



RESEARCH NOTE

Performance of Lipoarabinomannan Assay using Cerebrospinal fluid for the diagnosis of Tuberculous meningitis among HIV patients [version 1; peer review: awaiting peer review]

Richard Kwizera ¹, Fiona V. Cresswell ¹⁻³, Gerald Mugumya⁴, Micheal Okirwoth¹, Enock Kagimu¹, Ananta S. Bangdiwala ⁵, Darlisha A. Williams^{1,5}, Joshua Rhein ^{1,5}, David R. Boulware ⁵, David B. Meya^{1,4,5}

¹Infectious Diseases Institute, College of Health Sciences, Makerere University, Kampala, Uganda

²Clinical Research Department, London School of Hygiene and Tropical Medicine, London, WC1E 7HT, UK

³MRC-UVRI and LSHTM Uganda Research Unit, Entebbe, Uganda

⁴Department of Medicine, School of Medicine, College of Health Sciences, Makerere University, Kampala, Uganda

⁵Division of Infectious Diseases and International Medicine, Department of Medicine, University of Minnesota, Minneapolis, MN, 55455, USA

V1 First published: 19 Aug 2019, 4:123 (<https://doi.org/10.12688/wellcomeopenres.15389.1>)

Latest published: 19 Aug 2019, 4:123 (<https://doi.org/10.12688/wellcomeopenres.15389.1>)

Abstract

Background: The diagnostic utility of the *Mycobacteria tuberculosis* lipoarabinomannan (TB-LAM) antigen lateral flow assay on cerebrospinal fluid (CSF) for the diagnosis of tuberculous meningitis (TBM) has not been extensively studied and the few published studies have conflicting results.

Methods: Lumbar CSF from 59 HIV-positive patients with suspected TBM was tested with TB-LAM and Xpert MTB/Rif Ultra. The diagnostic performance of CSF TB-LAM was compared to positive CSF Xpert MTB/Rif Ultra (definite TBM) and a composite reference of probable or definite TBM according to the uniform case definition.

Results: Of 59 subjects, 12 (20%) had definite TBM and five (9%) had probable TBM. With reference to definite TBM, CSF TB-LAM assay had a diagnostic sensitivity of 33% and specificity of 96%. When compared to a composite reference of definite or probable TBM, the sensitivity was 24% and specificity was 95%. There were two false positive tests with TB-LAM (3+ grade). In-hospital mortality in CSF TB-LAM positive patients was 17% compared to 0% in those with definite TBM by Xpert MTB/Rif Ultra but negative LAM.

Conclusions: Lumbar CSF TB-LAM has a poor performance in diagnosing TBM. Both urine TB-LAM and Xpert Ultra should be further investigated in the diagnosis of TBM.

Keywords

Tuberculous meningitis, extra-pulmonary TB, lipoarabinomannan, TB-LAM, Xpert MTB/Rif Ultra, HIV, Diagnostics, cerebrospinal fluid

Open Peer Review

Reviewer Status AWAITING PEER REVIEW

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Richard Kwizera (kwizerarichard@gmail.com)

Author roles: **Kwizera R:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing; **Cresswell FV:** Conceptualization, Formal Analysis, Funding Acquisition, Methodology, Project Administration, Resources, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; **Mugumya G:** Formal Analysis, Investigation, Validation, Writing – Review & Editing; **Okirwoth M:** Formal Analysis, Investigation, Methodology, Writing – Review & Editing; **Kagimu E:** Data Curation, Investigation, Methodology, Writing – Review & Editing; **Bangdiwala AS:** Data Curation, Formal Analysis, Methodology, Validation, Writing – Review & Editing; **Williams DA:** Methodology, Project Administration, Resources, Supervision, Writing – Review & Editing; **Rhein J:** Project Administration, Resources, Supervision, Validation, Writing – Review & Editing; **Boulware DR:** Funding Acquisition, Project Administration, Resources, Supervision, Validation, Writing – Review & Editing; **Meya DB:** Funding Acquisition, Project Administration, Resources, Supervision, Validation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by the Wellcome Trust [107742, 107743 and 210772]. This research was also supported in part by the National Institute of Neurologic Diseases and Stroke (NINDS) and Fogarty International Center [R01NS086312, K01TW010268]. DBM and RK are currently supported through the DELTAS Africa Initiative grant [DEL-15-011] to THRiVE-2, from Wellcome Trust grant [107742] and the UK government. FVC is supported through a Wellcome Clinical PhD Fellowship [210772]. FVC is an honorary fellow of the Makerere University – UVRI Centre of Excellence for Infection and Immunity Research and Training (MUII-plus). MUII-plus is supported through the DELTAS Africa Initiative [107743]. The DELTAS Africa Initiative is an independent funding scheme of the African Academy of Sciences (AAS), Alliance for Accelerating Excellence in Science in Africa (AESA) and supported by the New Partnership for Africa's Development Planning and Coordinating Agency (NEPAD) with funding from the Wellcome Trust [107743] and the UK Government. The MRC/UVRI and LSHTM Uganda Research Unit is jointly funded by the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement and is also part of the EDCTP2 programme supported by the European Union.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2019 Kwizera R *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution Licence](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Kwizera R, Cresswell FV, Mugumya G *et al.* **Performance of Lipoarabinomannan Assay using Cerebrospinal fluid for the diagnosis of Tuberculous meningitis among HIV patients [version 1; peer review: awaiting peer review]** Wellcome Open Research 2019, 4:123 (<https://doi.org/10.12688/wellcomeopenres.15389.1>)

First published: 19 Aug 2019, 4:123 (<https://doi.org/10.12688/wellcomeopenres.15389.1>)

Introduction

In many human immunodeficiency virus (HIV) endemic countries, tuberculous meningitis (TBM) is the second most common cause of adult meningitis after cryptococcal meningitis¹, and accounts for 1–5% of all tuberculosis (TB) cases². TBM is the most severe form of TB and causes substantial morbidity and mortality in children and immunocompromised adults^{3,4}. HIV infection is known to increase the risk of death in patients with TBM, as does TBM stage at the time of treatment initiation^{2,5}. As is the case in cryptococcosis, high-quality nursing care is a critical component in managing TBM patients⁶.

Similarly, diagnosis of TBM is very challenging, especially in resource-limited settings where diagnosis relies on a combination of clinical, radiological and laboratory findings. The World Health Organisation (WHO) recommends Xpert MTB/RIF Ultra for the diagnosis of TBM using cerebrospinal fluid (CSF). Culture has many limitations related to turnaround time and sensitivity, and also requires considerable infrastructure and costs⁷. Therefore, the development of early point of care diagnosis for TBM is a priority. Recent studies have demonstrated that the next generation Xpert MTB/RIF Ultra is the most sensitive diagnostic test in HIV-positive adults⁷. However, Xpert MTB/RIF Ultra is not a bedside test, and thus access to same day results remain a challenge in many settings⁷.

Assays based on the detection of mycobacterial lipoparabinomanan (TB-LAM) antigen in urine have emerged as potential point-of-care tests for extra-pulmonary TB⁸. There is evidence that urine TB-LAM may help to reduce mortality and predict poor outcomes^{9,10}. The WHO recently added the TB-LAM assay onto its essential diagnostic list and recommended TB-LAM in hospitalised HIV positive adults with signs and symptoms of TB^{11,12}. However, there are conflicting results about TB-LAM assay sensitivity for TBM diagnosis¹³ in CSF. With reference to definite TBM, Cox *et al.* found a 75% sensitivity using CSF from the fourth ventricle in an autopsy cohort from 91 HIV-infected adults¹⁴. However, Bahr *et al.* had no positive TB-LAM tests using lumbar CSF from 67 HIV patients with meningitis¹³. In light of these results, and now that Xpert MTB/RIF Ultra is used instead of Xpert MTB/RIF, we aimed to further explore the utility of CSF TB-LAM test for the diagnosis of TBM among HIV-positive adults presenting with suspected meningitis.

Methods

Study setting and participants

Between April 2018 and June 2019, we assessed and performed diagnostic lumbar punctures on HIV-positive patients admitted to Mulago National Referral Hospital with suspected meningitis in Kampala, Uganda. Screening for TBM was performed cross-sectionally as part of the High Dose Rifampicin for Tuberculous Meningitis (RIFT) trial (ISRCTN registration number [ISRCTN42218549](https://www.isrctn.com/ISRCTN42218549), last updated 24/04/2018)¹⁵. Therefore, we did not calculate a sample size for the current study but included all participants that fit the screening criteria for the RIFT trial¹⁵. All included participants were HIV-infected adults (≥ 18 years old) who provided written informed consent by participant or surrogate, with a suspected

diagnosis of TBM (meningitis symptoms, clinical signs of meningism). Demographic information and baseline characteristics for participants were collected through clinical reviews using customized meningitis screening case report forms approved by the relevant ethics committees (Mulago Hospital Research Ethics Committee, Uganda National Council of Science and Technology, and the University of Minnesota). Opening pressures for CSF were measured using a manometer, followed by standard microbiology analysis (CrAg, cell count, protein, glucose, lactate, culture).

Diagnostic tests

In addition to standard microbiology analysis, CSF was tested with TB-LAM (Alere, Massachusetts, USA), and the test strip interpreted as per manufacturer's instructions. Briefly, the protective foil cover was removed from each test and the strip labelled with the participant's number. Two drops (or 60 μ L) of CSF were added to the sample pad. The test was then read after 25 minutes under standard indoor lighting conditions. The reference card was used in interpretation of the results by holding it alongside the patient window. For positive results, purple/gray bars appeared in both the control window and the patient window of the strip. For negative results, one purple/gray bar appeared in the control window of the strip and no bar appeared in the patient window of the strip. If there was no bar in the control window of the strip, the result was considered invalid and the test repeated. The strips were retained and cross checked by a second researcher to corroborate the finding.

CSF was also tested with Xpert MTB/Rif Ultra (Cepheid). Briefly, 2ml of sample reagent was added to 1ml of whole CSF and then left to stand at room temperature for 15 minutes. Then, 2ml of the sample mixture was transferred into the Xpert MTB/Rif Ultra cartridge and loaded into the Xpert machine. The test was run for 90 minutes and results from the assay indicate whether or not Mycobacteria TB (MTB) was detected in the sample. If MTB was detected, the results also stated whether resistance to rifampin was detected.

Test analysis

Data were analyzed using STATA version 14 (STATA, College Station, Texas). The disease prevalence, sensitivity, specificity, positive predictive values, negative predictive values and test accuracy were estimated at 95% confidence interval (CI). The diagnostic performance of CSF TB-LAM was compared to positive CSF Xpert MTB/Rif Ultra (definite TBM) and a composite reference of probable or definite TBM according to the uniform case definition¹⁶. Summaries were made in frequency & percentages for each baseline characteristic considered as a categorical, and medians (interquartile range) when each characteristic is considered as a continuous variable. For baseline variables with some missing data, we calculated the statistics using the available numbers.

Ethical statement

Institutional review board approvals for the study and the associated screening process were obtained locally in Uganda (Mulago Hospital Research Ethics Committee, approval number

MREC 1260); and from the London School of Hygiene and Tropical Medicine, UK (14388), University of Minnesota (1304M31361) and by the Uganda National Council of Science and Technology (HS136ES). Written informed consent for participation in the study and data publication was obtained from all participants or from their surrogates (e.g. family member or guardian) where the patient had altered mental status and did not have the capacity to provide consent.

Results

Overall, 59 HIV-positive hospitalized participants with suspected meningitis underwent diagnostic lumbar punctures, of which 20% (12/59) had definite TBM, 9% (5/59) had probable TBM, 25% (15/59) had possible TBM, and 46% (27/59) had no-TBM¹⁷. Of those with no TBM (n=27), 10 had cryptococcosis. Women comprised 50% of participants with an overall median age for all participants of 33 years (interquartile range [IQR]: 28, 40). Only 29% of the participants were receiving antiretroviral therapy at diagnosis. Among participants reporting a headache (n=57), the median duration of headache was 14 days (IQR: 14, 24). The CSF opening pressures at baseline (n=45) had a median of 200 mmH₂O

(IQR: 120, 260). Overall, 55% (n=36) had an acellular CSF, whilst those with a CSF lymphocytic pleocytosis had a median CSF white blood cell of 160 cells/ μ L (IQR: 135, 268) (Table 1).

With respect to the reference standard of definite TBM (positive CSF Xpert TB/Rif Ultra), the CSF TB-LAM assay had a sensitivity of 33% (4/12), specificity of 96% (45/47), positive predictive value (PPV) of 67% (4/6), and negative predictive value (NPV) of 85% (45/53). When compared to a composite reference of definite/probable TBM, the TB-LAM assay had a sensitivity of 24% (4/17), specificity of 95% (40/42), PPV of 67% (4/6), NPV of 76% (40/53) (Table 2). There were two false positive tests with TB-LAM (3+ grade), without any CSF pleocytosis, normal protein, normal glucose, negative cryptococcal antigen, and normal CSF opening pressure. One patient was discharged alive without TB therapy. The second patient had a headache for 60 days at presentation, but they were lost to follow up (i.e. self-discharged) without an etiologic diagnosis. In-hospital mortality in CSF TB-LAM positive patients was 17% (1/6) compared to 0% (0/8) in those with definite TBM by Xpert MTB/Rif Ultra but negative LAM.

Table 1. Characteristics of the study population.

Baseline characteristics	Data available for N participants*	Statistic
Women, n (%)	58	29 (50)
Age in years, median (IQR)	58	33 (28-40)
On ART, n (%)	47	29 (62)
Headache, n (%)	57	46 (81)
Duration of headache, median (IQR) days	45	14 (14-24)
Glasgow Coma Scale score, mean (SD)	55	13 (2.6)
CSF crag positive, n (%)	58	10 (17)
CSF Opening Pressure, median (IQR) mmH ₂ O	45	200 (120-260)
Acellular CSF, n (%)	55	36 (55)
CSF WBC in those with CSF WBC pleocytosis, median (IQR) cells/ μ L	55	160 (135-268)
CSF protein, median (IQR) mg/dL	52	57 (28-141)
CSF glucose, median (IQR) mg/dL	32	65 (34-82)
CSF lactate, median (IQR) mmol/L	36	3.9 (2.2-9)
Duration of hospitalization, median (IQR) days	46	7 (4-14)
Status at discharge	59	
Alive, n (%)		40 (68)
Dead, n (%)		9 (15)
Unknown, n (%)		10 (17)

Data presented are percentages (%), medians and interquartile ranges (IQR). N= number of participants with data for each parameter. *Some parameters have N<59 due to missing data. ART = antiretroviral therapy, CSF = cerebrospinal fluid, WBC = white blood cells.

Table 2. Summary of diagnostic performance of cerebrospinal fluid mycobacterial lipoarabinomannan assay for tuberculous meningitis.

Reference standard	Disease prevalence	Sensitivity	Specificity	PPV	NPV	Test Accuracy
Definite/probable TBM	28.8% (17/59)	23.5% (4/17)	95.2% (40/42)	66.7% (4/6)	75.5% (40/53)	74.6% (44/59)
95% CI	17.8 to 42.1%	6.8 to 49.9%	83.8 to 99.4%	28.7 to 90.8%	70.1 to 80.2%	61.6 to 85%
Definite TBM	20.3% (12/59)	33.3% (4/12)	95.7% (45/47)	66.7% (4/6)	84.9% (45/53)	83.1% (49/59)
95% CI	10.9 to 32.8%	9.9 to 65.1%	85.5 to 99.5%	29.3 to 90.6%	78.9 to 89.4%	71 to 91.6%

Data presented are the percentage, numerator/denominator, and 95% confidence intervals (CI). Test Accuracy = overall probability that a patient will be correctly classified. PPV = Positive predictive value, NPV = negative predictive value, TBM = tuberculous meningitis.

Conclusion

In conclusion, a rapid diagnosis of TBM could be achieved using a point of care test on CSF such as a TB-LAM antigen lateral flow assay; however, this study demonstrated a poor diagnostic performance of the existing Alere TB-LAM on CSF among HIV-associated tuberculous meningitis. While the relatively modest sample size is a limitation, a larger sample size is unlikely to fundamentally alter the findings of sensitivity. One explanation could be that TB-LAM is likely not be found in sufficient quantities in lumbar CSF. Further studies are required using larger number of cases to investigate the utility of urine TB-LAM in aiding the diagnosis of probable TBM or the diagnostic performance of the next generation of TB-LAM assay.

Data availability

Underlying data

Figshare: CSFLAM_data set revised.xlsx. <https://doi.org/10.6084/m9.figshare.9415853.v1>¹⁷

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

Grant information

This work was supported by the Wellcome Trust [107742, 107743 and 210772]. This research was also supported in part

by the National Institute of Neurologic Diseases and Stroke (NINDS) and Fogarty International Center [R01NS086312, K01TW010268]. DBM and RK are currently supported through the DELTAS Africa Initiative grant [DEL-15-011] to THRiVE-2, from Wellcome Trust grant [107742] and the UK government. FVC is supported through a Wellcome Clinical PhD Fellowship [210772]. FVC is an honorary fellow of the Makerere University – UVRI Centre of Excellence for Infection and Immunity Research and Training (MUII-plus). MUII-plus is supported through the DELTAS Africa Initiative [107743]. The DELTAS Africa Initiative is an independent funding scheme of the African Academy of Sciences (AAS), Alliance for Accelerating Excellence in Science in Africa (AESA) and supported by the New Partnership for Africa’s Development Planning and Coordinating Agency (NEPAD) with funding from the Wellcome Trust [107743] and the UK Government. The MRC/UVRI and LSHTM Uganda Research Unit is jointly funded by the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement and is also part of the EDCTP2 programme supported by the European Union.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements

We thank institutional support from the IDI research office.

References

- Jarvis JN, Meintjes G, Williams A, *et al.*: **Adult meningitis in a setting of high HIV and TB prevalence: findings from 4961 suspected cases.** *BMC Infect Dis.* 2010; 10: 67. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Thao LTP, Heemskerck AD, Geskus RB, *et al.*: **Prognostic Models for 9-Month Mortality in Tuberculous Meningitis.** *Clin Infect Dis.* 2018; 66(4): 523–32. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kalita J, Misra U, Ranjan P: **Predictors of long-term neurological sequelae of tuberculous meningitis: a multivariate analysis.** *Eur J Neurol.* 2007; 14(1): 33–7. [PubMed Abstract](#) | [Publisher Full Text](#)
- Saitoh A, Pong A, Waecker NJ Jr, *et al.*: **Prediction of neurologic sequelae in childhood tuberculous meningitis: a review of 20 cases and proposal of a novel scoring system.** *Pediatr Infect Dis J.* 2005; 24(3): 207–12. [PubMed Abstract](#) | [Publisher Full Text](#)
- Heemskerck AD, Bang ND, Thwaites GE: **Therapy for Tuberculous Meningitis.** *N Engl J Med.* 2016; 374(22): 2188–9. [PubMed Abstract](#) | [Publisher Full Text](#)
- Ndyetukira JF, Kwizera R, Kugonza F, *et al.*: **The conundrum of clinical trials and standard of care in sub-Saharan Africa – the research nurse perspective.** *J Res Nurs.* 2019. [Publisher Full Text](#)
- Bahr NC, Nuwagira E, Evans EE, *et al.*: **Diagnostic accuracy of Xpert MTB/RIF**

- Ultra for tuberculous meningitis in HIV-infected adults: a prospective cohort study.** *Lancet Infect Dis.* 2018; **18**(1): 68–75.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
8. Songkhla MN, Tantipong H, Tongsai S, *et al.*: **Lateral Flow Urine Lipoarabinomannan Assay for Diagnosis of Active Tuberculosis in Adults With Human Immunodeficiency Virus Infection: A Prospective Cohort Study.** *Open Forum Infect Dis.* Oxford University Press US. 2019; **6**(4): ofz132.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 9. Gupta-Wright A, Corbett EL, Wilson D, *et al.*: **Risk score for predicting mortality including urine lipoarabinomannan detection in hospital inpatients with HIV-associated tuberculosis in sub-Saharan Africa: Derivation and external validation cohort study.** *PLoS Med.* 2019; **16**(4): e1002776.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 10. Gupta-Wright A, Corbett EL, van Oosterhout JJ, *et al.*: **Rapid urine-based screening for tuberculosis in HIV-positive patients admitted to hospital in Africa (STAMP): a pragmatic, multicentre, parallel-group, double-blind, randomised controlled trial.** *Lancet.* 2018; **392**(10144): 292–301.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 11. WHO: **The use of lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis and screening of active tuberculosis in people living with HIV.** Geneva, Switzerland. 2015.
[Reference Source](#)
 12. Bongomin F, Govender NP, Chakrabarti A, *et al.*: **Essential *in vitro* diagnostics for advanced HIV and serious fungal diseases: international experts' consensus recommendations.** *Eur J Clin Microbiol Infect Dis.* 2019; **38**(9): 1581–1584.
[PubMed Abstract](#) | [Publisher Full Text](#)
 13. Bahr NC, Tugume L, Boulware DR: **A Word of Caution in Considering the Use of the Lipoarabinomannan Lateral Flow Assay on Cerebrospinal Fluid for Detection of Tuberculous Meningitis.** *J Clin Microbiol.* 2016; **54**(1): 241–2.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 14. Cox JA, Lukande RL, Kalungi S, *et al.*: **Accuracy of Lipoarabinomannan and Xpert MTB/RIF Testing in Cerebrospinal Fluid To Diagnose Tuberculous Meningitis in an Autopsy Cohort of HIV-Infected Adults.** *J Clin Microbiol.* 2015; **53**(8): 2667–73.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 15. Cresswell FV, Ssebambulidde K, Grint D, *et al.*: **High dose oral and intravenous rifampicin for improved survival from adult tuberculous meningitis: a phase II open-label randomised controlled trial (the RIFT study) [version 1; peer review: 2 approved].** *Wellcome Open Res.* 2018; **3**: 83.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 16. Marais S, Thwaites G, Schoeman JF, *et al.*: **Tuberculous meningitis: a uniform case definition for use in clinical research.** *Lancet Infect Dis.* 2010; **10**(11): 803–12.
[PubMed Abstract](#) | [Publisher Full Text](#)
 17. Kwizera R: **CSFLAM_data set revised.** *xlsx.* figshare.com. 2019.
<http://www.doi.org/10.6084/m9.figshare.9415853.v1>