

Performance of urinary diagnostic tests in inpatients treated for tuberculosis in a referral hospital in Rwanda

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Abstract

Background

Current diagnostic tests for tuberculosis (TB) among patients hospitalized in resource-poor settings are suboptimal. Sensitivity of the Determine™ TB LAM lateral flow Lipoarabinomannan test (LF-LAM) in urine, a low-cost point-of-care test, is too low in outpatients, but may be adequate in HIV co-infected TB inpatients.

Methods

Adult inpatients starting TB treatment at Kigali University Teaching Hospital were prospectively enrolled. Diagnostic workup for TB included sputum for auramine phenol microscopy and Xpert MTB/RIF, urine for Xpert MTB/RIF and LF-LAM assays, HIV testing and medical imaging.

Results

From November 2016 to September 2017, 114 patients with urine LF-LAM results, 57 (50%) HIV co-infected, were included. TB was confirmed by sputum auramine microscopy in 51 (45%), by sputum Xpert MTB/RIF in 46 (40%), by urine LF-LAM in 56 (46%) and by urine Xpert MTB/RIF in 17 (19%). All urine Xpert MTB/RIF positive samples were also LF-LAM positive. In addition to sputum analysis, urine LF-LAM increased diagnostic yield from 61/114 (54%) to 86/114 (75%). A positive urine LF-LAM test was not associated with HIV co-infection.

Conclusion

In hospitalized TB patients, irrespective of HIV status, urine LF-LAM provides a useful diagnostic test in addition to sputum samples. Sensitivity of urine Xpert MTB/RIF is disappointing and provides no additional sensitivity beyond urine LF-LAM.

Introduction

Sputum microscopy for Acid Fast Bacilli (AFB) with Ziehl-Neelsen stain, or the more sensitive and rapid auramine stain, is still the mainstay of TB diagnosis in much of sub-Saharan Africa, but confirms diagnosis in only half of patients suspected of having TB. HIV co-infection amplifies both emergence and dissemination of TB disease, often with low sputum mycobacterial load [1]. Sputum mycobacterial culture in liquid medium (Mycobacterial Growth Indicator Tube, MGIT) is the current diagnostic gold standard, and

is still too slow to be clinically useful in severe disease. Automated molecular techniques such as Xpert MTB/RIF can be tested rapidly, detect most rifampicin resistant strains and perform almost as well as mycobacterial culture but are relatively expensive and require sophisticated equipment [2]. Although the Xpert MTB/RIF test has proven its value in diagnosing TB infection in sputum, lymph node aspirate and tissue biopsy, test sensitivity is disappointing in body fluids such as pleural, pericardial, cerebrospinal fluids and ascites [3]. Detection of MTB by Xpert MTB/RIF in a centrifuged urine precipitate has shown promise among hospitalized HIV co-infected patients, but sensitivity is highest when patients are severely immune compromised [4].

Another new diagnostic technique detects lipo-arabinomannan (LAM), which is a water-soluble lipopolysaccharide component of the mycobacterial wall, in urine [4] but LAM is not specific for *Mycobacterium tuberculosis* [5]. In blood of patients with active TB, it forms immune complexes with LAM antibodies, and only free LAM molecules can pass through the glomerulus and become detectable in urine. Two key studies using a LAM antigen capture concentration method from urine samples have shown a high sensitivity in patients with active TB [6, 7]. Urinary LAM concentrations may be relative to the mycobacterial load, and are substantially higher in TB/HIV co-infected patients [7, 8]. Tests using an LAM ELISA technique also showed relatively high sensitivity and specificity [9]. The “Determine™ TB LAM” assay (Alere Inc.) is a lateral flow LAM (LF-LAM) test conceived as a point-of-care (POC) format that requires only a drop of urine and can be read within 30 minutes. Unfortunately, its sensitivity is insufficient to be clinically useful in outpatients [10, 11]. Specificity has increased in its format revised in 2016 that required a more prominent color bar reaction for a positive result, at the expense of sensitivity [4]. The LF-LAM test has been proposed as a rapid diagnostic test for TB among hospitalized, severely immune compromised HIV co-infected patients with suspected extensive TB disease who are unable to expectorate. However, test sensitivity never exceeded 60% [4, 12–14]. Infection with non-tuberculous mycobacteria, such as *Mycobacterium avium* complex (MAC), may also lead to false-positive LAM results in that target population [Reference: e.g. <https://doi.org/10.1016/j.ijid.2022.02.046>].

None of the current sputum-based diagnostic tests are sensitive enough to avoid the need for empirical TB treatment [15]. Combining these two novel urine-based techniques with sputum auramine microscopy and with sputum Xpert MTB/RIF might considerably increase diagnostic confirmation among hospitalized HIV co-infected patients with advanced TB disease [12, 13]. However, performance of these new techniques has not been extensively evaluated among HIV uninfected TB patients [7].

In Kigali, Rwanda, patients hospitalized for TB are often found to have disseminated TB imaging, irrespective of their HIV status [16]. The new urinary diagnostic tests for TB have been shown to perform well in patients with mycobacteremia and with disseminated

TB disease [17]. Therefore, the urine LF-LAM test may be a useful addition to speed up diagnostic confirmation and subsequent treatment for hospitalized patients. This study sought to determine the additional diagnostic benefit of urinary LF-LAM and Xpert in hospitalized patients with and without HIV.

Patients and method

Study design, population and site

This was a prospective cohort study of inpatients with confirmed or probable TB in the Internal Medicine wards of the Centre Hospitalier Universitaire de Kigali (CHUK), Rwanda. Diagnosis of TB was defined as: visualization of acid fast bacilli (AFB) by auramine microscopy and/or positive Xpert MTB/RIF from a sputum or lymph node aspirate (“confirmed TB”), or a composite definition combining clinical, radiographic and sonographic findings with empirical treatment started by the treating team (“probable TB”). The latter group comprised clinical elements such as patients with cryptococcus antigen (CrAg) negative mononuclear meningitis and patients with longstanding fever not responding to standard antibiotics for pulmonary infection in the presence of radiologic signs of pathology compatible with TB [16]. Consecutive, consenting hospitalized adult patients were recruited upon starting anti-TB treatment in hospital. Collected data included gender, HIV-1 status, CD4 count, pathological findings on chest x-ray (CXR) and abdominal ultrasound (US), and results from a set of four diagnostic methods to confirm mycobacterial infection. In expectorating patients, one or more sputum samples were collected for microscopy of AFB after auramine staining, and for MTB DNA detection using Xpert MTB/RIF. In addition, for each patient two urine samples (10ml and 40ml) were collected during the first week of TB treatment initiation and stored at -20°C for a maximum of 6 months, for determination of LAM using the second generation “Determine™ TB LAM” (Alere Inc.) lateral flow strip test (LF-LAM) on 60µL of pipetted urine. The LF-LAM strip was read after 25 minutes of reaction time, and interpreted according to the manufacturer’s instructions using an interpretation card alongside the LF-LAM test in indirect daylight conditions. Reading of the intensity of the reaction line was done by two independent persons, and graded from 0 to 4. A simultaneously obtained 40ml urine sample was centrifuged at 5000 RPM for 15 minutes, and the precipitate resuspended in 50µL of phosphate buffered saline for analysis by Xpert MTB/RIF (Cepheid Inc, Sunnyvale, CA USA), identical to the method described by Lawn *et al.* [4]. Patients were excluded if they had been treated for TB in the preceding 24 months, or if another diagnosis was established as the more likely cause of their illness during the follow-up period.

Disease status definitions

The main clinical TB categories (pulmonary TB: PTB; extrapulmonary TB: EPTB; concurrent pulmonary/extrapulmonary TB: PTB/EPTB) were established by current standards. Miliary TB was classified as concurrent PTB/EPTB, as described in our previous work [16]. Disseminated TB was defined based upon imaging data, without confirmation by tissue biopsy, bone marrow aspiration or blood culture. TB was disseminated when at least two noncontiguous anatomical sites identified on compounded CXR and US data, showed structural pathology compatible with TB, with at least one above, and one below the diaphragm. Miliary TB, and TB meningitis associated with any other noncontiguous TB focus were also considered as disseminated TB.

Data Analysis

Data were analyzed using Epi-info and Stata. Univariate analysis was done with Chi-square (Mantel Haenszel correction of Fisher exact) test for categorical variables, and Anova or Mann-Whitney-Wilcoxon nonparametric test comparing means of continuous variables, using a $p < 0.05$ as the threshold of significance.

Ethical aspects

Permission for this study was obtained from CHUK Research Ethics Committee, the Rwandan School of Medicine Ethics Committee, and the Institutional Review Board, Institute of Tropical Medicine, Antwerp, and the University of Antwerp, Belgium provided approval. Patients were counseled regarding the objectives of the study, and gave informed consent before inclusion in the study. Patient data obtained from the medical file, laboratory repository, TB registers and medical imaging protocols were noted down on a specific clinical record form, and transferred to a study database by the principal investigator and the data managers who had exclusive access to the data. All data were disassociated from patient identifiers. Results of urine LF-LAM and urine Xpert MTB/RIF tests had no bearing on the clinical decision to treat for TB.

Results

From November 2016 till October 2017, 120 patients were included in the study. Six were discarded because of another main diagnosis [2], violations of inclusion criteria [2], or lacking a urine sample [2]. Among 114 patients retained for analysis, 74 (65%) were male, 57 (50%) were HIV co-infected, 38 (33%) had isolated PTB, 47 (41%) concurrent PTB/EPTB, and 29 (25%) had isolated EPTB. CD4 count was $< 50/\mu\text{L}$ in 13/54 (24%) HIV co-infected patients. Abnormalities compatible with TB etiology were seen on chest x-ray in 95/104 (91%) and on ultrasound in 57/99 (58%). TB was disseminated in 46 (40%)

patients. LAM urine samples were obtained in the first three days after start of TB treatment in 108 patients, at day 8 in 3, and at day 15 or later in 3.

Sputum auramine microscopy was positive in 51/103 (50%), sputum Xpert MTB/RIF in 46/83 (55%), urine LF-LAM in 56/114 (49%) and urine Xpert MTB/RIF in 16/83 (19%) (Figure 1). All 16 positive urine Xpert MTB/RIF samples were also positive by LF-LAM. TB diagnosis was confirmed by at least one of the four microbiological detection methods in 86/114 (75%) patients. Adding the LF-LAM test increased diagnostic confirmation by 25/114 (21%) compared with the compounded sputum auramine/sputum Xpert MTB/RIF (61/114, 54%).

Figure: Relative contribution of sputum and urine tests to confirm TB diagnosis in 114 hospitalized patients on TB treatment

A positive urine LF-LAM result was not associated with a particular clinical TB definition: 19/38 (50%) in PTB, 11/29 (38%) in EPTB and 26/47 (55%) in PTB/EPTB ($p > 0.05$). Urine LF-LAM grade readings were significantly higher in patients with a positive urine XpertMTB/RIF test, with a positive auramine sputum sample, and with disseminated TB, but not with HIV co-infection (Table 1).

Performance of four TB diagnostic tests in relation with TB/HIV co-infection is shown in Table 2. A positive auramine sputum test and positive urine Xpert MTB/RIF test were associated with HIV co-infection, but a positive LF-LAM result was not.

Discussion

This was the first pilot study in Rwanda to investigate the contribution of LF-LAM and Xpert MTB/RIF in urine to corroborate TB diagnosis. It was not designed as a validation study of LF-LAM against a bacterial gold standard test for TB, but rather to assess the LAM test potential to confirm diagnosis in patients who were put on TB treatment during hospitalization. Contrary to most of the studies on new TB diagnostics conducted in sub-Saharan Africa so far, this study included both HIV uninfected patients as well as patients with a TB diagnosis made on clinical grounds only [18, 19].

Measuring the real-world performance of a new diagnostic test for TB on a clinical definition alone, or in comparison with a poorly sensitive bacteriological gold standard, is tricky [20]. Performing CXR and US may be a more sensitive, but admittedly less specific diagnostic method for TB disease [16, 21]. Our study results suggest that, in a setting highly endemic for TB where test specificity is of lesser concern, the current LF-LAM test could serve as an additional and useful test in hospitalized patients already suspected of having TB on clinical grounds. Reducing the time lapse to treatment decision compared with sputum tests and medical imaging may be its main advantage [22]. Although the

current LF-LAM test has been recommended in HIV infected patients only, this study supports the opinion that this restriction is unwarranted, and perhaps ethically undesirable, when dealing with patients with severe disease [23]. This was evidenced in pilot studies with the much more sensitive SILVAMP LAM (Fujifilm Inc) POC test and with a new LAM electrochemiluminescence test, showing high sensitivity in patients with confirmed TB, regardless of HIV status [24, 25].

The Determine™ TB LAM test (Alere, Inc) is proposed as a rapid point-of-care test and the format has been extensively tested before. Whether it adds diagnostic value to sputum Xpert MTB/RIF is still a matter of debate and may depend on the setting [8, 26]. A positive urine LF-LAM test was an independent predictor of a positive mycobacterial culture in sputum smear-negative HIV co-infected patients with radiological evidence of pulmonary disease compatible with TB [27]. Higher LAM concentrations may be a measure of a higher mycobacterial burden [7]. This could explain why in this study, LF-LAM grade readings were significantly higher in sputum AFB positive patients and in disseminated TB, but not in sputum Xpert MTB/RIF positive patients, the latter being a more sensitive test when mycobacterial burden is low [28]. Non-tuberculous mycobacteria (NTM), mostly *Mycobacterium avium* complex (MAC), were frequently isolated from sputum of HIV-infected patients with low CD4 count suspected of having pulmonary tuberculosis, but disseminated NTM is only seen in patients with very low CD4 counts, and account for less than 5% of all inpatients diagnosed with TB in South Africa [29]. In our study, only about 12% of all patients had very low CD4 counts. Therefore the impact of disseminated NTN infection on LF-LAM results is probably limited here.

In recent studies, the Xpert MTB/RIF test on centrifugated urine precipitate appeared to be a useful complement to sputum tests in HIV co-infected TB patients with low immunity [4]. It detects DNA from whole mycobacteria invading the urinary tract tissue as in renal TB. Large DNA fragments of MTB in blood are too big to pass through the glomerular filter system [30]. LAM should also be detectable in urine in high concentrations when MTB are present in the urinary system, as our study results confirm [31]. Because of its lower sensitivity, higher cost and perfect agreement with LF-LAM test results, the urine Xpert MTB/RIF test is probably inappropriate as a TB confirmation test when a LF-LAM test is concurrently performed.

Study limitations

The study was restricted to hospitalized patients from a highly TB endemic region, starting TB treatment, and most with advanced TB disease. The results may therefore not be generalizable to other settings, particularly where there is a lower prevalence of TB. TB diagnosis was based on clinical and/or microbiological data that did not include MGIT.

NTM could have produced some of LF-LAM readings turning positive, lowering LAM specificity for a MTB diagnosis.

Conclusion

In a high TB prevalence setting, the LF-LAM (“Determine™ TB LAM”) test may be a useful addition to sputum AFB and/or Xpert MTB/RIF tests in hospitalized patients suspected of having advanced TB disease, irrespective of their HIV status. As a diagnostic test, sensitivity of the urine Xpert MTB/RIF is disappointing even in HIV co-infected hospitalized TB patients with disseminated disease, and may be redundant when a LF-LAM test is available.

Declarations

Author Contribution

1. PI 2.Co-pi 3. writing4.Writing 5. promotor

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Data Availability

Publicly available in a repository

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Tables

Tables 1 and 2 are available in the supplementary files section

Figures

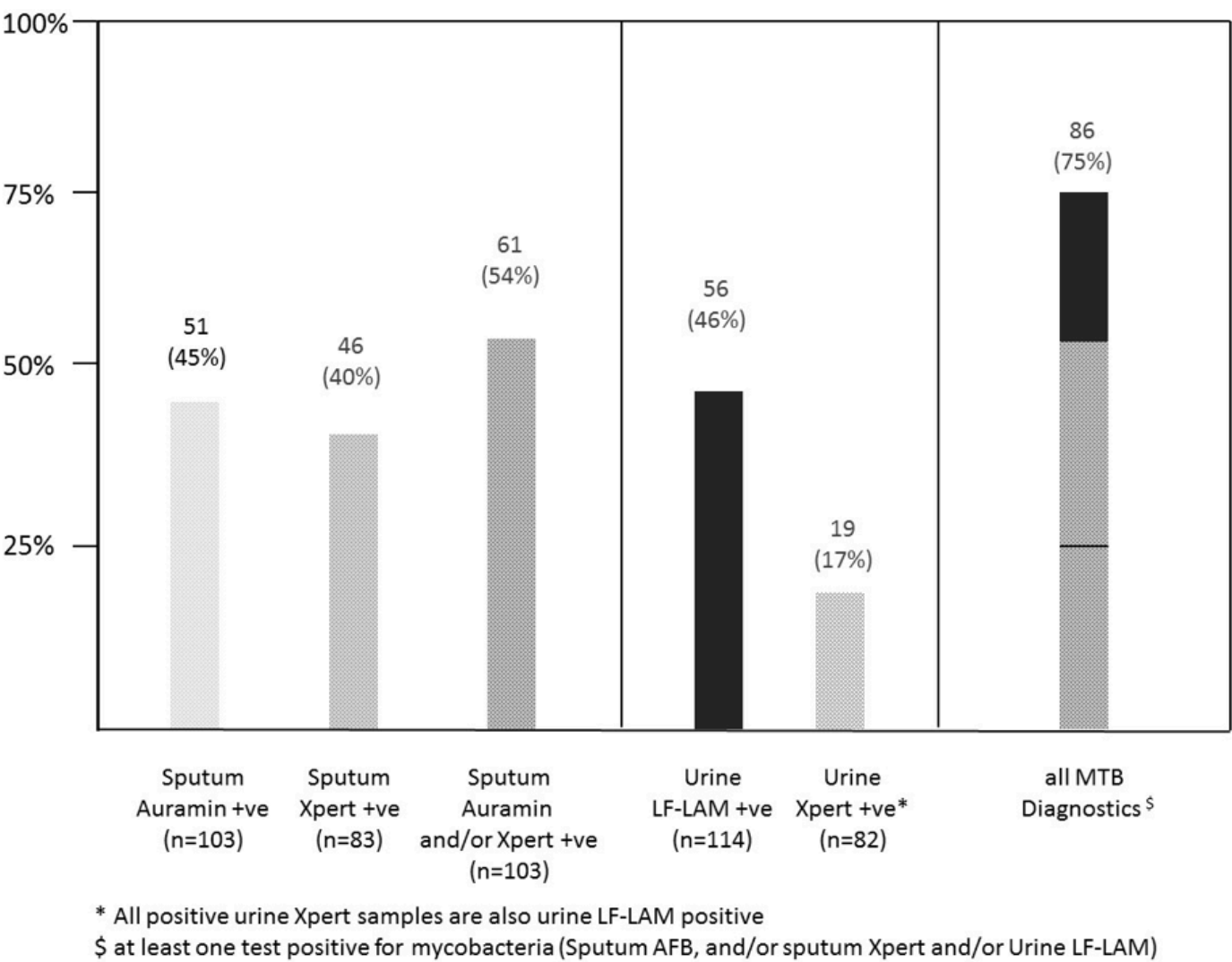


Figure 1

Relative contribution of sputum and urine tests to confirm TB diagnosis in 114 hospitalized patients on TB treatment

Supplementary Files

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