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RESEARCH NOTE

REVISED **Performance of Lipoarabinomannan Assay using Cerebrospinal fluid for the diagnosis of Tuberculous meningitis among HIV patients** [version 2; peer review: 2 approved]

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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 ✔ ✔

	Invited Reviewers	
	1	**2**
REVISED **version 2** published 30 Sep 2019	✔	
	↑	
version 1 published 19 Aug 2019	✔ report	✔ report

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

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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

REVISED **Amendments from Version 1**

Based on the two reviewers' comments to version 1, major changes were made to the conclusion and Table 1 while minor changes were made to the results section as seen in the tracked changes. We added the suggested reference in the discussion section. Table 1 was modified to present the characteristics of patients with diagnosis of definite, probable, possible and not-TBM. A sentence was added on cerebral imaging. We also added a sentence about the patients who were positive with urine LAM and those who were positive with urine Ultra. We added an explanation about the 17% of patients had unknown outcome. A limitation was added that TB culture was not used. However, we have found Ultra to be more sensitive than culture (70% versus 43% against definite/probable) for the diagnosis of TBM in our population (Bahr, Lancet ID, 2018). In the conclusion section, we have mentioned the Fuji LAM that is a new LAM POC test that has higher sensitivity in urine than the determine LAM POC and should also be evaluated for diagnosis of TB meningitis both in urine and CSF. Lastly, based on the second reviewer's comments, we would like to say that a cellular CSF is common in this population with advanced HIV disease even in the setting of a confirmed TB or CM infection (Cresswell, Int J Infect Dis 2018). The not-TBM group scored <6 points on the uniform case definition (n=16) or had a confirmed alternative diagnosis (n=10 with CM).

Any further responses from the reviewers can be found at the end of the article

Introduction

In many human immunodeficiency virus (HIV) endemic countries, tuberculous meningitis (TBM) is the second most common cause of adult meningitis after cryptococcal meningitis[1], and accounts for 1–5% of all tuberculosis (TB) cases[2]. 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 patients[6].

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 costs[7]. 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 adults[7]. However, Xpert MTB/RIF Ultra is not a bedside test, and thus access to same day results remain a challenge in many settings[7].

Assays based on the detection of mycobacterial lipoarabinomannan (TB-LAM) antigen in urine have emerged as potential point-of-care tests for extra-pulmonary TB[8]. 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 diagnosis[13] 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 adults[14]. However, Bahr *et al.* had no positive TB-LAM tests using lumbar CSF from 67 HIV patients with meningitis[13]. 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, 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 trial[15]. 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 60μL) 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 definition[16]. 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 not-TBM[17]. Of those with not-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 mmH$_2$O (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/μL (IQR: 135, 268) (Table 1). Only about 10% of the participants had cerebral imaging done as the CT scanner was dysfunctional for part of the study period. About twenty five percent of the patients had a positive TB-LAM while 20% had a positive urine MTB/Rif Ultra.

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. About 17% of patients had unknown outcome. This was because the study population included patients screened for a clinical trial but only a minority were subsequently enrolled into the trial. We endeavoured to follow screen failures through to hospital discharge but this was not possible in all cases.

Conclusion
In conclusion, a rapid CSF point of care test for TBM is needed; however, this study demonstrated a poor diagnostic performance of the existing Alere TB-LAM on CSF among HIV-associated tuberculous meningitis. Our results corroborate the findings of a recent Zambian study which demonstrated 22% sensitivity for CSF LAM against a reference standard of TB culture[18]. 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. TB culture was not used, which is also a limitation of the accuracy analysis. However, Xpert Ultra has a sensitivity that is greater than culture in our setting[7]. The novel Fujifilm SILVAMP TB-LAM (FujiLAM) assay has been shown to have higher sensitivity in urine than the Alere TB-LAM and

Table 1. Characteristics of the study population.

Baseline characteristics	Overall (N=59)		Definite TBM (n=12)		Probable TBM (n=5)		Possible TBM (n=15)		Not-TBM (n=27)	
	N*	Statistic	N*	Statistic	N*	Statistic	N*	Statistic	N*	Statistic
Women, n (%)	58	29 (50)	12	6 (50)	5	3 (60)	15	4 (26.7)	26	16 (61.5)
Age in years, median (IQR)	58	33 (28-40)	12	29 (28-33)	5	26 (24-34)	15	35 (32-43)	26	34 (26-46)
On ART, n (%)	47	29 (62)	11	7 (63.6)	2	2 (100)	11	3 (27.3)	23	17 (73.9)
Headache, n (%)	57	46 (81)	12	10 (83.3)	5	4 (80)	14	12 (85.7)	26	20 (76.9)
Duration of headache, median (IQR) days	45	14 (14-24)	10	17.5 (14-30)	4	14 (10.5-17.5)	12	17.5 (14-31.5)	19	14 (7-30)
Glasgow Coma Scale score, mean (SD)	55	13 (2.6)	12	12.5 (2.9)	5	11.8 (2.4)	14	12.7 (2.9)	24	14.3 (2.1)
CSF CrAg positive, n (%)	58	10 (17)	12	0 (0)	5	0 (0)	15	0 (0)	26	10 (38.4)
CSF Opening Pressure, median (IQR) mmH2o	45	200 (120-260)	7	180 (70-240)	3	260 (95-400)	13	190 (120-270)	22	215 (120-260)
Acellular CSF, n (%)	55	36 (55)	11	3 (27.3)	5	1 (20)	14	11 (78.6)	25	21 (84)
CSF WBC in those with CSF WBC pleocytosis, median (IQR) cells/µL	55	160 (135-268)	8	280 (162.5-575)	4	173 (130-237.5)	3	80 (35-160)	4	145 (87.5-210)
CSF protein, median (IQR) mg/dL	52	57 (28-141)	11	184 (107-316)	5	158 (147-215)	13	44 (35-72)	23	31 (22-61)
CSF glucose, median (IQR) mg/dL	32	65 (34-82)	7	44 (19.8-61)	3	90 (68-108)	8	86 (56.3-104)	14	61 (31-80)
CSF lactate, median (IQR) mmol/L	36	3.9 (2.2-9)	8	9.7 (8.2-11.2)	4	9.2 (6.3-11.1)	8	3.4 (2.3-8.1)	16	2.4 (1.9-3.8)
Duration of hospitalization, median (IQR) days	46	7 (4-14)	9	11 (9-14)	4	14.5 (10-16.5)	9	4 (4-14)	24	5 (2-12.5)
Status at discharge	59		12		5		15		27	
Alive, n (%)		40 (68)		8 (66.7)		2 (40)		8 (53.3)		22 (81.5)
Dead, n (%)		9 (15)		3 (25)		1 (20)		2 (13.3)		3 (11.1)
Unknown, n (%)		10 (17)		1 (8.3)		2 (40)		5 (33.3)		2 (7.4)

Data presented are percentages (%), medians and interquartile ranges (IQR). N= number of participants with data for each parameter. * Participants with data available. 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.

warrants evaluation for diagnosis of TB meningitis both in urine and CSF[19].

References

1. 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**

2. Thao LTP, Heemskerk 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**

3. 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**

4. 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**

5. Heemskerk AD, Bang ND, Thwaites GE: **Therapy for Tuberculous Meningitis.** *N Engl J Med.* 2016; **374**(22): 2188–9.
    PubMed Abstract | **Publisher Full Text**

6. 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

7. 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

18. Siddiqi OK, Birbeck GL, Ghebremichael M, *et al.*: **Prospective Cohort Study on Performance of Cerebrospinal Fluid (CSF) Xpert MTB/RIF, CSF Lipoarabinomannan (LAM) Lateral Flow Assay (LFA), and Urine LAM LFA for Diagnosis of Tuberculous Meningitis in Zambia.** *J Clin Microbiol.* 2019; **57**(8): pii: e00652-19.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

19. Broger T, Sossen B, du Toit E, *et al.*: **Novel lipoarabinomannan point-of-care tuberculosis test for people with HIV: a diagnostic accuracy study.** *Lancet Infect Dis.* 2019; **19**(8): 852–61.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Open Peer Review

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Version 2

Reviewer Report 02 October 2019

https://doi.org/10.21956/wellcomeopenres.16956.r36606

✔   **Maryline Bonnet** (iD)

Institute of Research for Development (IRD), Montpellier, France

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Clinical research on tuberculosis.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 16 September 2019

https://doi.org/10.21956/wellcomeopenres.16813.r36425

✔   **Vinod Patel**

Department of Neurology, Nelson R. Mandela School of Medicine, University of KwaZulu-Natal (UKZN), Durban, South Africa

The article is well written with no major flaws.

Regarding the non-TBM category, although 10 patient had cryptococcal meningitis, detail regarding the non-TBM diagnoses is important as this is a control group and a patient without a clinical meningitis may allow for better specificity. I note that some CSF's were acellular, what were these diagnoses?

Please provide detail (a table with confirmatory findings for TB such as CXR, abdominal ultrasound, scan findings, CSF findings etc.) regarding the aspects considered to arrive at a diagnosis of probable and possible TBM. A similar consideration for possible TBM. This would add clarity on the reliability of the test outcomes.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Assessing novel tests in the diagnosis of tuberculous meningitis.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 26 Sep 2019
Richard Kwizera, College of Health Sciences, Makerere University, Kampala, Uganda

The article is well written with no major flaws.
Response: Thank you.

Regarding the non-TBM category, although 10 patient had cryptococcal meningitis, detail regarding the non-TBM diagnoses is important as this is a control group and a patient without a clinical meningitis may allow for better specificity. I note that some CSF's were acellular, what were these diagnoses? Please provide detail (a table with confirmatory findings for TB such as CXR, abdominal ultrasound, scan findings, CSF findings etc.) regarding the aspects considered to arrive at a diagnosis of probable and possible TBM. A similar consideration for possible TBM. This would add clarity on the reliability of the test outcomes.
Response: A cellular CSF is common in this population with advanced HIV disease even in the setting of a confirmed TB or CM infection (Cresswell, Int J Infect Dis 2018). The not-TBM group scored <6 points on the uniform case definition (n=16) or had a confirmed alternative diagnosis (n=10 with CM).

Reviewer Report 09 September 2019

✔

Maryline Bonnet (iD)
Institute of Research for Development (IRD), Montpellier, France

This manuscript reports the results of a nested study in a large clinical trial, evaluating the accuracy of the CSF LAM for diagnosis of tuberculosis meningitis using both definite TB and composite reference of probable and definite TB based on standard case definitions. The study reports low sensitivity and high specificity and concludes on the modest role of the CSF LAM for diagnosis of TB meningitis. These results are important and the manuscript is well written.

No major comments.

Minor comments:
In the introduction, I suggest the authors adding the recent publication by Siddiqi *et al*.(2019)[1] in their references.

In Results, I would suggest the authors to present the characteristics of patients with diagnosis of probable TB using the uniform case definitions. I am surprised by the low proportion of probable TB as compared to definite TB. It would be interesting to know the score of patients with probable TB using the uniform case definition criteria. Were cerebral imaging criteria used? It would be also interesting to know the proportion of patients that fit the score of possible TB meningitis using the uniform case definitions. It is indeed important to know the proportion of possible TB meningitis cases based on the uniform cases definitions that were finally classified as non TB meningitis for the accuracy analysis.

It would be interesting to know the proportion of patients with TB positive results from another specimen than CSF, which is also an important criteria for diagnosis of probable TB meningitis. One option could be to present the patients' characteristics by definite TB, probable TB and others in Table 1.

How do the authors explain that 17% of patients had unknown outcome of death or alive at discharge? It is quite high in a context of a nested study in a clinical trial.

TB culture was not used, which is a limitation of the accuracy analysis. However, Xpert Ultra has a sensitivity that is very close to culture. This could be mentioned as a limitation.

It would be also very interesting to have the results of the urine LAM if used. In the study by Siddiqui *et al.* the urine LAM had higher sensitivity than the CSF LAM in patients with presumptive TB meningitis.

In the conclusion, the authors could mention the Fuji LAM that is a new LAM POC test that has higher

sensitivity in urine than the determine LAM POC and should also be evaluated for diagnosis of TB meningitis both in urine and CSF.

References

1. Siddiqi OK, Birbeck GL, Ghebremichael M, Mubanga E, Love S, Buback C, Kosloff B, Ayles H, Atadzhanov M, Dheda K, Koralnik IJ: Prospective Cohort Study on Performance of Cerebrospinal Fluid (CSF) Xpert MTB/RIF, CSF Lipoarabinomannan (LAM) Lateral Flow Assay (LFA), and Urine LAM LFA for Diagnosis of Tuberculous Meningitis in Zambia.*J Clin Microbiol*. 2019; **57** (8). PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Clinical research on tuberculosis.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 26 Sep 2019
Richard Kwizera, College of Health Sciences, Makerere University, Kampala, Uganda

This manuscript reports the results of a nested study in a large clinical trial, evaluating the accuracy of the CSF LAM for diagnosis of tuberculosis meningitis using both definite TB and composite reference of probable and definite TB based on standard case definitions. The study reports low sensitivity and high specificity and concludes on the modest role of the CSF LAM for diagnosis of TB meningitis. These results are important and the manuscript is well written.
 Response: Thank you
No major comments.

Minor comments:
Comment: In the introduction, I suggest the authors adding the recent publication by Siddiqi *et al*

.(2019)[1] in their references.
Response: We have now added this suggested reference in the discussion section.

Comment: In Results, I would suggest the authors to present the characteristics of patients with diagnosis of probable TB using the uniform case definitions.
Response: Table 1 has been modified to present the characteristics of patients with diagnosis of definite, probable, possible and not-TBM.

Comment: I am surprised by the low proportion of probable TB as compared to definite TB.
Response: This is likely to be because we used Ultra and not culture or Xpert as the reference standard. We have found Ultra to be better at confirming TBM in our population (Bahr, Lancet ID, 2018)

Comment: Were cerebral imaging criteria used?
Response: Only about 10% (n/N) of the participants had cerebral imaging done as the CT scanner was dysfunctional for part of the study period.

Comment: It would be also interesting to know the proportion of patients that fit the score of possible TB meningitis using the uniform case definitions.
Response: These have been added in Table 1.

Comment: It would be interesting to know the proportion of patients with TB positive results from another specimen than CSF, which is also an important criteria for diagnosis of probable TB meningitis.
Response: 25% of the patients were positive with urine LAM while 20% were positive with urine Ultra.

Comment: One option could be to present the patients' characteristics by definite TB, probable TB and others in Table 1.
Response: Thank you for this suggestion. This has been done in the modified Table 1.

Comment: How do the authors explain that 17% of patients had unknown outcome of death or alive at discharge? It is quite high in a context of a nested study in a clinical trial.
Response: This study population included patients screened for a clinical trial but only a minority were subsequently enrolled into the trial. We endeavoured to follow screen failures through to hospital discharge but this was not possible in all cases.

Comment: TB culture was not used, which is a limitation of the accuracy analysis. However, Xpert Ultra has a sensitivity that is very close to culture. This could be mentioned as a limitation.
Response: We have found Ultra to be more sensitive than culture (70% versus 43% against definite/probable) for the diagnosis of TBM in our population (Bahr, Lancet ID, 2018). However we agree addition to culture would have been positive so this has been added to the limitations.

Comment: It would be also very interesting to have the results of the urine LAM if used. In the study by Siddiqui *et al*. the urine LAM had higher sensitivity than the CSF LAM in patients with presumptive TB meningitis.
Response: 25% of the patients were positive with urine TB-LAM LFA (Alere). The details of the urine TB diagnostics are being analysed currently and will be presented separately.

Comment: In the conclusion, the authors could mention the Fuji LAM that is a new LAM POC test that has higher sensitivity in urine than the determine LAM POC and should also be evaluated for diagnosis of TB meningitis both in urine and CSF.
Response: Thank you for this suggestion. This has been added in the conclusion.

Competing Interests: No competing interests were disclosed.