Accuracy of Lipoarabinomannan and Xpert MTB/RIF Testing in Cerebrospinal Fluid To Diagnose Tuberculous Meningitis in an Autopsy Cohort of HIV-Infected Adults

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Point-of-care tests for tuberculous meningitis (TBM) are needed. We studied the diagnostic accuracy of the lipoarabinomannan (LAM) lateral flow assay (LFA), LAM enzyme-linked immunosorbent assay (ELISA), and Xpert MTB/RIF in cerebrospinal fluid (CSF) in an autopsy cohort of Ugandan HIV-infected adults. We obtained written informed consent postmortem from the next of kin. A complete autopsy was done and CSF obtained. We performed LAM LFA (on unprepared and supernatant CSF after heating and spinning), LAM ELISA, and Xpert MTB/RIF on the CSF samples. Accuracy parameters were calculated for histopathological TBM and also for the composite standard, including Xpert MTB/RIF-positive cases. We tested CSF of 91 patients. LAM LFA had a sensitivity of 75% for definite histopathological TBM, ELISA a sensitivity of 43%, and Xpert MTB/RIF a sensitivity of 100% and specificities of 87%, 91%, and 87%, respectively. LAM LFA had a sensitivity of 50% for definite and probable histopathological TBM, ELISA a sensitivity of 38%, and Xpert MTB/RIF a sensitivity of 86% and specificities of 70%, 91%, and 87%, respectively. LAM LFA had a sensitivity of 68% for the composite standard and ELISA a sensitivity of 48% and specificities of 78% and 98%, respectively. The rapid diagnostic tests detected TBM in 22% to 78% of patients not on anti-TB treatment. Point-of-care tests have high accuracy in diagnosis of TBM in deceased HIV-infected adults. LAM LFA in CSF is a useful additional diagnostic tool.

In 2012, an estimated 8.6 million people developed tuberculosis (TB) worldwide. Of these, 1.1 million were coinfecting with HIV (1). TB-HIV-coinfecting patients have an increased risk of developing extrapulmonary TB (EPTB), including TB meningitis (TBM) (2–4). TBM is the most severe form of EPTB, with in-hospital mortality rates of 13% to 72% (5). Prompt treatment with anti-TB drugs improves patient outcome (6). Therefore, sensitive and rapid diagnostic tests to diagnose TBM are vital.

The diagnosis of TBM is made through microscopic visualization of acid-fast bacilli (AFB) or culturing *Mycobacterium tuberculosis* in cerebrospinal fluid (CSF) (7). The sensitivity of AFB microscopy in CSF is highly variable (0% to 87%) irrespective of HIV coinfection and depends on the case definition, the quantity of CSF examined, and the experience of the technician (5, 8). In resource-limited settings, the clinical use of culture is hindered by limited availability, long turnaround times, laboratory safety issues, and relatively high cost (8). Therefore, in clinical practice, TBM is often diagnosed presumptively on the basis of a combination of clinical, laboratory, and radiological findings (7).

Other technologies have become available to diagnose TB. Lipoarabinomannan (LAM), a *M. tuberculosis* cell wall component, can be detected in multiple bodily fluids, including CSF, of TB patients (9–12). A LAM enzyme-linked immunosorbent assay (ELISA) (Clearview TB ELISA; Alere, Waltham, MA, USA) developed for urinary LAM detection was used in studies of CSF, an off-label use, in 2 South African cohorts of clinical meningitis patients. The studies reported sensitivity of 64% to 69% and spec-

ificity of 62% to 65% for diagnosis of culture- or PCR-positive TBM. Higher sensitivity and specificity were noted in HIV-infected patients with CD4 cell counts below 100 cells/mm³ (11, 13). Recently, a point-of-care lateral flow assay (LFA) was developed (Determine TB LAM; Alere, Waltham, MA, USA) that detects LAM in unprocessed urine (14). This test offers important advantages over the ELISA in terms of speed, simplicity, and costs.

The Xpert *M. tuberculosis*/RIF assay (Cepheid, CA, USA) is a commercial nucleic acid amplification test that uses real-time PCR to detect *M. tuberculosis* and identify rifampin resistance. The World Health Organization recommends the use of Xpert MTB/

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Definite TBM

- Microscopic changes and acid fast bacilli (AFB) on ZN stain OR
- Typical microscopic changes (granulomas and/or meningeal exudate containing lymphocytes and macrophages with epithelioid and Langerhans giant cells) and clinical and/or macroscopic evidence of meningitis and/or TB elsewhere

Probable TBM

- TB elsewhere + 2 of the following: clinical, macroscopic or microscopic changes not typical for but consistent with TBM

Possible TBM

- TB elsewhere + 1 of the following: clinical, macroscopic or microscopic changes not typical for but consistent with TBM

No TBM

FIG 1 Definition of the histopathology reference standard.

RIF rather than conventional microscopy and culture as the initial diagnostic test for CSF specimens in patients suspected to be harboring TBM (15). Xpert MTB/RIF in CSF has sensitivity of 80% and specificity of 99%, based on pooled data from 13 studies, including 709 CSF samples; a minority of these samples were from HIV-infected patients (15–19).

LFA LAM could be the urgently needed rapid test for TBM. However, its use in CSF for the diagnosis of TBM is not yet investigated. Moreover, data on CSF LAM ELISA and Xpert MTB/RIF performance in high-prevalence-TB and -HIV settings are limited. We therefore studied the diagnostic accuracy for TBM of all 3 modalities in an autopsy cohort of HIV-infected adults in Uganda.

MATERIALS AND METHODS

Setting and population. This study was a substudy of an autopsy study that is described in detail elsewhere (20). In brief, we included HIV-infected adults (>18 years old) who died in one of the medicine wards of Mulago Hospital, Kampala, Uganda, during the period from February to June 2013. Written informed consent for study participation was obtained from the next of kin. Postpartum deaths and deaths after trauma were excluded. The HIV serological status was based on documentation in the hospital chart.

Autopsy procedure. CSF was obtained postmortem from the fourth ventricle after opening the skull and carefully lifting the brain. CSF (10 to 15 ml) was collected in a sterile container and stored at -20°C . The macroscopic appearance of the CSF (clear, cloudy, lightly bloodstained, or heavily bloodstained) was noted.

A complete autopsy took place within 4 h after consent was obtained. The autopsy included visual inspection of the spine, routine sampling of the left and right cerebrum, the cerebellum, and the brainstem, and additional sampling of any macroscopically abnormal lesion. The tissue was fixed in 10% formalin. Hematoxylin and eosin (H&E) staining slides were made for each tissue section and analyzed by 2 experienced pathologists. Additional Ziehl-Neelsen (ZN) staining was done on samples with histopathology suggestive of TB. The pathologists reading the slides were blinded to the CSF test results.

CSF LAM testing. (i) **CSF preparation.** Frozen CSF samples were thawed to the ambient temperature and batch tested. To separate antigen-antibody complexes, 1 ml of CSF was heated to 95 to 100°C for 30 min and, after cooling, spun (10,000 rpm) for 15 min. The supernatant was collected. LAM-antigen testing was performed on the supernatant and on an unprepared defrosted sample, referred to here as “unprepared CSF.” The Determine TB LAM and the Clearview TB ELISA were provided by Alere (Waltham, MA, USA).

(ii) **Lateral-flow TB LAM antigen testing.** For each CSF sample, 60 μl of unprepared CSF and 60 μl of supernatant CSF were applied to a LFA test strip. After 25 min, 2 experienced laboratory technicians independently read the test strip by comparing the LFA test result to the manufacturer-supplied reference card (grading intensity from +1 to +5).

(iii) **LAM-ELISA.** Testing procedures were conducted according to the manufacturer’s instructions as follows. Supernatant (0.1 ml) was applied onto a 96-well plate in duplicate for each sample. Immediately after processing, the optical density (OD) was measured at 450 nm. The average OD for each sample was compared to the cutoff (CU) value for positivity or negativity. Per the manufacturer’s instruction, the CU value was determined by adding 0.1 OD units to the average negative-control OD value.

The laboratory technicians were blinded to the autopsy results.

Xpert MTB/RIF assay. One ml of thawed CSF was mixed with 2 ml of test reagent, and the reaction mixture was incubated at room temperature for 15 min. A 2-ml volume of the reaction mixture was transferred to the Xpert MTB/RIF cartridge, and the automated test procedure was initiated according to the manufacturer’s instructions. The automated software reported whether *M. tuberculosis* was detected and, if so, in what quantity, high (cycle threshold [C_T] = <16), medium (C_T = 16 to 22), low (C_T = 23 to 28), or very low (C_T = >28), and whether rifampin resistance was detected.

Reference standard for TBM. The following 5 items were assessed for each patient: (i) clinical symptoms of meningoencephalitis; (ii) macroscopic pus at the brain base and/or intracerebral granuloma; (iii) typical microscopic changes (granulomas and/or meningeal exudate containing lymphocytes and macrophages with epithelioid and Langerhans giant cells); (iv) microscopic changes not typical for but consistent with TBM (inflammatory infiltrate composed of lymphocytes, mononuclear cells, and epithelioid histiocytes, sometimes associated with endarteritis obliterans, but without true granuloma formation); and (v) TB elsewhere.

We defined a histopathology reference standard differentiating definite TBM, probable TBM, possible TBM, and no TBM (Fig. 1). Because of the diagnostic uncertainty, possible TBM cases were excluded from analysis. We calculated the sensitivity, specificity, and positive predictive values and negative predictive values (PPV and NPV, respectively) of LAM LFA, LAM ELISA, and Xpert MTB/RIF for definite TBM and for definite and probable TBM using no TBM as the denominator.

We defined a second, composite standard that included the CSF Xpert MTB/RIF results. Definite TBM was defined as histopathologically definite TB or a positive CSF Xpert MTB/RIF result. The definition for absence of TBM remained unchanged. We calculated the sensitivity, specificity, PPV, and NPV of LAM LFA and LAM ELISA for definite TBM using no TBM as the denominator.

Statistical methods. Sensitivity, specificity, PPV, and NPV are reported with Wilson 95% confidence intervals (95% CI). Comparison of accuracy parameters was done by application of the exact McNemar’s test, with significance defined at a P value of <0.05. Data were analyzed using STATA version 11.0 (Stata Corp., College Station, TX, USA). Study reporting and analysis are consistent with the Standards of the Reporting of Diagnostic Accuracy (STARD) criteria (21).

Ethical approval. The study received ethical approval from the Joint Clinical Research Center Research and Ethics Committee (Uganda), the Mulago Internal Review Board (Uganda), and the Institute of Tropical Medicine Institutional Review Board (Belgium). The study received final

FIG 2 Summary flow chart. *, definite TBM, including all histopathologically definite TBM and all Xpert MTB/RIF-positive patients; CSF, cerebrospinal fluid; n, number; TBM, TB meningitis.

approval and registration by the Uganda National Council of Science and Technology (HS 1300).

RESULTS

During the study period, we performed 96 autopsies and obtained adequate CSF for further testing from 91 patients (Fig. 2). The median age of the patients was 35 years (interquartile range [IQR], 28 to 40), and 57% were female. The median CD4 cell count (available for 45 patients) was 47 cells/mm³ (IQR, 21 to 165), 55 (60%) were on antiretroviral therapy (ART) for a median duration of 49 days (IQR, 21 to 183), and 26 (30%) were on anti-TB treatment for a median duration of 30 days (IQR, 8 to 60). The CSF was clear in 36 (39%) patients, cloudy in 28 (31%), lightly bloodstained in 17 (18%), and heavily bloodstained in 10 (11%).

Histopathological TBM. Histopathologically definite TBM was diagnosed in 8 (9%) patients, 5 of whom had AFB on ZN staining of the brain tissue. Six (7%) patients had probable TBM, 8 (9%) patients possible TBM, and 69 (76%) no TBM (Fig. 2). The

no-TBM group included 11 patients with *Cryptococcus neoformans* meningitis, 2 patients with bacterial meningitis, and 1 patient with candidal meningitis.

Lipoarabinomannan testing using the histopathological reference standard. (i) **LFA in unprepared CSF.** The LFA in unprepared CSF had a sensitivity of 75% (95% CI, 35 to 97) to diagnose definite TBM and of 50% (95% CI, 23 to 77) to diagnose definite and probable TBM using +1 as the cutoff (CU) value for test positivity (Table 1). Specificity was 70% (95% CI, 58 to 79) both for definite TBM and for definite and probable TBM. Increasing the cutoff point for positivity to +2, sensitivity dropped to 38% (95% CI, 9 to 49, $P = 0.25$) for definite TBM and to 29% (95% CI, 8 to 58, $P = 0.25$) for definite and probable TBM. Specificity significantly increased to 87% (95% CI, 79 to 95; $P = 0.0005$).

(ii) **LFA in supernatant CSF.** The LFA in supernatant CSF had a sensitivity of 88% (95% CI, 47 to 100) for definite TBM and of 71% (95% CI, 42 to 92) for definite and probable TBM using +1

TABLE 1 Lipoarabinomannan lateral flow assay, lipoarabinomannan ELISA, and Xpert MTB/RIF accuracy for histopathologically definite TBM and definite and probable TBM^a

Assay and disease category	% sensitivity (95% CI) n/N	% specificity (95% CI) n/N	% PPV (95% CI) n/N	% NPV (95% CI) n/N
Definite TBM				
LFA of unprepared CSF CU +1	75 (35–97) 6/8	70 (59–80) 48/69	22 (11–41) 6/27	96 (87–99) 48/50
LFA of unprepared CSF CU +2	38 (9–49) 3/8	87 (79–95) 60/69	25 (9–53) 3/12	92 (83–97) 60/65
LFA of supernatant CU +1	88 (47–100) 7/8	70 (58–79) 48/69	25 (13–43) 7/28	98 (89–100) 48/49
LFA of supernatant CU +2	75 (41–93) 6/8	83 (72–90) 57/69	33 (16–56) 6/18	97 (88–99) 57/59
LAM-ELISA	43 (10–82) 3/7*	91 (82–96) 63/69	33 (7–70) 3/9	94 (86–98) 63/67
Xpert MTB/RIF	100 (63–100) 8/8	87 (77–93) 60/69	47 (23–72) 8/17	100 (94–100) 60/60
Definite and probable TBM				
LFA of unprepared CSF CU +1	50 (23–77) 7/14	70 (59–80) 48/69	27 (12–48) 7/26	87 (76–94) 48/55
LFA of unprepared CSF CU +2	29 (8–58) 4/14	87 (79–95) 60/69	31 (13–58) 4/13	86 (76–92) 60/70
LFA of supernatant CU +1	71 (42–92) 10/14	70 (58–79) 48/69	32 (19–50) 10/31	92 (82–97) 48/52
LFA of supernatant CU +2	50 (27–73) 7/14	83 (72–90) 57/69	37 (19–59) 7/19	89 (79–95) 57/64
LAM-ELISA	38 (14–68) 5/13	91 (82–96) 63/69	45 (17–77) 5/11	89 (79–94) 63/71
Xpert MTB/RIF	86 (57–98) 12/14	87 (77–93) 60/69	57 (34–78) 12/21	97 (89–99) 60/62

^a CI, confidence interval; n, number of observations; N, total number; PPV, positive predictive value; NPV, negative predictive value; TBM, tuberculous meningitis; LFA, lateral flow assay; CU +1, cutoff point +1 used for lateral flow assay positivity; CU +2, cutoff point +2 used for lateral flow assay positivity; *, one patient excluded because of missing LAM-ELISA results.

as the CU for test positivity. Specificity was 70% (95% CI, 58 to 79) both for definite TBM and for definite and probable TBM. Increasing the CU for positivity to +2, sensitivity dropped to 75% (95% CI, 41 to 93, $P = 1.0$ compared to +1 CU) for definite TBM and to 50% (95% CI, 27 to 73, $P = 0.25$ compared to +1 CU) for definite and probable TBM. Specificity significantly increased to 83% (95% CI, 72 to 90, $P = 0.0039$ compared to +1 CU). Sensitivity and specificity for unprepared versus supernatant CSF were not significantly different for both cutoff points for positivity.

(iii) ELISA. The test accuracy for the LAM ELISA was not significantly different from the highest sensitivity obtained with LFA (88% in supernatant using +1 CU) and the highest specificity obtained with LFA (87% in unprepared CSF +2 CU).

Xpert MTB/RIF using the histopathological reference standard. Xpert MTB/RIF had a sensitivity of 100% (95% CI, 63 to 100) for definite histopathological TBM and of 86% (95% CI, 57 to 98) for definite and probable TBM (Table 1). The 2 Xpert MTB/RIF-negative cases with probable TBM were patients with TB (one with pulmonary TB and one with disseminated TB), not on anti-TB treatment, with symptoms compatible with meningitis, pus at the brain base ($n = 1$), and microscopic changes consistent with TBM (Table 2).

The specificity of Xpert MTB/RIF was 87% (95% CI, 77 to 93) both for definite TBM and for definite and probable TBM. The 9 Xpert MTB/RIF-positive cases with a negative histopathological outcome were all patients with TB but without clinical, macroscopic, or microscopic evidence of TBM (Table 2). None of the CSF samples of these 9 were bloodstained. Xpert MTB/RIF intensity was very low (56%) or low (44%).

Untreated TBM. Nine (69%) patients (95% CI, 42 to 87) with histopathologically definite or probable TBM were not on anti-TB treatment at the moment of death. All 9 had clinical symptoms of meningoencephalitis. Of these, 7 (78%) had a positive CSF Xpert MTB/RIF result, 5 (56%) a positive LFA result in supernatant CSF (using +1 as the CU), 3 (33%) a positive LFA result in unprepared CSF (using +1 as the CU), and 2 (22%) a positive ELISA result.

Lipoarabinomannan testing using the composite reference standard. Definite TBM was diagnosed according to the composite standard in 22 cases: 8 cases with histopathologically definite TBM plus a positive CSF Xpert MTB/RIF result and 14 cases with only a positive CSF Xpert MTB/RIF result (Fig. 2). One patient was found by Xpert MTB/RIF to harbor rifampin-resistant *M. tuberculosis*.

LFA in unprepared CSF. The sensitivity of the LFA in unprepared CSF was 68% (95% CI, 47 to 84) for definite TBM using the +1 CU for test positivity, and the specificity was 78% (95% CI, 66 to 87). Increasing the CU to +2, sensitivity significantly decreased to 41% (95% CI, 23 to 61, $P = 0.03$) and specificity significantly increased to 93% (95% CI, 84 to 97, $P = 0.004$) (Table 3). Of the 4 patients with a positive CSF LAM result (+2 CU) but a negative Xpert MTB/RIF result, one had TB-adenitis without clinical or macro- or microscopic results suggestive of TBM. The remaining 3 did not have TB or cryptococcal, candidal, or bacterial meningitis found during the complete autopsy.

LFA in supernatant CSF. The LFA in supernatant CSF had a sensitivity for definite TBM of 77% (95% CI, 57 to 90) using the +1 CU for test positivity and a specificity of 78% (95% CI, 65 to 86). Increasing the CU to +2, sensitivity decreased to 59% (95% CI, 39 to 77, $P = 0.125$) and specificity significantly increased to 90% (95% CI, 79 to 95, $P = 0.0156$) (Table 3). Sensitivity and

specificity in unprepared CSF versus supernatant CSF were not significantly different irrespective of the cutoff point for positivity. Of the 6 patients with a positive supernatant LAM (+2 CU) result but a negative Xpert MTB/RIF result, 4 had TB elsewhere and one had both TB elsewhere and cryptococcal meningitis found in a complete autopsy. Four of them were on anti-TB treatment, with the duration ranging from 8 days to 3 months.

ELISA. LAM ELISA accuracy was not significantly different from the highest sensitivity obtained with LFA (77% with supernatant using +1 CU) and the highest specificity obtained with LFA (93% with unprepared CSF at +2 CU).

Tables S1 to S6 in the supplemental material present the accuracy results for patients with a CD4 count below 100 cells/mm³ ($n = 29$), patients not on anti-TB treatment ($n = 61$), and patients with clinical symptoms of meningoencephalitis ($n = 40$).

For those with symptoms of meningoencephalitis, the LFA PPV for histopathologically definite TBM and for definite and probable TBM were a maximum of 71% (95% CI, 36 to 92, supernatant CSF, +2 CU) and a maximum of 75% (95% CI, 41 to 93, supernatant CSF, +2 CU), respectively. The LFA PPV for the composite reference was 89% (95% CI, 57 to 98, unprepared CSF, +1 CU). Other accuracy parameters were similar to those for the whole study cohort.

The accuracy of the tests that included possible TBM in the histopathological gold standard was calculated. As expected, the specificity was lower, but none of the outcomes were significantly different from those seen using definite TBM and definite and probable TBM as the gold standard (see Table S7 in the supplemental material).

DISCUSSION

This study had 3 major findings. First, we showed that the off-label use of lipoarabinomannan LFA in CSF has a diagnostic yield that is at least comparable to that of the lipoarabinomannan ELISA (11, 13). Second, results of lipoarabinomannan testing in CSF were highly specific in our cohort of deceased HIV-infected adults. Last, our report contributes to the limited body of evidence available on the use of Xpert MTB/RIF in analysis of the CSF of HIV-coinfected patients and shows that Xpert MTB/RIF sensitivity in HIV-infected patients, using a histopathological gold standard, is similar to that reported in HIV-uninfected individuals (15).

The main advantages of the LAM LFA are its simplicity, low cost (\$1.50 per test), and rapidity (25 min per test). The LFA is a point-of-care diagnostic test that can be used at the bedside. Its performance in CSF may seem modest; depending on the cutoff point, the preparation of the CSF, and the gold standard used, a wide sensitivity range (29% to 71%) and a relative modest PPV range (54% to 68%) were observed. Nevertheless, LFA sensitivity is substantially better than the literature-reported sensitivity (10% to 20%) for AFB smear diagnosis, a modality still widely used (17, 22, 23). Xpert MTB/RIF has a higher sensitivity but is not yet universally available and has technical and logistic requirements that are not met in every setting (15, 24). We observed a specificity of LAM LFA of as high as 93%. In patients with symptoms of meningitis, the PPV was as high as 75%, indicating its clinical potential as a rule-in test. False-positive results may be partially explained by the inclusion of actual TBM cases that were missed using the histopathological standard, as specificity improved when Xpert MTB/RIF-positive cases were included. Cross-reac-

TABLE 2 Detailed overview of the histopathologically definite, histopathologically probable, and composite standard definite TBM cases^a

Case category	Result(s) for histopathological standard criterion				Lipoarabinomannan testing result(s)					Patient characteristic and result(s)			
	Clinical	Macroscopic	Microscopic 1	Microscopic 2	TB elsewhere	Xpert result/intensity	LFA of unprepared CSF/intensity	LFA of supernatant/intensity	ELISA/OD	CD4 cell count (cells/mm ³)	On ART/duration (no. of days)	On TB-tx/duration (no. of days)	CSF aspect
Histopathologically definite TBM	Y	N	Y, AFB+	NA	Y	P/medium	P/+1	P/+2	N	63	N	N	Clear
	Y	Y	Y, AFB-	NA	Y	P/medium	P/+2	P/+3	N	30	Y/-	-	Clear
	Y	N	Y, AFB-	NA	Y	P/low	P/+1	P/+2	N	-	Y/183	N	Clear
	Y	Y	Y, AFB+	NA	Y	P/low	P/+1	P/+3	-	-	N	Y/1	Lightly BS
	Y	N	Y, AFB-	NA	Y	P/low	N	P/+1	P/0.281*	295	Y/-	Y/1	Lightly BS
	Y	N	Y, AFB+	NA	Y	P/low	P/+3	P/+4	P/0.519*	-	N	N	Heavily BS
Histopathologically probable TBM	N	N	Y, AFB+	NA	Y	P/very low	N	N	N	-	N	N	Clear
									P/0.283**	-	Y/196	Y/5	Clear
	Y	N	N	Y	Y	P/low	P/+5	P/+5	P/1.17**	24	Y/21	Y/42	Cloudy
	Y	Y	N	Y	Y	P/low	N	P/+1	N	172	N	N	Clear
	Y	N	N	Y	Y	P/low	N	N	N	44	N	N	Clear
	Y	Y	N	Y	Y	P/low	N	P/+1	P/0.556*	101	Y/-	N	Lightly BS
Positive Xpert result and histopathologically possible TBM	Y	N	N	Y	Y	N	N	N	N	155	Y/140	N	Clear
													Lightly BS
Positive Xpert result and histopathological absence of TBM	N	N	N	N	Y	P/very low	N	N	N	-	N	N	Lightly BS
	N	N	N	N	Y	P/low	P/+3	P/+3	P/0.344*	10	N	N	Clear
	N	N	N	N	Y	P/low	P/+2	P/+3	P/0.447**	21	Y/84	N	Cloudy
	N	N	N	N	Y	P/low	P/+1	P/+2	N	-	N	N	Clear
	N	N	N	N	Y	P/low	P/+4	P/+4	P/0.6**	-	N	N	Clear
	N	N	N	N	Y	P/very low	P/+2	P/+3	P/0.286*	-	Y/-	N	Clear
	N	N	N	N	Y	P/very low	P/+4	P/+5	P/0.739**	29	N	Y/2	Cloudy
	N	N	N	N	Y ^b	P/very low	P/+1	P/+1	N	2	N	N	Cloudy
	N	N	N	N	Y	P/very low	P/+1	P/+1	N	12	Y/21	N	Cloudy
	N	N	N	N	Y	P/very low	N	N	N	13	Y/14	N	Clear
													Clear
													Clear

^a Clinical, symptoms of meningoencephalitis; Macroscopic, pus at the brain base and/or intracerebral granulomas; Microscopic 1, typical microscopic changes (granulomas and/or meningeal exudate containing lymphocytes and macrophages with epithelioid and Langhans giant cells); Microscopic 2, changes not typical of but consistent with TBM; LFA, lateral flow assay; OD, optical density; ART, antiretroviral therapy; TB-tx, antituberculosis treatment; CSF, cerebrospinal fluid; Y, yes; N, no; AFB, acid-fast bacilli; P, positive; N, negative; -, information missing; NA, not applicable; BS, bloodstained; *, optical density cutoff (ODCU) for positivity value of 0.2615; **, ODCU for positivity value of 0.280; ***, ODCU for positivity value of 0.291.

^b Symptoms explained by cryptococcal meningitis.

TABLE 3 Lipoarabinomannan lateral flow assay and lipoarabinomannan ELISA accuracy for composite standard definite TBM^a

Assay	% sensitivity (95% CI) <i>n/N</i>	% specificity (95% CI) <i>n/N</i>	% PPV (95% CI) <i>n/N</i>	% NPV (95% CI) <i>n/N</i>
LFA of unprepared CSF CU +1	68 (47–84) 15/22	78 (66–87) 47/60	54 (36–70) 15/28	91 (62–98) 47/54
LFA of unprepared CSF CU +2	41 (23–61) 9/22	93 (84–97) 56/60	69 (42–87) 9/13	81 (70–89) 56/69
LFA of supernatant CU +1	77 (57–90) 17/22	78 (66–87) 47/60	57 (39–73) 17/30	90 (79–96) 47/52
LFA of supernatant CU +2	59 (39–77) 13/22	90 (80–95) 54/60	68 (46–85) 13/19	86 (75–92) 54/63
LAM-ELISA	48 (28–68) 10/21*	98 (91–100) 59/60	91 (62–98) 10/11	84 (74–91) 59/70

^a CI, confidence interval; *n*, number of observations; *N*, total number; PPV, positive predictive value; NPV, negative predictive value; TBM, tuberculous meningitis; LFA, lateral flow assay; CU +1, cutoff point +1 used for lateral flow assay positivity; CU +2, cutoff point +2 used for lateral flow assay positivity; *, one patient excluded because of missing LAM-ELISA results.

tivity of the lipoarabinomannan assay with antigens from *Candida* sp. or *Cryptococcus neoformans* has been hypothesized as the reason for false-positive lipoarabinomannan ELISA results in urine but was not shown in our study (25, 26).

LFA test accuracy may be further improved by CSF preparation and/or assay alterations. We used supernatant in order to separate LAM antigen-antibody complexes, which seemed to improve sensitivity, although not significantly. Further study of CSF preparation methods to maximize test accuracy should be undertaken, but the simplicity is an important advantage of the LAM LFA and should be considered in study design.

Our study of 91 autopsies had 8 definite and 6 probable histopathological TBM cases, and our report contributes to the limited data on Xpert MTB/RIF performance in CSF of HIV-infected patients (17, 27). The largest study on the accuracy of Xpert MTB/RIF in CSF was in 178 South-African HIV-infected adults and reported a sensitivity of 51% (95% CI, 35 to 86) using culture or Amplicor PCR positivity as the gold standard (17). We found a sensitivity range of 86% to 100%. This difference may be explained by the nature of our study population (severely immunocompromised patients), by the mode by which CSF was obtained (by extraction from the 4th ventricle instead of by lumbar puncture), and by the gold standard that was used (histopathology versus culture and PCR).

The South African study showed a significant increase in the sensitivity of Xpert MTB/RIF to 82% using a CSF pellet after centrifugation (17). Also, subjecting the CSF to vortex mixing before application in the cartridge has been reported to increase sensitivity (27). Optimization of CSF preparation should be investigated further in order to maximize the Xpert MTB/RIF diagnostic yield. Blood has been postulated to be a PCR inhibitor, limiting the sensitivity of Xpert MTB/RIF. We observed no PCR inhibition, despite inclusion of 27 bloodstained CSF samples. This is in line with a study that included 1,175 extrapulmonary samples (none CSF) reporting no difference in inhibition of Xpert MTB/RIF in bloodstained versus clear samples (28).

We found Xpert MTB/RIF specificity lower than that reported by others (15–19, 29). We obtained the CSF at the brain base, an anatomic location commonly affected by TBM (30). Moreover, Xpert MTB/RIF has a lower limit of detection of 80 to 100 CFU of *M. tuberculosis* per ml of CSF (17). The level of mycobacterial load at which histopathological changes consistent with TBM appear in severely immunosuppressed HIV-infected patients like the ones we studied is not known. One might postulate that, in our “false-positive” Xpert MTB/RIF cases, histopathological changes had not (yet) appeared due to impaired inflammatory responses, despite mycobacteria being present, and therefore that those cases actually were true positives. In support of this hypothesis, all Xpert

MTB/RIF-positive patients in our study had disseminated TB beyond the lung parenchyma. Another reason for the lower specificity may have been a sampling error with respect to the brain lesions, despite our thorough sampling protocol.

In this population of severely immunosuppressed HIV-infected adults, CSF Xpert MTB/RIF detected 78% of untreated definite and probable TBM cases and the LAM LFA in supernatant 55%. All of these patients had symptoms of meningoencephalitis and likely would have undergone a lumbar puncture as part of their clinical assessment. Moreover, subanalysis of those with clinical symptoms of meningoencephalitis showed similar sensitivity and specificity values with increased PPV. This indicates the potential clinical benefit of these rapid tests in hospitalized, severely immunosuppressed HIV-infected patients.

Our study had several shortcomings. We performed the diagnostic tests in a deceased, HIV-positive population irrespective of meningeal symptoms and obtained the CSF from the 4th ventricle. This has to be taken into account when translating our findings to a clinical setting. Moreover, we did not perform mycobacterial cultures of the CSF. This hinders interpretation of the Xpert MTB/RIF-positive, histopathology-negative cases and complicates comparison with other studies. Finally, we relied on the inpatient ward charts for clinical information, as the patients were not prospectively enrolled while alive. This prevented us from conducting subgroup analysis at different CD4 strata.

We have studied the off-label use of the lipoarabinomannan lateral flow assay to diagnose TBM in an autopsy cohort of HIV-infected adults. The assay is a rapid, point-of-care test and had reasonable diagnostic accuracy. Further evaluation of this promising point-of-care test in a clinical setting is warranted to evaluate its diagnostic potential in a population with high TB-HIV coinfection. Moreover, we showed excellent sensitivity of Xpert MTB/RIF for diagnosis of TBM using CSF from a population of severely immunosuppressed HIV-infected adults.

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REFERENCES

- World Health Organization. 2013. Global tuberculosis report 2013. World Health Organization, Geneva, Switzerland. http://apps.who.int/iris/bitstream/10665/91355/1/9789241564656_eng.pdf.
- Wilkinson D, Moore DA. 1996. HIV-related tuberculosis in South Africa—clinical features and outcome. *S Afr Med J* 86:64–67.
- Yang Z, Kong Y, Wilson F, Foxman B, Fowler AH, Marrs CF, Cave MD, Bates JH. 2004. Identification of risk factors for extrapulmonary tuberculosis. *Clin Infect Dis* 38:199–205. <http://dx.doi.org/10.1086/380644>.
- Azuaje C, Fernandez Hidalgo N, Almirante B, Martin-Casabona N, Ribera E, Diaz M, Prats G, Pahissa A. 2006. Tuberculous meningitis: a comparative study in relation to concurrent human immunodeficiency virus infection. *Enferm Infecc Microbiol Clin* 24:245–250. (In Spanish.) [http://dx.doi.org/10.1016/S0213-005X\(06\)73770-3](http://dx.doi.org/10.1016/S0213-005X(06)73770-3).
- Marais S, Pepper DJ, Marais BJ, Török ME. 2010. HIV-associated tuberculous meningitis—diagnostic and therapeutic challenges. *Tuberculosis (Edinb)* 90:367–374. <http://dx.doi.org/10.1016/j.tube.2010.08.006>.
- Verdon R, Chevrete S, Laissy JP, Wolff M. 1996. Tuberculous meningitis in adults: review of 48 cases. *Clin Infect Dis* 22:982–988. <http://dx.doi.org/10.1093/clinids/22.6.982>.
- Marais S, Thwaites G, Schoeman JF, Török ME, Misra UK, Prasad K, Donald PR, Wilkinson RJ, Marais BJ. 2010. Tuberculous meningitis: a uniform case definition for use in clinical research. *Lancet Infect Dis* 10: 803–812. [http://dx.doi.org/10.1016/S1473-3099\(10\)70138-9](http://dx.doi.org/10.1016/S1473-3099(10)70138-9).
- Ho J, Marais BJ, Gilbert GL, Ralph AP. 2013. Diagnosing tuberculous meningitis—have we made any progress? *Trop Med Int Health* 18:783–793. <http://dx.doi.org/10.1111/tmi.12099>.
- Mishra AK, Driessen NN, Appelmek BJ, Besra GS. 2011. Lipoarabinomannan and related glycoconjugates: structure, biogenesis and role in Mycobacterium tuberculosis physiology and host-pathogen interaction. *FEMS Microbiol Rev* 35:1126–1157. <http://dx.doi.org/10.1111/j.1574-6976.2011.00276.x>.
- Sada E, Aguilar D, Torres M, Herrera T. 1992. Detection of lipoarabinomannan as a diagnostic test for tuberculosis. *J Clin Microbiol* 30:2415–2418.
- Patel VB, Bhigjee AI, Paruk HF, Singh R, Meldau R, Connolly C, Ndung'u T, Dheda K. 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. <http://dx.doi.org/10.1186/1743-8454-6-13>.
- Dheda K, Davids V, Lenders L, Roberts T, Meldau R, Ling D, Brunet L, van Zyl Smit R, Peter J, Green C, Badri M, Sechi L, Sharma S, Hoelscher M, Dawson R, Whitelaw A, Blackburn J, Pai M, Zumla A. 2010. Clinical utility of a commercial LAM-ELISA assay for TB diagnosis in HIV-infected patients using urine and sputum samples. *PLoS One* 5:e9848. <http://dx.doi.org/10.1371/journal.pone.0009848>.
- Patel VB, Singh R, Connolly C, Kasprowitz V, Zumla A, Ndung'u T, Dheda K. 2010. Comparison of a clinical prediction rule and a LAM antigen-detection assay for the rapid diagnosis of TBM in a high HIV prevalence setting. *PLoS One* 5:e15664. <http://dx.doi.org/10.1371/journal.pone.0015664>.
- Nakiyingi L, Moodley VM, Manabe YC, Nicol MP, Holshouser M, Armstrong DT, Zemanay W, Sikhondze W, Mbabazi O, Nonyane BA, Shah M, Joloba ML, Alland D, Ellner JJ, Dorman SE. 2014. Diagnostic accuracy of a rapid urine lipoarabinomannan test for tuberculosis in HIV-infected adults. *J Acquir Immune Defic Syndr* 66:270–279. <http://dx.doi.org/10.1097/QAI.0000000000000151>.
- World Health Organization. 2013. Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children. Policy update. World Health Organization, Geneva, Switzerland. http://apps.who.int/iris/bitstream/10665/112472/1/9789241506335_eng.pdf.
- Causse M, Ruiz P, Gutierrez-Aroca JB, Casal M. 2011. Comparison of two molecular methods for rapid diagnosis of extrapulmonary tuberculosis. *J Clin Microbiol* 49:3065–3067. <http://dx.doi.org/10.1128/JCM.00491-11>.
- Patel VB, Theron G, Lenders L, Matinyena B, Connolly C, Singh R, Coovadia Y, Ndung'u T, Dheda K. 2013. Diagnostic accuracy of quantitative PCR (Xpert MTB/RIF) for tuberculous meningitis in a high burden setting: a prospective study. *PLoS Med* 10:e1001536. <http://dx.doi.org/10.1371/journal.pmed.1001536>.
- Tortoli E, Russo C, Piersimoni C, Mazzola E, Dal Monte P, Pascarella M, Borroni E, Mondo A, Piana F, Scarparo C, Coltellà L, Lombardi G, Cirillo DM. 2012. Clinical validation of Xpert MTB/RIF for the diagnosis of extrapulmonary tuberculosis. *Eur Respir J* 40:442–447. <http://dx.doi.org/10.1183/09031936.00176311>.
- Zeka AN, Tasbakan S, Cavusoglu C. 2011. Evaluation of the GeneXpert MTB/RIF assay for rapid diagnosis of tuberculosis and detection of rifampin resistance in pulmonary and extrapulmonary specimens. *J Clin Microbiol* 49:4138–4141. <http://dx.doi.org/10.1128/JCM.05434-11>.
- Cox JA, Lukande RL, Kalungi S, Van Marck E, Van de Vijver K, Kambugu A, Nelson AM, Manabe YC, Colebunders R. 2014. Needle autopsy to establish the cause of death in HIV-infected hospitalized adults in Uganda: a comparison to complete autopsy. *J Acquir Immune Defic Syndr* 67:169–176. <http://dx.doi.org/10.1097/QAI.0000000000000290>.
- Bossuyt PM, Reitsma JB, Bruns JJ, Gatsonis CA, Glasziou PP, Irwig LM, Moher D, Rennie D, de Vet HC, Lijmer JG. 2003. The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. *Ann Intern Med* 138:W1–W12. <http://dx.doi.org/10.7326/0003-4819-138-1-200301070-00012-w1>.
- Thwaites G, Chau TT, Mai NT, Drobniewski F, McAdam K, Farrar J. 2000. Tuberculous meningitis. *J Neurol Neurosurg Psychiatry* 68:289–299. <http://dx.doi.org/10.1136/jnnp.68.3.289>.
- Thwaites G, Chau TT, Farrar JJ. 2004. Improving the bacteriological diagnosis of tuberculous meningitis. *J Clin Microbiol* 42:378–379. <http://dx.doi.org/10.1128/JCM.42.1.378-379.2004>.
- World Health Organization. 2014. WHO monitoring of Xpert MTB/RIF roll-out. World Health Organization, Geneva, Switzerland. <http://who.int/tb/laboratory/mtbrifrollout/en/>.
- Kroidl I, Clowes P, Mwakyelu J, Maboko L, Kiangi A, Rachow A, Reither K, Jung J, Nsojo A, Saathoff E, Hoelscher M. 2014. Reasons for false-positive lipoarabinomannan ELISA results in a Tanzanian population. *Scand J Infect Dis* 46:144–148. <http://dx.doi.org/10.3109/00365548.2013.853133>.
- Boehme C, Molokova E, Minja F, Geis S, Loscher T, Maboko L, Koulchin V, Hoelscher M. 2005. Detection of mycobacterial lipoarabinomannan with an antigen-capture ELISA in unprocessed urine of Tanzanian patients with suspected tuberculosis. *Trans R Soc Trop Med Hyg* 99:893–900. <http://dx.doi.org/10.1016/j.trstmh.2005.04.014>.
- Nhu NT, Heemskerk D, Thu do DA, Chau TT, Mai NT, Nghia HD, Loc PP, Ha DT, Merson L, Thinh TT, Day J, Chau N, Wolbers M, Farrar J, Caws M. 2014. Evaluation of GeneXpert MTB/RIF for diagnosis of tuberculous meningitis. *J Clin Microbiol* 52:226–233. <http://dx.doi.org/10.1128/JCM.01834-13>.
- Scott LE, Beylis N, Nicol M, Nkuna G, Molapo S, Berrie L, Duse A, Stevens WS. 2014. Diagnostic accuracy of Xpert MTB/RIF for extrapulmonary tuberculosis specimens: establishing a laboratory testing algorithm for South Africa. *J Clin Microbiol* 52:1818–1823. <http://dx.doi.org/10.1128/JCM.03553-13>.
- Denkinger CM, Pai M. 2014. Using cerebrospinal fluid for the diagnosis of tuberculous meningitis with GeneXpert. *Eur Respir J* 44:1095–1096. <http://dx.doi.org/10.1183/09031936.00106914>.
- Dastur DK, Lalitha VS, Udani PM, Parekh U. 1970. The brain and meninges in tuberculous meningitis-gross pathology in 100 cases and pathogenesis. *Neurol India* 18:86–100.