Studies on the prevention and control of human immune deficiency virus associated tuberculosis

Chakezha, T.

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Chapter 7: Diagnostic accuracy of a urine lipoarabinomannan enzyme-linked immunosorbent assay for screening ambulatory HIV-infected persons for tuberculosis


1 Center for TB Research, Division of Infectious Diseases, Department of Medicine, School of Medicine, Johns Hopkins University, Baltimore, MD;
2 Aurum Institute for Health Research, Johannesburg, South Africa;
3 Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD;
4 Division of Diagnostic Radiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa;
5 Queen Mary, University of London, London, United Kingdom;
6 Department of Infectious Disease Epidemiology, Faculty of Epidemiology and Population Health; London School of Hygiene and Tropical Medicine, London, United Kingdom.
7 Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom.

Key Words: HIV, lipoarabinomannan, sensitivity, specificity, predictive value, screening, tuberculosis


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ABSTRACT

Objective: To assess the diagnostic accuracy of the urine lipoarabinomannan (LAM) test among ambulatory HIV-infected persons.

Design: Cross-sectional.

Methods: HIV-infected persons consecutively presenting to the HIV Clinic at a primary care facility in Ekurhuleni, South Africa, were screened for symptoms of tuberculosis (TB) and asked to provide sputum and blood samples for smears for acid-fast bacilli and mycobacterial culture and a urine specimen for a LAM enzyme-linked immunosorbent assay. Fine needle aspirates were obtained from participants with enlarged lymph nodes and sent for histopathology. Non-pregnant participants underwent chest x-ray.

Results: Four hundred twenty-two HIV-infected participants were enrolled with median age 37 years (interquartile range: 31-44 years), median CD4+ T-cell count 215 cells/µl (interquartile range: 107–347 cells/µl), and 212 (50%) receiving antiretroviral therapy. Thirty (7%) had active TB: 18 with only pulmonary TB, five with only extra-pulmonary TB, and seven with both pulmonary TB and extra-pulmonary TB. Twenty-seven percent [95% confidence interval (CI): 12% - 48%] of TB cases were sputum acid-fast bacilli positive. The sensitivity and specificity of the urine LAM compared with the gold standard of positive bacteriology or histopathology were 32% (95% CI: 16% - 52%) and 98% (95% CI: 96% - 99%), respectively. Urine LAM had higher sensitivity in TB cases with higher bacillary burdens, though these differences were not statistically significant.

Conclusions: The sensitivity of urine LAM testing is inadequate to replace mycobacterial culture. In contrast to prior research on the urine LAM, this study was conducted among less sick, ambulatory HIV-infected patients presenting for routine care.
INTRODUCTION

Tuberculosis (TB) in HIV-infected persons is pre-dominantly smear negative, necessitating a multistep diagnostic algorithm to confirm or exclude TB in smear-negative TB suspects. Completing this algorithm is onerous and costly for patients and providers alike. Moreover, considerable uncertainty remains even when the full diagnostic algorithm is followed. A rapid, sensitive, point-of-care test for TB, allowing TB disease status to be accurately assigned on the day of initial presentation could transform TB diagnosis and screening, allowing for prompt initiation of TB treatment and for intensified case finding, isoniazid preventive therapy, and antiretroviral therapy (ART) to be readily integrated into the first days of HIV care. The ideal TB diagnostic test would be accurate, rapid, point-of-care, safe, robust, widely applicable to different populations and forms of TB; affordable and acceptable to clinicians, laboratory technicians, and patients; and should either replace sputum smear microscopy or increase microbiologic confirmation of TB among sputum smear-negative cases.

Lipoarabinomannan (LAM) is a cell wall lipopolysaccharide only found in mycobacteria and can be detected in urine of patients with mycobacterial infections. Enzyme-linked immunosorbent assays (ELISAs) have been developed to detect LAM in urine. The aims of this study were to assess the sensitivity and positive predictive value of urine LAM to screen HIV-infected persons for TB and the specificity and negative predictive value of the urine LAM to exclude TB among HIV-infected persons before initiation of ART and/or isoniazid preventive therapy.

METHODS

Study Site and Population

This study was conducted at a primary care clinic in Ekurhuleni, South Africa. Consecutive patients presenting to the clinic between October 2009 and May 2010 for HIV-related care were asked to participate in the study. Inclusion criteria were age >18 years, HIV infection (testing was provided by routine clinical services, not the study), ability to communicate in English, Zulu, or Sepedi, and written informed consent. Patients who were already on treatment for TB or who had discontinued TB treatment in the past three months, patients on dialysis, and prisoners were excluded.
Study Procedures

Demographic information and clinical history were obtained through interview and medical record review. All study participants provided two sputum, one blood, and one urine specimen regardless of symptoms. Participants unable to expectorate spontaneously underwent percussion, and if necessary, sputum induction with 3% hypertonic saline. All participants were examined for lymphadenopathy and a fine needle aspiration was performed on all superficial easily accessible lymph nodes >1 cm in size. Additional diagnostic work up and all treatment was provided at the discretion of the treating clinicians who were given the results of all study-related diagnostic testing except the urine LAM test.

Urine specimens were refrigerated after collection, transported to the laboratory in cooler boxes at 2–8 °C on the day of collection, heat inactivated, spun down, and stored at 22°C until testing was performed. All other specimens were transported to the laboratory at ambient temperature and processed on the day of collection. Sputum smears were decontaminated with N-acetyl-L-cysteine-sodium hydroxide. A Ziehl-Neelsen–stained smear was prepared, examined under 1003 magnification using a light microscope and graded according to the World Health Organization grading system. Processed sputum sediment from both specimens was cultured using the BACTEC mycobacteria growth indicator tube 960 system (BD Diagnostics, Sparks, MD).

Blood was sent for complete blood count and CD4+ T-cell counts and inoculated directly into BACTEC Myco-F/Lytic bottles at the time of collection. Smears of lymph node aspirates were prepared at the time of collection and sent for histopathology. Mycobacterial species identification was performed using the Capilia MPB64 monoclonal antibody test (TAUNS, Numazu, Japan). Urine was tested for LAM using the Clearview TB ELISA (Inverness Medical Innovations, Waltham, MA) according to the manufacturer’s instructions in batches. The laboratory technicians reading the urine LAM ELISA optical densities were blinded to the participants’ histories and other diagnostic test results. A rapid urine pregnancy test was also performed on all female participants not already known to be pregnant. All participants who were not pregnant underwent a chest x-ray. The chest x-ray was read by a single reader (V.M.) for active TB.

Case Definitions

Participants were classified as having pulmonary TB (PTB) if they had at least one sputum culture that was positive for Mycobacterium tuberculosis, or at least one sputum smear that was acid-fast bacilli (AFB) positive, but with culture not performed or contaminated. Participants were classified as
having extra-pulmonary TB (ETB) if they had at least one blood culture that was positive for M. tuberculosis, or at least one fine needle aspiration from an enlarged lymph node that was AFB positive and/or histologically consistent with TB.

**Data Management and Statistical Analyses**

We assumed that the urine LAM test would have a sensitivity of 50%–80%, and thus that we would be able to estimate the sensitivity of the test with 95% confidence intervals (CIs) +/- 15% if we enrolled at least 400 participants with a TB prevalence of 10%–15%. We assumed that the urine LAM test would have a specificity of 85%–100%, and thus that we would be able to estimate the specificity of the test with 95% CIs +/- 15% if we enrolled at least 400 participants with a TB prevalence of 10%–15%. The statistical software package SAS 9.2 (SAS Institute Inc, Cary, NC) was used for data analysis. The performance characteristics of the urine LAM as compared with the gold-standard case definitions, and the prevalence of TB was calculated along with 95% CIs using the exact binomial method.

**Ethics**

The study was approved by the Gauteng Province Department of Health, the Ekurhuleni Metropolitan Municipality Executive Director for Health, the Ekurhuleni Metropolitan Municipality Ethics Committee, the University of the Witwatersrand Human Research Ethics Committee, the Johns Hopkins University School of Medicine Institutional Review Board, and the London School of Hygiene and Tropical Medicine Ethics Committee.

**RESULTS**

Between October 2009 and May 2010, 443 persons presenting to the study clinic for routine HIV-related care were invited and consented to participate and were screened for eligibility; 21 (5%) were ineligible [HIV-uninfected (8), currently being treated for TB (12), and completion of TB treatment, three months before eligibility screening (1)] (Fig. 1). One hundred forty-four (34%) of participants were male, the median age was 37 years [interquartile range (IQR): 31–44 years], median CD4+ T-cell count was 215 cells/µl (IQR: 107–347 cells/µl), and 212 (50%) were receiving ART. The median CD4+ T-cell count among those not receiving ART was 160 cells/µl (IQR: 65–315 cells/µl). Participants receiving ART had a median CD4+ T-cell count of 258 cells/µl (IQR: 165–357 cells/µl) and had been receiving ART for a median duration of 247 days (IQR: 99–848 days) excluding those who has just been initiated on ART the day of enrollment in the study.
Three hundred sixty-one (86%) of participants reported having any duration of cough, fever, night sweats, or weight loss. A total of 30 TB cases (7%, 95% CI: 5% to 10%) was identified, 18 (60%) had only PTB, 7 (23%) had PTB and ETB, and 5 (17%) had only ETB (Fig. 1). Seven PTB cases were diagnosed on the basis of a positive sputum smear and 25 on the basis of a positive sputum culture. Of the 12 cases with ETB, seven had a positive blood culture, three had lymph node histopathology consistent with TB, and two had both. Twenty-seven percent (95% CI: 12% - 48%) of TB cases were sputum AFB positive.

The performance characteristics of symptoms, body mass index, routine laboratories and urine LAM are shown in Table 1. The sensitivity, specificity, positive predictive value, and negative predictive value of the urine LAM test were 32% (95% CI: 16% to 52%), 98% (95% CI: 96% - 99%), 53% (95% CI: 28% - 77%), and 95% (95% CI: 93% - 97%), respectively. The urine LAM test had sensitivities of 40% (5% - 85%) and 20% (95% CI: 6% - 44%) among sputum AFB-positive and AFB-negative TB cases. The urine LAM test was more sensitive in TB cases with night sweats (42%, 95% CI: 20% - 67%), weight loss (36%, 95% CI: 18% - 57%), body mass index <18.5 kg/m² (67%, 95% CI: 30% - 93%), anemia (43%,
95% CI: 22% - 66%), CD4⁺ T-cell counts <50 cells per microliter (56%, 95% CI: 21% - 86%), moderate/advanced disease on chest x-ray (38%, 95% CI: 14% - 68%), or ETB (64%, 95% CI: 31% - 89%), but none of these differences achieved statistical significance.

Having either a positive urine LAM test or sputum smear was 36% sensitive (95% CI: 18% to 57%), 98% specific (95% CI: 95% to 99%), and had positive and negative predictive values of 64% (95% CI: 35% to 87%) and 93% (95% CI: 89% to 96%). Four of seven participants who grew non-tuberculous mycobacteria and did not grow TB from sputum and/or blood had positive urine LAM tests.

DISCUSSION
The sensitivity of the urine LAM among HIV-infected ambulatory patients in this study was only 50% among sputum AFB-positive and 16% among AFB-negative TB cases, respectively, making it inadequate to replace sputum smear microscopy in AFB-positive TB cases or mycobacterial culture in AFB-negative TB cases, even if the urine LAM is formatted into a rapid lateral flow assay. Our study differed from prior studies in that we studied the urine LAM test to screen a high-risk population for TB rather than to confirm a diagnosis of TB among symptomatic persons; our participants were ambulatory patients presenting for routine HIV-related care, who were less immunosuppressed than populations in which this test has previously been studied; and a highly sensitive standard diagnostic work up for TB was performed in all study participants regardless of symptoms reported.

The prevalence of active TB among ambulatory HIV-infected adults in our study was 7%, which was lower than previously found by Bassett et al and Houlihan et al in KwaZulu Natal and by Lawn et al in the Western Cape, South Africa, where the prevalence of TB was 20%–30% among HIV-infected ambulatory patients presenting for initiation of ART; however, the HIV-infected patients in these prior studies had a lower median CD4⁺ T-cell counts (100–125 cells/mL) than the median CD4⁺ T-cell count of participants in our study (215 cells/mL) and were not receiving ART in contrast to the 50% in our study who were on ART.⁹⁻¹¹

The sensitivity of the urine LAM in our study (32%, 95% CI: 16%–52%) was comparable with or lower than that reported in previous studies (20%–80%),⁷,⁸,¹²⁻¹⁶ likely because our study participants were ambulatory, relatively less sick persons with lower bacillary burdens of TB. Prior studies have
demonstrated that the urine LAM has greater sensitivity for detecting TB among patients with a higher bacillary burden: sputum AFB-positive versus AFB-negative TB patients;\textsuperscript{7,8,13-19} patients with definite rather than probable or possible TB;\textsuperscript{7,8,15,16} HIV-infected versus HIV-uninfected TB patients;\textsuperscript{7,14-16} HIV-infected patients with lower CD4\textsuperscript{+} T-cell counts;\textsuperscript{7,12,14,17} PTB versus ETB patients;\textsuperscript{17} and TB patients with mycobacteremia versus TB patients without mycobacteremia.\textsuperscript{7,17}
### Table 1: Performance characteristics of BMI, routine laboratories, and urine LAM for diagnosis of TB

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity [x/n] = % (95% CI)</th>
<th>Specificity [x/n] = % (95% CI)</th>
<th>Positive Predictive Value [x/n] = % (95% CI)</th>
<th>Negative Predictive Value [x/n] = % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI, 18.5 kg/m²</strong></td>
<td>[9/30] = 30 (15 to 49)</td>
<td>[364/387] = 94 (91 to 96)</td>
<td>[9/32] = 28 (14 to 47)</td>
<td>[364/385] = 95 (92 to 97)</td>
</tr>
<tr>
<td><strong>Anemia</strong></td>
<td>[22/30] = 73 (54 to 88)</td>
<td>[196/388] = 51 (45 to 56)</td>
<td>[22/214] = 10 (7 to 15)</td>
<td>[196/204] = 96 (92 to 98)</td>
</tr>
<tr>
<td>Sputum smear microscopy</td>
<td>[7/26] = 27 (12 to 48)</td>
<td>[228/228] = 100</td>
<td>[7/7] = 100</td>
<td>[228/247] = 92 (88 to 95)</td>
</tr>
<tr>
<td>Chest x-ray (CXR)†</td>
<td>[21/22] = 95 (77 to 100)</td>
<td>[58/123] = 47 (38, 56)</td>
<td>[21/86] = 24 (16 to 35)</td>
<td>[58/59] = 98 (91 to 100)</td>
</tr>
<tr>
<td>Urine LAM</td>
<td>[9/28] = 32 (16 to 52)</td>
<td>[378/386] = 98 (96 to 99)</td>
<td>[9/17] = 53 (28 to 77)</td>
<td>[378/397] = 95 (93 to 97)</td>
</tr>
<tr>
<td><strong>Site of TB</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTB‡</td>
<td>[5/23] = 22 (7 to 44)</td>
<td>[378/386] = 98 (96 to 99)</td>
<td>[5/13] = 38 (14 to 68)</td>
<td>[378/396] = 95 (93 to 97)</td>
</tr>
<tr>
<td>ETB§</td>
<td>[7/11] = 64 (31 to 89)</td>
<td>[378/386] = 98 (96 to 99)</td>
<td>[7/15] = 47 (21 to 73)</td>
<td>[378/382] = 99 (97 to 100)</td>
</tr>
<tr>
<td>Smear positivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFB+</td>
<td>[3/6] = 50 (12 to 88)</td>
<td>[0/0] = —</td>
<td>[3/3] = 100</td>
<td>[0/0] = 0</td>
</tr>
<tr>
<td>AFB2</td>
<td>[3/19] = 16 (4 to 40)</td>
<td>[222/227] = 98 (95 to 99)</td>
<td>[3/8] = 38 (9 to 76)</td>
<td>[222/238] = 93 (89 to 96)</td>
</tr>
<tr>
<td>Absolute CD4+ T-cell count (cells/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤50</td>
<td>[5/9] = 56 (21 to 86)</td>
<td>[36/40] = 90 (76 to 97)</td>
<td>[5/9] = 56 (21 to 86)</td>
<td>[36/40] = 90 (76 to 97)</td>
</tr>
<tr>
<td>≤200</td>
<td>[8/23] = 35 (16 to 57)</td>
<td>[164/170] = 96 (92 to 99)</td>
<td>[8/14] = 57 (29 to 82)</td>
<td>[164/179] = 92 (87 to 95)</td>
</tr>
<tr>
<td>201–350</td>
<td>[1/5] = 20 (1 to 72)</td>
<td>[113/114] = 99 (95 to 100)</td>
<td>[1/2] = 50 (1 to 99)</td>
<td>[113/117] = 97 (91 to 99)</td>
</tr>
<tr>
<td>351–500</td>
<td>[0/0] = —</td>
<td>[55/56] = 98 (90 to 100)</td>
<td>[0/0] = 0</td>
<td>[55/55] = 100</td>
</tr>
<tr>
<td>&gt;500</td>
<td>[0/0] = —</td>
<td>[46/46] = 100</td>
<td>[0/0] = —</td>
<td>[46/46] = 100</td>
</tr>
<tr>
<td>Currently receiving ART</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>[6/19] = 32 (13 to 57)</td>
<td>[178/183] = 97 (94 to 99)</td>
<td>[6/11] = 55 (23 to 83)</td>
<td>[178/191] = 93 (89 to 96)</td>
</tr>
<tr>
<td>Yes</td>
<td>[3/3] = 33 (7 to 70)</td>
<td>[199/202] = 99 (96 to 100)</td>
<td>[3/6] = 50 (12 to 88)</td>
<td>[199/205] = 97 (94 to 99)</td>
</tr>
<tr>
<td>Urine LAM plus BMI, 18.5 kg/m²</td>
<td>[12/28] = 43 (24 to 63)</td>
<td>[354/384] = 92 (89 to 95)</td>
<td>[12/42] = 29 (16 to 45)</td>
<td>[354/370] = 96 (93 to 98)</td>
</tr>
<tr>
<td>Urine LAM plus anemia*</td>
<td>[21/28] = 75 (55 to 89)</td>
<td>[192/385] = 50 (45 to 55)</td>
<td>[21/214] = 10 (6 to 15)</td>
<td>[192/199] = 96 (93 to 99)</td>
</tr>
<tr>
<td>Urine LAM plus sputum smear microscopy†</td>
<td>[9/25] = 36 (18 to 57)</td>
<td>[222/227] = 98 (95 to 99)</td>
<td>[9/14] = 64 (35 to 87)</td>
<td>[222/238] = 93 (89 to 96)</td>
</tr>
<tr>
<td>Urine LAM plus CXR†</td>
<td>[20/20] = 100</td>
<td>[55/123] = 45 (36 to 54)</td>
<td>[20/88] = 23 (14 to 33)</td>
<td>[55/55] = 100</td>
</tr>
</tbody>
</table>

*Anemia: hemoglobin, 12 g/dL in women, 13 g/dL in men.
†Evidence of active TB on chest x-ray.
‡The performance of the urine LAM test was calculated after excluding the 5 participants who had only ETB.
§The performance of the urine LAM test was calculated after excluding the 18 participants who had only PTB.
kExcluding participants for whom one of the test results was missing. BMI, body mass index.
The sensitivity of the urine LAM alone did not exceed 64% in any subgroup, and thus the urine LAM was not a useful test to rule out TB in any subgroup. The specificity of the urine LAM exceeded 90% in all subgroups and may be a useful test for ruling in TB among sick hospitalized HIV-infected persons with a high burden of TB, but in this out-patient population, the positive predictive value reached only 57%, even among those with CD4⁺ T-cell counts ≤200 cells per microliter.

In the past year, there has been much excitement about the Cepheid GeneXpert MTB/RIF assay, a molecular test for TB that is run within two hours, could be used at the point of care and which has >98% and >72% sensitivity for sputum smear-positive and smear-negative TB, respectively, and >98% specificity, significantly better performance characteristics than that of the urine LAM.20
One of the main benefits of active TB case finding is to detect patients early in the natural history of disease, and such programs will have limited impact if they rely on TB diagnostics with poor sensitivity in this group. Development of TB diagnostics should focus on rapid point-of-care technologies that have significantly higher sensitivity, specificity, and predictive value among patients with both early and advanced TB disease.
REFERENCES


