Quantitative Analysis of a Urine-Based Assay for Detection of Lipoarabinomannan in Patients with Tuberculosis\textsuperscript{7}

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Urinary lipoarabinomannan (LAM) detection is a promising approach for rapid diagnosis of active tuberculosis (TB). In microbiologically confirmed TB patients, quantitative LAM detection results increased progressively with bacillary burden and immunosuppression. Patients with disseminated TB and/or advanced HIV are target populations for whom urine LAM detection may be particularly useful.

Effective control of tuberculosis (TB) is hindered by the lack of rapid, accurate diagnostic modalities. Current tools have serious limitations when applied to HIV-infected patients, and clinical diagnosis can be challenging (2, 6, 8, 13). Sputum smear microscopy is widely utilized to diagnose active TB but has impaired sensitivity and detects less than half of HIV-TB coinfection cases (2, 6, 8). Mycobacterial culture is the laboratory standard for diagnosis but is not widely available in resource-constrained settings and can take weeks to determine a positive result.

The detection of lipoarabinomannan (LAM), a 17.5-kDa glycolipid component of the mycobacterial cell wall, is an attractive approach to diagnosing active TB (1, 7, 9, 15, 16). LAM is released from metabolically active mycobacteria and is detectable intact in urine (1, 7). The Clearview TB enzyme-linked immunosorbent assay (ELISA) (“urine LAM test”; Inverness Medical Innovations, Waltham, MA) is a direct antigen sandwich immunoassay in a 96-well-plate format that provides both quantitative and qualitative results. Our group and others have recently reported on the qualitative diagnostic accuracy of this urine LAM test (9, 10, 16). Urine LAM test sensitivity, while imperfect, appears to be higher than that of sputum smear microscopy, and the test performs with a high positive predictive value in populations with high HIV and TB prevalence (9, 12, 16). However, quantitative urine LAM test results have not been studied fully. Quantitative analysis allows a more complete understanding of test performance and may offer insight into optimal test usage. Preclinical and limited regression modeling from clinical studies have shown that quantitative test results positively correlate with increasing bacillary burden (1, 16). We therefore examined the relationship between quantitative urine LAM test results and TB characteristics among patients with culture-confirmed TB.

A full description of the study design has been published previously (16). Briefly, we conducted a nested prospective cohort study at three hospitals in South Africa to evaluate the diagnostic accuracy of the urine LAM test. Hospitalized adult TB suspects were enrolled after informed consent. Study-directed testing included sputum smear microscopy for acid-fast bacilli (AFB), sputum mycobacterial culture, mycobacterial blood cultures, HIV and CD4 testing, urine LAM testing using the Clearview TB ELISA kit, and additional mycobacterial cultures (e.g., from enlarged lymph nodes) when clinically indicated. Participants had a follow-up study visit at 2 months. Participants with at least one positive AFB smear or mycobacterial culture positive for Mycobacterium tuberculosis were determined to have “confirmed TB” and were included in the current analysis. Individuals with M. tuberculosis isolated from a blood culture (mycobacteremia) were considered to have disseminated disease. Pulmonary TB (PTB) was defined as the presence of one or more spuata positive for AFB by microscopy and/or positive for M. tuberculosis by culture. Localized extrapulmonary TB was defined as isolation of M. tuberculosis from pleural culture or fine-needle aspiration of a lymph node but not from other respiratory specimens or blood. Quantitative urine LAM test results were expressed as optical density (OD) readings. The final sample OD was determined by subtracting the OD of the negative control from the sample reading, with a minimum value of 0 (i.e., a value of 0 was reported if the OD of the negative control was greater than the OD of the sample). An OD above 0.1 was considered positive per manufacturer specifications. Multiple linear regression models were used to determine predictors of higher quantitative urine LAM test results. Wilcoxon rank sum and Kruskal-Wallis tests were used to compare nonnormal continuous outcomes; chi-square (\(\chi^2\)) tests were used to compare categorical outcomes. A \(P\) value of \(\leq0.05\) was considered statistically significant, and 95% confidence intervals (95% CI) were used. Statistical calculations were performed using Stata 10.1 (StataCorp). This study was approved by the institutional review boards of Johns Hopkins University School of Medicine and the University of Witwatersrand.

Full characteristics of the study population are published elsewhere (16). Overall, 499 participants were enrolled, and 193 (39%) had confirmed TB (185 with positive cultures for M.
Localized extrapulmonary TB (5)*

Pulmonary TB only (145)†

Mycobacteremia only (18)‡

Pulmonary TB with mycobacteremia (25)

**P** value 0.0001

Pulmonary TB with Mycobacteremia only (18)

1.04 (0.14–1.43) 0.99 (1.02) 14 (78, 52–94)

1.4 (0.43–2.8) 1.6 (1.1) 21 (84, 64–96)

Localize extrapulmonary TB, PTB alone, mycobacteremia compared to those with disseminated disease (Table 1). Localized extrapulmonary TB was patients with localized extrapulmonary TB, PTB alone, mycobacteremia isolated from a lymph node. The urine LAM test was positive in five patients who had M. tuberculosis isolated from pleural cultures, in 18 cases (9%), and combined PTB with mycobacteremia (13%). The overall LAM test sensitivity was 59% (95% CI, 52 to 66) in participants with confirmed TB, and the specificity was 96% (95% CI, 91 to 99) among individuals without TB. Among participants with confirmed TB, the range of quantitative LAM test results was 0 to 4.87 (median, 0.19; interquartile range [IQR], 0.03 to 1.3).

Among participants with confirmed TB, there was a significant difference in median OD for those with more localized disease compared to those with disseminated disease (P = 0.0001) (Table 1). The median OD increased progressively from 0.01 (IQR, 0.01 to 0.06) to 0.13 (IQR, 0.02 to 0.70) to 0.64 (IQR, 0.14 to 1.43) to 1.4 (IQR, 0.43 to 2.8) in those with localized extrapulmonary TB, PTB alone, mycobacteremia without PTB, and combined PTB with mycobacteremia, respectively. Among 161 participants with sputum culture-positive PTB, those that were AFB smear positive had higher ODs than those that were smear negative (median ODs of 0.33 versus 0.13, respectively; P = 0.03) (Table 2). There was a trend toward higher OD with increasing grades of smear positivity (median ODs of 0.13 [IQR, 0.02 to 0.62], 0.18 [IQR, 0.1 to 1.3], 0.26 [IQR, 0.03 to 2.8], and 0.38 [IQR, 0.048 to 1.8] for smear-negative, smear-positive grade 1+, smear-positive grade 2+, and smear-positive grade 3+ cases, respectively; P = 0.18). Among confirmed TB patients with HIV infection, the urine LAM test OD increased with decreasing strata of CD4 count (P = 0.0001) (Table 3). The median OD increased progressively from 0.10 (IQR, 0.02 to 0.5) to 0.20 (IQR, 0.03 to 0.8) to 0.42 (IQR, 0.04 to 1.0) to 1.3 (IQR, 0.2 to 2.8) in those with CD4 counts of <150, 101 to 150, 50 to 100, and <50, respectively (P = 0.0001).

Multiple linear regression models were used to further explore factors associated with higher quantitative urine LAM test results. Covariates included in the models were age, sex, HIV status, mortality at 2 months, TB treatment, mycobacteremia, sputum smear positivity, fever, Karnofsky score, chest X-ray (CXR) cavitation, hypoxia, pulse, and CD4 strata. Adjusting for all other factors, individuals with mycobacteremia had an average OD that was 0.64 OD units higher than that for individuals with negative blood cultures (P = 0.002); individuals with smear-positive pulmonary TB had an average OD that was 0.33 OD units higher than that for individuals with smear-negative pulmonary TB (P = 0.05). Among HIV-infected patients, individuals with CD4 counts of <50 had an average OD that was 1.05 OD units higher than that for individuals with CD4 counts of >150 (P < 0.0001). No other covariates were significantly associated with higher quantitative urine LAM test results.

Taken together, our findings show that quantitative urine LAM test results correlate with degree of bacillary burden in patients with microbiologically confirmed TB. Further, the absolute urine LAM test OD increased dramatically as immuno-

### Table 1. Optical density by type of disease (determined by site) among participants with confirmed TB

<table>
<thead>
<tr>
<th>Type of disease (no. of cases)</th>
<th>OD median (IQR)</th>
<th>OD mean (SD)</th>
<th>No. of cases with positive urine LAM test result (% sensitivity, 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Localized extrapulmonary TB</td>
<td>0.01 (0.01–0.06)</td>
<td>0.09 (0.16)</td>
<td>1 (20, 1–72)</td>
</tr>
<tr>
<td>Pulmonary TB only (145)</td>
<td>0.13 (0.02–0.70)</td>
<td>0.63 (0.97)</td>
<td>78 (54, 45–62)</td>
</tr>
<tr>
<td>Mycobacteremia only (18)</td>
<td>0.64 (0.14–1.43)</td>
<td>0.99 (1.02)</td>
<td>14 (78, 52–94)</td>
</tr>
<tr>
<td>Pulmonary TB with mycobacteremia (25)</td>
<td>1.4 (0.43–2.8)</td>
<td>1.6 (1.1)</td>
<td>21 (84, 64–96)</td>
</tr>
<tr>
<td><strong>P</strong> value 0.0001</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Four of five participants had M. tuberculosis isolated from pleural cultures, and 1 of 5 had M. tuberculosis isolated from a lymph node. The urine LAM test was positive in the participant with M. tuberculosis isolated from the lymph node but negative in the 4 participants with localized pleural TB.

† M. tuberculosis in sputum but not blood.

‡ M. tuberculosis in blood but not sputum.

§ —, analysis of variance (ANOVA) was not performed, due to nonnormal distribution.

### Table 2. Optical density by sputum AFB smear microscopy status among participants with sputum culture-positive pulmonary TB

<table>
<thead>
<tr>
<th>Sputum AFB smear microscopy status (no. of cases)**</th>
<th>OD median (IQR)</th>
<th>OD mean (SD)</th>
<th>No. of cases with positive urine LAM test result (% sensitivity, 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (88)</td>
<td>0.13 (0.02–0.62)</td>
<td>0.60 (0.93)</td>
<td>47 (53, 0.42–0.64)</td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any grade (73)</td>
<td>0.33 (0.052–1.71)</td>
<td>0.94 (1.15)</td>
<td>47 (64, 0.52–0.75)</td>
</tr>
<tr>
<td>Grade 1+ (13)</td>
<td>0.18 (0.1–1.3)</td>
<td>0.68 (0.89)</td>
<td>10 (77, 0.46–0.94)</td>
</tr>
<tr>
<td>Grade 2+ (15)</td>
<td>0.26 (0.03–2.8)</td>
<td>1.13 (1.3)</td>
<td>10 (67, 0.38–0.88)</td>
</tr>
<tr>
<td>Grade 3+ (45)</td>
<td>0.38 (0.048–1.8)</td>
<td>0.95 (1.15)</td>
<td>27 (60, 0.44–0.74)</td>
</tr>
</tbody>
</table>
| **P** = 0.18 for comparison of AFB-negative, AFB 1+, AFB 2+, and AFB 3+ results by the Kruskal-Wallis test.

### Table 3. Optical density by CD4 status among participants with HIV infection and confirmed TB

<table>
<thead>
<tr>
<th>CD4 count (no. of cases)</th>
<th>OD median (IQR)</th>
<th>OD mean (SD)</th>
<th>No. of cases with positive urine LAM test result (% sensitivity, 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;150 (63)</td>
<td>0.10 (0.02–0.5)</td>
<td>0.42 (0.72)</td>
<td>32 (51, 0.38–0.64)</td>
</tr>
<tr>
<td>101–150 (16)</td>
<td>0.2 (0.03–0.8)</td>
<td>0.65 (0.96)</td>
<td>9 (56, 0.30–0.80)</td>
</tr>
<tr>
<td>50–100 (25)</td>
<td>0.42 (0.04–1.0)</td>
<td>0.72 (0.9)</td>
<td>20 (71, 0.51–0.87)</td>
</tr>
<tr>
<td>&lt;50 (60)</td>
<td>1.3 (0.2–2.8)</td>
<td>1.4 (1.12)</td>
<td>51 (85, 0.73–0.93)</td>
</tr>
<tr>
<td><strong>P</strong> value</td>
<td>0.0001</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

* Chi-square (χ²) test comparing proportions of positive urine LAM test results (sensitivities) between types of disease.

** Chi-square (χ²) test comparing proportions of positive urine LAM test results (sensitivities) between CD4 count strata.

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**Note:** The data and analysis provided are based on the information available in the document. Further statistical tests and considerations are noted where applicable.
suppression progressed among HIV-coinfected TB patients, probably due to higher mycobacterial burden and greater likelihood of disseminated TB. Strikingly, the median quantitative urine LAM test result was over 10-fold higher than the current threshold for test positivity in those with CD4 counts less than 50 and 5-fold higher than this threshold in those with CD4 counts between 51 and 100. Usage of quantitative urine LAM test results may thus offer additional clinical insight into the degree of TB disease severity that cannot be gleaned from qualitative results alone.

To date, studies of the qualitative accuracy of the urine LAM test have yielded varied results. In heterogeneous populations with low HIV prevalence, the sensitivity has ranged from 13 to 51% (3, 4, 10, 12, 14). Alternatively, sensitivity rises among HIV-positive patients and could reach 67 to 85% in those with low CD4 counts (9, 16). This quantitative analysis provides evidence that urine LAM test performance may be best in patients with disseminated TB disease and high mycobacterial disease burden. HIV-infected TB suspects with advanced immunosuppression, a group in which sputum microscopy is of low yield (5, 8, 11, 17), may be a target population for whom the urine LAM test would be particularly useful. Of note, the combination of LAM testing and sputum smear microscopy may have an additive benefit in this target group (16). While the urine LAM test is unlikely to stand alone for definitive TB diagnostic testing, its usage is attractive as a rapid diagnostic modality that complements smear microscopy for HIV-prevalent, resource-constrained settings where mycobacterial culture is unavailable.

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REFERENCES
