0.03$). The sensitivity and specificity in those with a CD4<100 cells/µl was 50% (27;73) and 95% (74;99), respectively. A clinical-prediction rule $ \geq 6$ derived from multivariate analysis had a sensitivity and specificity (95%CI) of 47% (31;64) and 98% (90;100), respectively. When LAM was combined with the clinical-prediction-rule, the sensitivity increased significantly ($ p < 0.001$) to 63% (47;68) and specificity remained high at 93% (82;98).

Conclusions: Despite its modest sensitivity the LAM ELISA is an accurate rapid rule-in test for TBM that has incremental value over smear-microscopy. The rule-in value of LAM can be further increased by combination with a clinical-prediction rule, thus enhancing the rapid diagnosis of TBM in HIV-infected persons with advanced immunosuppression.

Introduction

Although the tuberculosis (TB) epidemic has plateaued in several regions of the world, in Africa, fuelled by poverty and HIV co-infection, TB is out of control. South Africa has the fifth highest burden of TB and the largest number of HIV-infected residents in any one country worldwide [1]. Given the high rate of HIV-TB co-infection, extra-pulmonary TB (EPTB) and hence central nervous system TB, which comprises 1 to 18% of EPTB [2,3,4,5], is a common clinical problem.

HIV-infected patients with TB meningitis (TBM) are particularly challenging to manage because there are no accurate tools to rapidly establish a diagnosis and delay in establishing treatment is associated with mortality [6,7,8]. Smear-microscopy in an ideal research setting, and using high volumes of processed CSF, may have a modest sensitivity [8,9]. However, in a programmatic setting in Africa the yield is dismal and recent studies in HIV-infected populations reveal a sensitivity of less than 5% [10]. Polymerase chain reaction (PCR), which may be used as a confirmatory test for TBM [11], is a good rapid rule-in test for TBM with a sensitivity of ~40 to 50% but this technology is unavailable in most hospitals in Africa [12]. We recently showed that CSF antigen-specific quantitative T cell assays may be a rapid and accurate rule-in test for TBM but the available ELISPOT...
Categorisation of patients

Patients were categorised as definite TBM (either CSF culture or PCR positive for M. tb) [11], probable TBM (clinical features of meningitis, an LP consistent with an aseptic bacterial meningitis, negative for other causes of meningitis, and two of the following: a chest X-ray consistent with active PTB, a CT scan consistent with TBM (basal enhancement or hydrocephalus), and a response to anti-tuberculous therapy), or non TBM (an alternate definite cause for meningitis identified and response to appropriate non-tuberculous therapy) [11,30]. The reference standard was thus PCR or culture positivity for M. tb [11].

The laboratory technician was blinded to the clinical diagnosis and clinician blinded to the laboratory result. Tests were done in duplicate. Standard curves were derived by serial dilution of LAM antigen using CSF from a patient with benign intracranial hypertension.

Laboratory processing

CSF samples were processed for the detection of M. tb using a standardized PCR assay (Roche AMPLICOR) as per manufacturer instructions. LAM antigen was measured using an ELISA kit (Clearview® TB ELISA, Inverness Medical Innovations, USA). The samples were thawed and allowed to equilibrate to room temperature. After an initial heating step (95–100 °C for 30 min) to separate antigen-antibody complexes, CSF samples (0.2 ml) were seeded, in duplicate, into 96 well plates coated with anti-LAM antibodies. Following this an ELISA was done to measure optical density (OD) determined by a trained technician blinded to patient details. The LAM OD values were extrapolated from a standard curve constructed from two-fold serial dilutions (8 in total ranging from 10 to 0.08 mg/ml) of the LAM antigen (20 mg/ml) in CSF (Inverness Medical Innovations, USA).

Methods

Patient recruitment and processing

One hundred and fifty consecutive patients were prospectively recruited over a period of 15 months, between January 2008 and April 2009, at Inkosi Albert Luthuli Central Hospital (IALCH), a tertiary referral center in Durban, South Africa. This study was approved by the biomedical research ethics committee of the University of KwaZulu-Natal. Patients presenting with a meningal illness indicating the need for a lumbar puncture (LP) were recruited from referring regional hospitals. Detailed recruitment and patient processing methods were recently described [28]. Informed written consent was obtained from all patients (in patients who were unable provide consent at initial presentation, due to an abnormal mental state, consent was obtained from a first degree relative or from the Head of Department when a lumbar puncture was clinically justified) [29]. After excluding contraindications to a lumbar puncture (LP), CSF samples were processed for microscopy (auramine staining of centrifuged samples using a mercury vapour fluorescent microscope), *Mycobacterium tuberculosis* (*M. tb*), bacterial, and fungal culture, and tests were performed to exclude other locally prevalent causes of meningitis including microscopy (Gram stain and for acid-fast bacilli), routine chemistry (protein, glucose, chloride), TB PCR (Roche Amplicor, Roche Diagnostics GmbH, Roche Applied Science, 68298 Mannheim, Germany), viral PCR (Roche Amplicor) for [cytomegalovirus (CMV), herpes simplex (HSV type 1) and varicella zoster virus (VZV)], fluorescent treponemal antibody (FTA) test and venereal disease research laboratory (VDRL) for neurosyphilis if FTA was positive, cysticercal enzyme linked immunosorbent assay (ELISA), and a cryptococcal antigen latex agglutination test (CLAT) which has a high specificity and sensitivity. Detailed methods were described in a previous publication [28]. Blood for CD4 counts was taken and HIV status noted in all patients. Clinical information recorded included demographic information, duration of symptoms and anti-tuberculous therapy, HIV status, duration of steroid therapy, past history of tuberculosis and history of tuberculosis (TB) contact.

Statistical Analysis

Gli square tests or Fisher exact tests were used to compare categorical variables between TBM and non TBM patients. Numeric variables were compared using a t-test or Wilcoxon Rank sum test/or Kruskal Wallis test if normality could not be assumed. Diagnostic performance, including 95% confidence intervals was assessed using sensitivity, specificity, agreement (proportion in whom both sample-specific results were concordant), predictive values and area under the receiver operating characteristic (ROC) curve where the combined results of culture or PCR was used as the gold standard to classify patients as definite TBM or non TBM. Three cut-off points were used: the laboratory standard, Youden index [31] and maximum specificity.

A clinical index was generated using a stepwise logistic regression model. Continuous variables such as clinical and laboratory parameters were dichotomised using ROC curves to identify cut-off points which maximised specificity prior to inclusion in the model. Rounded β-coefficients from the reduced model of significant variables were used to create a weighted clinical index. The index was then dichotomised and the sensitivity and specificity calculated. The sensitivity and specificity were recalculated using the revised clinical plus LAM index. A one sample z statistic was used to determine if adding LAM to the clinical index resulted in a significant change in diagnostic performance.

Results

Patient characteristics

Of the 150 consecutively recruited patients, two patients were excluded (in 1 patient no definite diagnosis made and in one other no CSF obtained). Thus, 148 samples were processed for LAM antigen (39 definite TBM [31 culture positive, 8 additional PCR positive patients], 55 probable TBM and 54 non TBM; Figure 1.
There were 14 PCR positive samples within the cohort of 31 culture positive patients. 84% of the cohort was HIV-infected, 15.5% HIV uninfected, 5% declined HIV testing. The median inter-quartile range (IQR) CD4 count was 132 cells/μl (54;241). Socio-demographic and a comparison of CSF findings between definite TBM and non-TBM groups findings are shown in Table 1. Alternate diagnoses in the non TBM group included cryptococcal meningitis (n = 30), bacterial meningitis (n = 5), viral meningitis (n = 14; 4 VZV, 1 CMV, 9 unknown), neoplastic meningitis (n = 2), mucormycosis (n = 1), venous sinus thrombosis with CSF change (n = 1), and neurosyphilis (n = 1).

LAM antigen detection test outcomes

Performance outcomes were derived using the manufacturer’s recommended cut-point (OD value of 0.1295), an optimal cut-point using Youden’s index (a point on the ROC curve yielding maximal sensitivity matched with the corresponding specificity) and the AUC-derived cut point selecting for high specificity at the expense of sensitivity. Outcomes are shown when the definite TBM group (n = 39) was compared to: (i) the entire non TBM group; i.e. including patients with cryptococcal meningitis (n = 30), bacterial meningitis (n = 5), viral meningitis (n = 14; 4 VZV, 1 CMV, 9 unknown), neoplastic meningitis (n = 2), mucormycosis (n = 1), venous sinus thrombosis with CSF change (n = 1), and neurosyphilis (n = 1).

Relationship to CD4 count

When patients were stratified according to CD4 count, there was a greater likelihood of definite TBM when the CD4 count was <100 cells/μl versus ≥100 cells/μl; p = 0.01; Table 3. A comparison of CD4 counts <200 to ≥200 cells/μl also showed significance; p = 0.03. No significant difference was seen when sensitivity was compared in the in the HIV-infected and HIV uninfected groups (p = 0.3; Table 3). However, there were very few patients in the HIV negative group.

There was no distinction when comparing probable TBM and non TBM groups when stratified according to a CD4 count <100 cells/μl versus ≥100 cells/μl; p = 0.4.

Derivation of a clinical prediction rule and comparison to CSF LAM levels

We derived a clinical index using univariate and multivariate analysis (shown in Table 4). Factors significantly associated with a diagnosis of TBM in the multivariate analysis were CSF to serum glucose ratio, lymphocyte count, CD4 count and a negative CLAT result. The clinical prediction rule was then calculated using the

Figure 1. Summary flow chart of patient categorisation and investigations performed at recruitment.

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formula based on the coefficients from the multivariate model and examined using various cut-points for the diagnosis of TBM. The cut-point of $6 provided the best rule-in value. Table 5 shows outcome data using the clinical prediction rule alone and when the clinical prediction rule was combined with the LAM result: there was a significant improvement in sensitivity (31% to 63%; $p = 0.001$) and agreement (68% to 80% ($p = 0.01$) but specificity remained high at 93%.

Comparison of rapid tests for the diagnosis of TBM

Three tests were applied for a rapid diagnosis. When examining for yield for rapid diagnosis using only culture positive patients as the denominator (n = 31 of the 39 definite TBM patients) the yields for smear microscopy, LAM and PCR were, 0 (0%), 9 (29%), at cut-point $\geq 0.18$) and 14 (45%), respectively. Thus, there was a significantly improved yield over smear microscopy when using both LAM ($p\leq 0.001$) and PCR ($p = <0.001$). There was no significant difference between the LAM and PCR yield ($p = 0.22$).

Discussion

This study has three major findings. Firstly a newly developed clinical prediction rule, suited to resource-poor high HIV prevalence settings, is a useful rule-in test for the rapid diagnosis of TBM. Secondly, LAM antigen, which has not previously been prospectively evaluated in CSF, is useful as a rapid rule-in test for the diagnosis of TBM in HIV-infected individuals with advanced immunosuppression. Thirdly, combining the prediction rule with LAM antigen detection further increases the rule-in value for TBM.

Table 1. Comparison of the clinical and laboratory parameters in the definite TB meningitis (culture or PCR positive; n = 39) and non TB meningitis (n = 54) groups.

| Characteristic | Definite TBM (%) [IQR] | Non TBM (%) [IQR] | P value |
|---------------|------------------------|-------------------|---------|
| Number        | 39 (42%)               | 54 (36%)          | 0.7     |
| Mean age (± S.D) | 33.5 (9.5)                | 32.9 (9.7)        | 0.7     |
| Age $<36/\geq 36$ years* | 24/15 (61.5/38.5)        | 35/19 (29.6/70.4) | 0.7     |
| Sex           |                         |                   |         |
| Male/Female   | 18/21 (46.2/53.9)       | 16/38 (29.6/70.4) | 0.1     |
| Ethnic Group  |                         |                   |         |
| BA/M/E/I      | 38/1/0/0 (97.4/2.6/0/0) | 53/0/0/1 (98.2/0/0/1.9) | 0.3     |
| HIV status    |                         |                   |         |
| P/N/Unknown   | 34/4/1 (87.2/10.3/2.6)  | 47/6/187.0/11.1/1.9 |         |
| Previous TB   |                         |                   |         |
| Yes/No/Unknown| 8/27/4 (20.6/69.2/10.3) | 24/30/0 (44.4/55.6/0) | 0.007   |
| TB contact (within 2 years) |                       |                   |         |
| Yes/No/Unknown| 9/26/4 (23.1/66.6/10.3) | 14/40/0 (25.9/74.1/0) | 0.06    |
| Duration of illness (days) |               |                   |         |
| $<6/\geq 6$ days* | 6/31 (16.2/83.8)      | 9/45 (16.7/83.3)  | 0.9     |
| Steroid treatment |                     |                   |         |
| Yes/No        | 12/27 (30.8/69.2)      | 8/46 (14.8/85.2)  | 0.07    |
| CLAT          |                         |                   |         |
| Yes/no        | 4/35 (10.3/89.7)       | 27/27 (50/50)     | <0.001  |
| CD4 cells/µl IQR | 84 [53-173]          | 161 [54-261]     | 0.04    |
| Hydrocephalus (CT/MRI) |                |                   |         |
| Yes/no        | 17/13 (56.7/43.3)     | 10/13 (43.5/56.5) | 0.3     |
| CSF parameters |                         |                   |         |
| Lymphocytes (cells/µl) | 99 [16-42]           | 28 [10-82]       | 0.01    |
| Neutrophils (cells/µl) | 67 [20-134]         | 9 [0-66]         | 0.002   |
| Protein g/l   | 1.8 [1.14-2.7]        | 1.0 [0.8-1.9]    | <0.001  |
| CSF Glucose mmol/l | 1 [1.0-1.7]         | 2.1 [1.5-2.7]   | <0.001  |
| CSF: serum Glucose ratio | 0.2 [0.16-0.3]   | 0.4 [0.2-0.5] | <0.001  |
| Lymphocytes: total ratio | 0.6 [0.2-0.8]   | 0.8 [0.2-1.0]  | 0.2     |

*We chose a 36 year and 6 day cut off as this was a significant discriminator between acute septic and aseptic meningitis [9].

BA (Black African), M (mixed race), E (European), I (Indian).

P (positive), N (negative).

* = Median and inter-quartile ranges.

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Given that standardised PCR assays have modest sensitivity [12], are expensive, and not widely available in high TB and HIV burden settings, smear microscopy remains the only diagnostic test that can rapidly and confidently establish a diagnosis of TB. Thus, although at first glance, the LAM sensitivity of 31% may seem modest the high specificity confers rule-in value enabling a rapid diagnosis in approximately a third more of patients than could have been obtained with microscopy. Furthermore, in HIV-infected persons with a CD4 count of less than 100 cells/\mu l the sensitivity rose to 50%. Several studies have now confirmed the rule-in utility of the LAM ELISA in HIV co-infected subjects with advanced immunosuppression when using urine samples [19,21,24,25,32,33]. These studies have also confirmed that urine LAM positivity is associated for HIV positivity and advanced immunosuppression characterised by low CD4 counts [19,24,32]. Similarly, we confirm that in CSF LAM positivity is associated with a positive Gram stain or CLAT (2B), or those non-TBM patients who had a negative Gram stain or CLAT (2C). Each of the tables (2A, 2B, 2C) has results specific using the manufacturer’s cut-point, optimal cut point using Youdens index and an AUC derived cut point.

![Table 2. Performance outcomes (sensitivity, specificity, predictive values and accuracy) of the LAM ELISA (95% CI), at different cut-points in the definite TBM and non-TBM groups, using CSF.](https://www.plosgone.org/doi/10.1371/journal.pone.0015664.t002)

### 2A. Definite TBM (n = 39) compared to unselected Non TBM (n = 54) AUC\(^2\) = 0.74 (0.64;0.84)

| Cut Point (OD) | Sens (CI) | Spec (CI) | PPV (CI) | NPV (CI) | Agreement (CI) |
|---------------|-----------|-----------|----------|----------|----------------|
| \(\geq 0.1295\)\(^*\) | 69% (52;83) | 65% (51;77) | 59% (43;73) | 74% (60;86) | 67% (56;76) |
| \(\geq 0.148\)\(^\dagger\) | 46% (30;63) | 89% (77;96) | 75% (53;90) | 70% (57;80) | 71% (61;80) |
| \(\geq 0.18\)\(^\ddagger\) | 31% (17;48) | 94% (85;99) | 80% (52;96) | 65% (45;76) | 68% (57;77) |

### 2B. Definite TBM (n = 39) compared to non TBM (n = 35) where non-TB samples were Gram stain or CLAT positive AUC 0.73 (0.61;0.84)

| Cut Point (OD) | Sens (CI) | Spec (CI) | PPV (CI) | NPV (CI) | Agreement (CI) |
|---------------|-----------|-----------|----------|----------|----------------|
| \(\geq 0.1295\)\(^*\) | 69% (52;83) | 63% (45;79) | 68% (51;81) | 65% (46;80) | 66% (54;77) |
| \(\geq 0.148\)\(^\dagger\) | 46% (30;63) | 86% (70;95) | 78% (56;93) | 59% (44;72) | 65% (53;76) |
| \(\geq 0.18\)\(^\ddagger\) | 31% (17;48) | 94% (81;99) | 86% (57;98) | 55% (42;68) | 61% (49;72) |

### 2C. Definite TBM (n = 39) compared to non-TBM (n = 19) where non-TB samples were not Gram stain or CLAT positive AUC 0.77 = (0.63;0.89)

| Cut Point (OD) | Sens (CI) | Spec (CI) | PPV (CI) | NPV (CI) | Agreement (CI) |
|---------------|-----------|-----------|----------|----------|----------------|
| \(\geq 0.1295\)\(^*\) | 69% (52;83) | 68% (43;87) | 82% (65;93) | 52% (31;72) | 69% (55;80) |
| \(\geq 0.148\)\(^\dagger\) | 46% (30;63) | 95% (74;100) | 95% (74;100) | 46% (30;63) | 62% (48;74) |
| \(\geq 0.18\)\(^\ddagger\) | 31% (17;48) | 95% (74;100) | 92% (64;100) | 40% (26;56) | 52% (38;65) |

*Values expressed as percentages using manufacturers cut point for urine.

\(^{\dagger}\)Optimal cut-point as defined by Youden’s index [31].

\(^{\ddagger}\)Cut points chosen from the ROC curve to derive greater utility of LAM as a rule-in test.

\(^{\ast}\)AUC = Area under the curve.

Sens = sensitivity, Spec = specificity, PPV = positive predictive value, NPV = negative predictive value.

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Thus, although at first glance, the LAM sensitivity of 31% may seem modest the high specificity confers rule-in value enabling a rapid diagnosis in approximately a third more of patients than could have been obtained with microscopy. Furthermore, in HIV-infected persons with a CD4 count of less than 100 cells/\mu l the sensitivity rose to 50%. Several studies have now confirmed the rule-in utility of the LAM ELISA in HIV co-infected subjects with advanced immunosuppression when using urine samples [19,21,24,25,32,33]. These studies have also confirmed that urine LAM positivity is associated for HIV positivity and advanced immunosuppression characterised by low CD4 counts [19,24,32]. Similarly, we confirm that in CSF LAM positivity is associated with a positive HIV status and low CD4 count. We hypothesise that HIV-infected patients, particularly those with advanced immunosuppression, have a higher mycobacterial load and hence higher levels on LAM antigen, at least, in some of these patients. Although comprehensive follow-up was not available, these patients were observed as in-patients for a period of 7 to 10 days for...
Figure 2. Lipoarabinomannan antigen performance outcomes using CSF when comparing definite, probable and non-TB meningitis groups. (A) shows the definite TBM compared with the unselected non-TB meningitis group and the corresponding ROC curve (B). Responses when the non-TB meningitis group was stratified by rapid test results (Gram stain or CLAT positive, versus, Gram stain and CLAT both negative) are shown in (C) with the corresponding ROC curve (D). Note (C) for the sake of clarity does not show the probable TB meningitis group. doi:10.1371/journal.pone.0015664.g002
Table 3. LAM performance outcomes in definite TBM and non-TBM patients when stratified by HIV status and CD4 count.

| Characteristic | All (95% CI) | HIV* (95% CI) | CD4 (95% CI) |
|---------------|-------------|---------------|--------------|
|               | Negative    | Positive      | <100         | 100–199      | ≥200         |
|               | (n = 93)    | (n = 10)      | (n = 81)     | (n = 39)     | (n = 31)     |
| Sensitivity‡ | 31 (17;48)  | 0% (0;60)     | 35 (20;53)   | 50 (27;73)   | 18 (2;52)    |
| Specificity‡ | 94 (85;99)  | 100 (54;100)  | 96 (86;99)   | 95 (74;99)   | 100 (74;100) |
| PPV‡         | 80 (52;96)  | N/A           | 86 (57;98)   | 91 (59;99)   | 100 (16;100) |
| NPV‡         | 65 (54;76)  | 60 (26;88)    | 67 (55;78)   | 64 (44;81)   | 57 (34;78)   |
| Agreement    | 68 (57;77)  | 60 (26;88)    | 70 (59;80)   | 72 (55;85)   | 61 (39;80)   |

*Comparison between HIV positive and HIV negative patients was not significant; p value was 0.3.
†Expressed as percentages.
‡Comparison between sensitivity values for CD4 counts <100 with ≥100, the p value was 0.01.
§Comparison between sensitivity values for CD4 counts <200 with ≥200, the p value was 0.03.

Note: HIV status was unknown for 1 patient in the definite TBM group and 1 patient in the non TBM group.

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Table 4. Univariable and multivariable analysis for the prediction of definite TB meningitis.

| Characteristic | OR (95%CI) | P value | β coefficient | Score |
|---------------|------------|---------|---------------|-------|
| **Univariable analysis** | | | | |
| Lymphocyte count >200 (cells/μl) | 6.5 (2–22) | 0.003 | | |
| Neutrophil count ≥36 (cells/μl) | 5.0 (2–12) | <0.001 | | |
| Protein Level ≥2.5 g/l | 3.6 (1–10) | 0.02 | | |
| CSF glucose ≤1 mmol/l | 8.4 (3–24) | <0.001 | | |
| Ratio of CSF/serum glucose ≤0.2 | 9.3 (3–28) | <0.001 | | |
| CD4 count (<200 cells/μl) | 2.9 (1–7) | 0.03 | | |
| CLAT test (NEG) | 8.7 (3–28) | <0.001 | | |
| Previous TB (no) | 3.1 (1.2–8.0) | 0.02 | | |
| **Multivariable analysis** | | | | |
| Ratio of CSF/serum glucose ≤0.2 | 7.1 (1.8–29) | 0.006 | 2 | 2 |
| Lymphocyte count >200 (cells/μl) | 7.6 (1.5–40) | 0.017 | 2 | 2 |
| CD4 count (<200 cells/μl) | 6.8 (1.9–24) | 0.003 | 1.9 | 2 |
| CLAT test (NEG) | 12.9 (3–52) | <0.001 | 2.6 | 3 |

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Table 5. Comparative performance outcomes of the clinical prediction rule, LAM, and a combination of LAM and the clinical prediction rule for the diagnosis of definite TB meningitis.

| Cut Point | Sens (CI) | Spec (CI) | PPV (CI) | NPV (CI) | Agreement (CI) | AUC (CI) |
|-----------|-----------|-----------|----------|----------|----------------|---------|
| CPR ≥4 (excluding LAM) | 87% (72;96) | 70% (56;82) | 67% (52;80) | 88% (75;96) | 77% (67;85) | 86% (79;94) |
| CPR ≥4 (including LAM) | 47% (31;64) | 98% (70;100) | 95% (61;82) | 73% (74;100) | 77% (67;85) | 0.86 (0.79;0.94) |
| LAM (OD) ≥0.18 | 31% (17;48) | 94% (85;99) | 80% (52;96) | 65% (54;76) | 66% (57;77) | 0.74 (0.64;0.84) |
| CPR ≥4 + LAM | 89% (75;97) | 65% (51;77) | 64% (50;77) | 90% (76;97) | 75% (65;83) | 77% (69;85) |
| CPR ≥4 + LAM & LAM | 63% (46;78) | 93% (82;98) | 86% (67;96) | 78% (66;87) | 80% (71;88) | 80% (0.71; 0.88) |

*One patient did not have lymphocyte count and was excluded.
1 Clinical prediction rule.
2 p value comparing sensitivity of the clinical prediction rule alone (47%) vs. the clinical prediction rule plus the LAM result (63%) = 0.07.
3 p value comparing sensitivity of LAM 31% vs. clinical prediction rule combined with LAM (63%) =0.001.

Sens = sensitivity, Spec = specificity, PPV = positive predictive value, NPV = negative predictive value.

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References

1. World Health Organisation (2008) Global tuberculosis control: Surveillance, planning, financing. Available: http://www.who.int/tb/publications/global_report/2008/en/index.html.
2. Centers for Disease Control (2005) Extrapulmonary tuberculosis cases and percentages by site of disease: reporting areas. Atlanta, GA: Centers for Disease Control and Prevention. Available: www.cdc.gov/tb/surv/surv2005/PDF/table27.pdf.
3. Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, et al. (2001) Harrison’s Principles of Internal Medicine 15th Edition: McGraw-Hill p1028.
4. Ozbay B, Uzun K (2002) Extrapulmonary tuberculosis in high prevalence of tuberculosis and low prevalence of HIV. Clin Chest Med 23: 351–354.
5. Sumi MG, Annamma M, Sarada C, Radhakrishnan VV (2000) Rapid diagnosis of tuberculous meningitis by Western blotting. Clin Immunol Immunopathol 61: 35–40.
6. Garcia-Moncó J (1999) Central nervous system tuberculosis. Neurol Clin 17: 737–759.

Author Contributions

Conceived and designed the experiments: VBP KD. Performed the experiments: RS VK. Analyzed the data: CG. Contributed reagents/materials/analysis tools: VBP VK TN KD. Wrote the paper: VBP KD. Supervised the study: KD AZ TN.
diagnostic yield and association with immune reconstitution disease. AIDS 23: 1875–1880.

25. Mutetwa R, Boehme C, Dimairo M, Bandason T, Manyati SS, et al. (2009) Diagnostic accuracy of commercial urinary lipoarabinomannan detection in African tuberculosis suspects and patients. Int J Tuberc Lung Dis 13: 1253–1259.

26. Tessema TA, Hamusur B, Bujm G, Svenson S, Bjorvatn B (2001) Diagnostic evaluation of urinary lipoarabinomannan at an Ethiopian tuberculosis centre. Scand J Infect Dis 33: 279–284.

27. Patel VB, Bhigjee AI, Paruk HF, Singh R, Meldau R, et al. (2009) Utility of a novel lipoarabinomannan assay for the diagnosis of tuberculous meningitis in a resource-poor high-HIV prevalence setting. Cerebrospinal Fluid Res 6: 13.

28. Patel VB, Singh R, Connolly C, Coovadia Y, Peer AK, et al. Cerebrospinal T Cell Responses Aid the Diagnosis of Tuberculous Meningitis in a HIV and TB Endemic Population. Am J Respir Crit Care Med.

29. Simmons CP, Thwaites GE, Quyen NT, Torok E, Hoang DM, et al. (2006) Pretreatment intracerebral and peripheral blood immune responses in Vietnamese adults with tuberculous meningitis: diagnostic value and relationship to disease severity and outcome. J Immunol 176: 2007–2014.

30. Thwaites G, Chau TT, Mai NT, Drobniewski F, McAdam K, et al. (2000) Tuberculous meningitis. J Neurol Neurosurg Psychiatry 68: 289–299.

31. Schisterman EF, Perkins NJ, Liu A, Bondell H (2005) Optimal cut-point and its corresponding Youden Index to discriminate individuals using pooled blood samples. Epidemiology 16: 73–81.

32. Shah M, Martinson NA, Chaisson RE, Martin DJ, Variava E, et al. (2010) Quantitative analysis of a urine-based assay for detection of lipoarabinomannan in patients with tuberculosis. J Clin Microbiol 48: 2972–2974.

33. Shah M, Variava E, Holmes CB, Coppin A, Golub JE, et al. (2009) Diagnostic accuracy of a urine lipoarabinomannan test for tuberculosis in hospitalized patients in a High HIV prevalence setting. J Acquir Immune Defic Syndr 52: 145–151.

34. Moghtaderi A, Alavi-Naini R, Izadi S, Cuevas LE (2009) Diagnostic risk factors among adults with and without HIV infection. Experience in an urban public hospital. Arch Intern Med 156: 1710–1716.

35. Marais S, Thwaites G, Schoeman JF, Torok ME, Mira UK, et al. (2010) Tuberculous meningitis: a uniform case definition for use in clinical research. Lancet Infect Dis 10: 803–812.

36. Weinberg A, Bloch KC, Li S, Taub YW, Palmer M, et al. (2005) Dual infections of the central nervous system with Epstein-Barr virus. J Infect Dis 191: 234–237.

37. Lipovsky MM, Toenova L, Crennaerts FE, Kaplan G, Chemiak R, et al. (2000) Cryptococcal glucuronoxylomannan delays translocation of leukocytes across the blood-brain barrier in an animal model of acute bacterial meningitis. J Neuroimmunol 111: 10–14.

38. Chau ED, Reves R, Belile JT, Brennan PJ, Hahn WE (2000) Diagnosis of tuberculosis by a visually detectable immunoassay for lipoarabinomannan. Am J Respir Crit Care Med 161: 1713–1719.

39. Thwaites GE, Chau TT, Farrar J (2004) Improving the bacteriological diagnosis of tuberculous meningitis. J Clin Microbiol 42: 378–379.

40. Kennedy DH, Fallon RJ (1979) Tuberculous meningitis. JAMA 241: 264–268.

41. Ogawa SK, Smith MA, Bremsnessel DJ, Lowy FD (1987) Tuberculous meningitis in an urban medical center. Medicine (Baltimore) 66: 317–326.

42. Stewart SM (1953) The bacteriological diagnosis of tuberculous meningitis. J Clin Pathol 6: 241–242.

43. Verdon R, Chevet S, Laissy JP, Wolff M (1996) Tuberculous meningitis in adults: review of 48 cases. Clin Infect Dis 22: 982–988.

44. Girgis NI, Sultan Y, Farid Z, Mansour MM, Erian MW, et al. (1998) Tuberculous meningitis, Abbassia Fever Hospital-Naval Medical Research Unit No. 3-Cairo, Egypt, from 1976 to 1996. Am J Trop Med Hyg 58: 28–34.

45. Kashyap RS, Ramteke SS, Morey SH, Purohit HJ, Taori GM, et al. (2009) Diagnostic Value of Early Secreted Antigenic Target-6 for the Diagnosis of Tuberculous Meningitis Patients. Infection.

46. Berenguer J, Moreno S, Laguna F, Vicente T, Adrados M, et al. (1992) Tuberculous meningitis in patients infected with the human immunodeficiency virus. N Engl J Med 326: 668–672.

47. Dube MP, Holm N, Larsen RA (1992) Tuberculous meningitis in patients with and without human immunodeficiency virus infection. Am J Med 93: 520–524.

48. Kilpatrick ME, Girgis NI, Yasmin MW, Abu el Ella AA (1986) Tuberculous meningitis-clinical and laboratory review of 100 patients. J Hyg (Lond) 96: 231–238.

49. Porckert MT, Sotir M, Parrott-Moore P, Blumberg HM (1997) Tuberculous meningitis at a large inner-city medical center. Am J Med Sci 313: 325–331.

50. Ter Pou A, Beus I (1982) Characteristics of cerebrospinal fluid in tuberculous meningitis. Acta Cytol 26: 678–680.

51. Thwaites GE, Duc Bang N, Thi Quy H, Thi Tuong Oanh D, et al. (2005) The influence of HIV infection on clinical presentation, response to treatment, and outcome in adults with Tuberculous meningitis. J Infect Dis 192: 2134–2141.