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Diagnostic accuracy of a rapid urine lipoarabinomannan test for tuberculosis in HIV-infected adults

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Abstract

Objective—In settings of high HIV prevalence, tuberculosis control and patient management are hindered by lack of accurate, rapid tuberculosis diagnostic tests that can be performed at point-of-care. The Determine TB LAM Ag (‘TB LAM’) test is a lateral flow immunochromatographic test

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AUTHOR CONTRIBUTIONS

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for detection of mycobacterial lipoarabinomannan (LAM) in urine. Our objective was to determine sensitivity and specificity of the TB LAM test for tuberculosis diagnosis.

Design—Prospective diagnostic accuracy study.

Setting—Hospital and outpatient settings in Uganda and South Africa.

Participants—HIV-infected adults with tuberculosis symptoms and/or signs.

Methods—Participants provided a fresh urine specimen for TB LAM testing, blood for mycobacterial culture, and two respiratory specimens for smear microscopy and mycobacterial culture.

Main outcome measures—For the TB LAM test, sensitivity in participants with culture-positive tuberculosis and specificity in participants without tuberculosis.

Results—1013 participants were enrolled. Among culture-positive tuberculosis patients, the TB LAM test identified 136/367 (37.1%) overall and 116/196 (59.2%) in the group with CD4 100 cells/mm³. The test was specific in 559/573 (97.6%) of patients without tuberculosis. Sensitivity of the urine TB LAM test plus sputum smear microscopy was 197/367 (53.7%) overall and 133/196 (67.9%) among those with CD4 100. CD4 50 (adjusted odds ratio [AOR] 6.2, P<0.001) or 51–100 (AOR 7.1, P<0.001), mycobacteremia (AOR 6.1; P<0.01) and hospitalization (AOR 2.6, P=0.03) were independently associated with a positive TB LAM test.

Conclusions—In HIV-positive adults with CD4 100, the TB LAM urine test detected over half of culture-positive tuberculosis patients, in less than 30 minutes and without the need for equipment or reagents.

Keywords

sensitivity and specificity; tuberculosis; opportunistic infections; diagnosis; HIV

INTRODUCTION

The HIV pandemic has driven resurgence of tuberculosis, a leading cause of death in HIV-infected persons in sub-Saharan Africa.^{1,2} In individuals co-infected with HIV and *Mycobacterium tuberculosis*, the two pathogens are tragically synergistic. Among the clinical consequences are high mortality and ‘atypical’ tuberculosis disease presentations such as disseminated tuberculosis and non-cavitary pulmonary tuberculosis, especially with advancing immunosuppression.^{3,4} These alterations in tuberculosis clinical manifestations impair the sensitivity and specificity of chest radiography, and the sensitivity of sputum smear microscopy, which are two of the most widely available diagnostic tools.⁵ Clinical sensitivities of sputum mycobacterial culture and nucleic acid amplification tests are less affected by HIV, but these tests are not widely available at point-of-care, and culture takes weeks for a result.⁶ Xpert MTB/RIF, a closed cartridge nucleic acid amplification TB test, is an advance with respect to ease of operator use but has limitations including requirements for electricity and climate/dust control, and a minimum testing time of about two hours.⁷ Further, Xpert MTB/RIF is optimized for testing of sputum, a specimen type that is sometimes not obtainable or informative and is biohazardous. In HIV-infected individuals with advanced immunosuppression and tuberculosis, the clear survival benefit from prompt

tuberculosis treatment plus early antiretroviral therapy makes imperative the timely, accurate diagnosis of both diseases.^{8–10}

Lipoarabinomannan (LAM), a glycolipid component of the *Mycobacterium tuberculosis* cell wall, is an attractive diagnostic target.¹¹ As a bacterial product, LAM has the theoretical potential to discriminate active tuberculosis disease from latent tuberculosis infection independent of human immune responses. Diagnostic accuracy studies using a plate-based enzyme-linked immunosorbence assay for detection of LAM have shown sensitivities that are higher in HIV-infected versus HIV-uninfected tuberculosis patients, and that are inversely correlated with degree of HIV-associated immunosuppression.^{12–17} The Determine™ TB LAM Ag test ('TB LAM' test, Alere, Waltham, MA) is a lateral flow immunochromatographic assay for detection of urinary LAM. This point-of-care test requires a drop of unprocessed urine and no other equipment or supplies. Results are read by visual inspection 25 minutes after applying the urine to the test strip.

We reasoned that a simple, rapid, truly point-of-care test having increasing tuberculosis diagnostic sensitivity with increasing HIV-associated immunosuppression might be useful in diagnosing the subset of patients with the highest mortality from untreated tuberculosis and the greatest potential survival benefit from prompt treatment of both diseases. We performed a multicenter study to assess the accuracy of the TB LAM test, performed in real-time on fresh urine, for diagnosis of tuberculosis in HIV-infected adults.

METHODS

Design and settings

This diagnostic accuracy study was cross-sectional with limited longitudinal follow-up. Outpatient recruitment settings were the Infectious Diseases Institute clinic in Kampala, Uganda and Town Two clinic, Khayelitsha, near Cape Town, South Africa. Inpatient settings were Mulago Hospital in Kampala and G.F. Jooste Hospital in Cape Town. The enrollment period was January 2011 through November 2011. Data collection was planned before the investigational tests and reference standards were performed, and all study procedures were performed according to the study protocol and a written procedures manual.

Population

The target population was HIV-positive adults suspected of having tuberculosis. Inclusion criteria were age ≥18 years; suspected to have active tuberculosis based on having at least one of cough, fever, night sweats, or weight loss; HIV-positive; and informed consent. Exclusion criteria were administration of more than two days of anti-tuberculosis treatment within sixty days prior to enrollment, and inability to provide a urine specimen.

Clinical and laboratory assessments

At enrollment, participants were interviewed for medical and demographic information, had a chest X-ray, and then provided a spontaneously voided urine specimen for LAM testing, blood for mycobacterial culture and CD4 T cell count, and two respiratory specimens for

smear microscopy and mycobacterial culture. If a participant could not spontaneously expectorate sputum, then sputum induction was performed using nebulized hypertonic saline. Participants who had a positive urine TB LAM test but no enrollment culture positive for *M. tuberculosis* underwent the same panel of tests and interview two months later, and medical records from the time of enrollment to the time of follow-up were reviewed. For all other participants medical records from enrollment to two months post-enrollment were reviewed or an interview was conducted.

All laboratory tests were performed on site in Uganda or South Africa in designated labs with existing external quality assurance programs, and testing was initiated immediately following specimen collection (i.e. specimens were not stored prior to testing). Unprocessed sputum was smeared and stained using the Ziehl-Neelsen (ZN) method; smears were examined microscopically for acid fast bacilli (AFB) and graded.¹⁸ Sputa were decontaminated using N-acetyl-L-cysteine-NaOH¹⁹; a 0.5-ml portion of the sputum sediment was cultured using the BACTEC MGIT 960 system (Becton and Dickinson, Franklin Lakes, NJ), and 0.2 ml was inoculated onto Lowenstein Jensen media. Cultures were incubated at 37°C for up to eight weeks. Mycobacterial blood cultures were performed using the MYCO/F LYTIC (Becton and Dickinson) or BacT/ALERT MB (bioMérieux, Marcy-l'Etoile, France) systems, which previously have been shown to have similar diagnostic yields.^{20,21} Positive cultures were assessed for AFB using ZN staining and light microscopy, and for *M. tuberculosis complex* using an anti-MPB64 antibody assay (Capilia TB-Neo, TAUNS Laboratories, Numazu, Japan) or the GenoType MTBDRplus assay (Hain Lifesciences GmbH, Nehren, Germany).

TB LAM tests were performed and graded by trained study staff according to manufacturer recommendations.²² Staff interpreting the TB LAM assays were blinded to clinical data and results of other diagnostic testing. Sixty µl of fresh urine was applied to the test strip which was incubated at room temperature for 25 minutes. The strip was visually inspected, and the intensity of any visualized test band was graded by comparing test band color intensity to the color intensity of a series of bands on a manufacturer-supplied paper reference card. Test band intensity was graded as zero if no band was visualized, and grade 1 through 5 for visualized bands. A blinded second reader immediately conducted an independent reading. The initial reading was considered the study result for that test, except for analyses of inter-reader agreement for which results of the first and second readers were considered. TB LAM results were neither provided to clinicians caring for enrolled participants nor used for clinical care decision-making.

Final diagnostic classification of participants

Participants were categorized into one of three diagnostic categories based on microbiological and clinical criteria.

Culture-positive tuberculosis—*M. tuberculosis* cultured from any specimen

Possible tuberculosis—No culture positive for *M. tuberculosis*, but at least one of the following: a) sputum smear microscopy positive but no sputum culture positive for any mycobacteria, b) started on anti-tuberculosis treatment with subsequent documented clinical

improvement within two months of enrollment, c) a diagnosis of active tuberculosis within two months of enrollment by a non-study clinician, d) death within two months of enrollment reported to be due to tuberculosis per a death certificate, autopsy report, or medical record.

No evidence of tuberculosis—Does not meet criteria for either ‘Culture-positive tuberculosis’ or ‘Possible tuberculosis’.

Statistical methods

Sample size—We calculated that 100 culture-positive tuberculosis cases/site would provide, for each of the two study sites, 95% confidence intervals (CI) for investigational test sensitivity of about $\pm 10\%$ over a range of investigational test sensitivities of 30% to 70%. Assuming a 20% prevalence of culture-positive tuberculosis among participants, the enrollment target was 500 participants/site (1000 total).

Analysis—Descriptive statistics were used to characterize the study population. Results for two TB LAM readers were compared and proportionate agreement and κ statistics were calculated. Nonparametric receiver operator characteristic (ROC) analysis was performed to evaluate sensitivity and specificity based on different band intensity thresholds for TB LAM test positivity. To construct ROC curves, results of participants classified as ‘Culture-positive tuberculosis’ or ‘No evidence of tuberculosis’ were used; results of participants classified as ‘probable TB’ were excluded. Sensitivities, specificities, and predictive values were calculated, with 95% CIs generated using the exact binomial distribution for the proportions. Unless stated otherwise, results shown for TB LAM testing are those using a test band positivity threshold of grade 2 (i.e. any test band of intensity grade 2 or higher was considered positive, and any band of lesser intensity was considered negative). Sensitivities were calculated separately for participants with ‘Culture-positive tuberculosis’ and ‘Possible tuberculosis’. Specificities were calculated for participants classified as ‘No evidence of tuberculosis’. A multivariable logistic regression model was used to investigate predictors of TB LAM test positivity. All statistical tests were 2-sided. Statistical calculations were performed using Stata 12 software (Statacorp, 2011, Texas).

Ethics

This study was approved by ethics committees of Johns Hopkins University School of Medicine, University of Cape Town, Joint Clinical Research Centre (Kampala, Uganda), and Uganda National Council for Science and Technology. Participants provided written informed consent.

Role of the funding source

This study was designed and implemented by investigators of the TB-Clinical Diagnostics Research Consortium (TB-CDRC). Neither the sponsor nor Alere was involved in study design, implementation, analysis, or decision to submit results for publication.

RESULTS

Participants

1037 individuals were screened and 1013 (97.7%) were enrolled (Figure 1). Sputum could not be obtained from 16/1013 (1.6%) of participants, who were excluded from the analysis. Characteristics of 997 analyzed participants are in Table 1. Median CD4 (cells/mm³) was 152 (IQR 41, 337), and was lower in participants enrolled in Uganda (97; IQR 21, 290) versus South Africa (217; IQR 87, 389; $P<0.001$ by nonparametric Wilcoxon rank-sum test).

630/997 (63.2%) participants had no specimen (neither respiratory nor blood) that was culture-positive for *M. tuberculosis*, and 367/997 (36.8%) had at least one specimen that was culture-positive for *M. tuberculosis*. Among those 367 participants, in 243 (66.2%) only respiratory specimen(s) were culture-positive, and in 108 (29.4%) respiratory and blood cultures were positive for *M. tuberculosis* (resulting in 351/997 [35.2%]) participants with pulmonary TB based on respiratory cultures); in 16 (4.4%) only the blood culture was positive. 128/367 (34.9%) culture-positive participants had at least one respiratory specimen that was positive by smear microscopy. As shown in Figure 1, 367/997 (36.8%) participants were classified as 'Culture-positive tuberculosis'; 57/997 (5.7%) as 'Possible tuberculosis'; and 573/997 (57.5%) as 'no evidence of tuberculosis' (Figure 1).

TB LAM test operating characteristics and distribution of results

A valid TB LAM result was obtained on the first attempt for all tests (997/997, 100.0%). The distribution of band intensity results was: no band (grade 0), 657/997 (65.9%); grade 1, 183/997 (18.4%); grade 2, 42/997 (4.2%); grade 3, 38/997 (3.8%); grade 4, 55/997 (5.5%); grade 5 or greater, 22/997 (2.2%). Between two independent readers, agreement as to presence versus absence of a test band of intensity grade 2 or higher was 96.1% (kappa 0.92), agreement as to presence versus absence of a test band of any intensity was 95.7% (kappa 0.90), and agreement as to test band grade was 83.4% (kappa 0.77).

TB LAM sensitivity among participants with culture-positive tuberculosis, and specificity among participants with no evidence of tuberculosis

Receiver operator characteristic curve analysis showed that a test band intensity positivity threshold of grade 2 maximized sensitivity and specificity (Figure 2A and Supplemental Digital Content 1–2). Using a positivity threshold of grade 2, overall TB LAM sensitivity was 136/367 (37.1%) (Table 2). Sensitivity was higher in Uganda than in South Africa (83/182 [45.6%] versus 53/185 [28.7%] $p=0.0008$). Overall specificity was 559/573 (97.6%). Specificities were 268/282 (95.0%) and 291/291 (100%) in Uganda and South Africa, respectively ($P=0.0001$). In a secondary analysis we explored the potential impact of incomplete gold-standard mycobacteriologic assessment on TB LAM specificity by requiring that, in order to be classified as 'no evidence of tuberculosis', a participant have at least one evaluable (non-contaminated) respiratory culture and a non-contaminated blood culture in addition to not meeting the criteria for 'Culture-positive tuberculosis' or 'Possible tuberculosis'. In this secondary analysis, specificity was 530/541 (98.0%; 95% CI 96.4, 99.0). TB LAM accuracy results stratified by hospitalization status at the time of enrollment are shown in Supplemental Digital Content 3.

TB LAM test sensitivity and specificity using a positivity threshold of grade 2, stratified by CD4 T cell count, are shown in Table 2 and Figure 2B. Sensitivity was 116/196 (59.2%) among culture-positive participants with CD4 ≤ 100 , and 20/169 (11.8%) among those with CD4 >100 ($P < 0.0001$). Sensitivity remained slightly higher in Uganda than in South Africa among participants with CD4 ≤ 100 (71/109 [65.1%] versus 45/87 [51.7%]; $P = 0.06$) but sensitivities were similar between the two sites when only hospitalized participants with CD4 ≤ 100 were considered (Uganda 61/92 [66.3%] versus South Africa 38/61 [62.3%]; $P = 0.60$). Specificity was slightly lower in the group with CD4 ≤ 100 than in the group with CD4 >100 (156/165 [94.5%] versus 399/403 [99.0%]; $P = 0.001$). Among 57 participants with 'possible tuberculosis', the TB LAM test was positive in seven (sensitivity 12.3%; 95% CI 5.1, 23.8). For reference, Supplemental Digital Content 4 shows TB LAM accuracy results if any visualized band was considered a positive test.

Factors associated with a positive TB LAM test (positivity threshold grade 2)

Among participants with 'Culture-positive tuberculosis', the adjusted odds ratio (AOR) for TB LAM positivity among participants with CD4 ≤ 50 and CD4 51–100 were 6.2 ($P < 0.001$) and 7.1 ($P < 0.001$), respectively versus CD4 >200 (Table 3); the AOR for mycobacteremia versus no mycobacteremia was 6.1 ($P < 0.001$), and for being hospitalized versus not hospitalized at the time of enrollment was 2.6, ($P = 0.03$). Among 124 participants with *M. tuberculosis* isolated from blood cultures, the TB LAM test was positive in 91 (73.4%). Within two months after enrollment 128/997 (12.8%) participants died, including 42/157 (26.8%) of participants who were TB LAM positive and 86/840 (10.2%) of participants who were TB LAM negative (OR 3.2, 95% CI 2.1, 4.9).

TB LAM test sensitivity (positivity threshold grade 2) compared with that of respiratory specimen direct smear microscopy

TB LAM test sensitivity was compared with that of respiratory specimen smear microscopy performed on two sputa at baseline. Among participants with culture-positive tuberculosis, 67/367 (18.3%) were positive by both the TB LAM test and ZN smear microscopy, 170/367 (46.3%) were negative by both tests, 69/367 (18.8%) were positive by the TB LAM test only, and 61/367 (16.6%) were positive by ZN smear microscopy only. TB LAM sensitivity was similar to that of smear microscopy (136/367 [37.1%] versus 128/367 [34.9%], $P = 0.53$). The combined sensitivity of TB LAM plus smear microscopy (either or both tests positive, 197/367 [53.7%]) was higher than the sensitivity of either test alone (Supplemental Digital Content 5). In participants with CD4 ≤ 100 , the combined sensitivity of TB LAM plus smear microscopy was 133/196 (67.9%).

DISCUSSION

Our multicenter study shows that the TB LAM test detected well over half of tuberculosis patients with advanced HIV-related immunosuppression – the group in which tuberculosis mortality is highest and the benefits of early ART initiation are most marked. Test specificity exceeded the target of 95% proposed by an international expert committee for a point-of-care tuberculosis test.²³ For the TB LAM test, agreement between two trained readers was high, and all tests yielded a valid result on the first attempt. TB LAM sensitivity

correlated with advancing immunosuppression, and a positive TB LAM test was associated with mycobacteremia and hospitalization. These features suggest that the target population for testing is clinically very ill adults who have advanced HIV disease and CD4 < 100. The availability of HIV rapid testing and expanding availability of point-of-care CD4 testing will facilitate identification of this target population, and the potential programmatic impact of TB LAM would be expected to be greatest in settings of high TB/HIV burden and low coverage and/or uptake of antiretroviral therapy. The combination of TB LAM and direct smear microscopy had higher sensitivity than did either test used alone, since these tests – one performed on urine and the other on sputum – detected tuberculosis in largely non-overlapping subsets of patients.

TB LAM test sensitivity was higher at the Uganda site than at the South African site. This likely reflects differences between study sites with respect to tuberculosis and HIV disease severity; in support of this are our findings of similar sensitivities between sites among hospitalized participants with CD4 < 100, and that enrollment site was not associated with TB LAM positivity when adjusted for other factors. However, we cannot fully exclude a role for potential differences between sites in *M. tuberculosis* bacterial characteristics, test interpretation after visualization, or test batch-to-batch variability. Test specificity was lower at the Uganda site than at the South African site. The specificity difference was most pronounced in the group with CD4 < 50, and could be related to failure to make a ‘gold standard’ culture diagnosis in some participants with tuberculosis and advanced immunosuppression.

Whether it is possible to improve the clinical sensitivity of urine LAM assays is an unresolved issue, and there are technical and biological aspects for consideration. For the TB LAM test the lower limit of LAM detection is reported to be 0.25 ng/ml (Alere, personal communication), and is limited by the polyclonal antibodies and detection system used. Optimization of antibodies and use of detection systems with better analytical sensitivity might favorably decrease the lower limit of LAM detection in clinical specimens, and point to the need for continued research in these areas.^{24,25} From a biological standpoint, the pathophysiological processes that result in LAM antigenuria are unclear but have implications with respect to whether assays that can detect exquisitely low antigen concentrations in urine will be useful in non-immunocompromised tuberculosis patients.²⁶ For example, if LAM antigenuria is the consequence of glomerular filtration of circulating LAM, then assays with high analytical sensitivity may be useful in detecting physically ‘distant’ pulmonary disease with relatively low total pathogen burden. On the other hand, if LAM antigenuria reflects clinically unrecognized renal tract tuberculosis, then application of more sensitive LAM assays to patients with only pulmonary tuberculosis will not be useful.²⁷ In our study, urine cultures and/or urine Xpert MTB/RIF testing might have helped to resolve this issue but were not performed. Available evidence shows that a high proportion of urine specimens that are TB LAM positive are also positive by Xpert MTB/RIF.^{28–30} Since detection of *M. tuberculosis* by Xpert MTB/RIF requires intact bacilli, the available evidence, while not conclusive, supports the hypothesis that LAM antigenuria reflects renal tract TB.

We are aware of three other published studies using the TB LAM lateral flow test, all of which used urine that had been previously collected and frozen. Lawn et al. tested urine specimens from 542 ambulatory HIV-infected adults being screened for tuberculosis using a rigorous protocol-specified battery of tests prior to initiation of antiretroviral therapy in South Africa.³¹ Using a grade 1 positivity threshold, test sensitivity was 28.2% overall, but was 66.7% in the group with CD4<50; specificity was 98.6% overall. Peter et al. have reported two diagnostic accuracy assessments using frozen urine from hospitalized HIV-positive adults in South Africa whose tuberculosis diagnostic work-up had been directed by routine care clinicians.^{32,33} They found, as we did in our study, that using a grade 2 positivity threshold optimized TB LAM sensitivity and specificity. The first study included 242 participants, and among those with culture-positive tuberculosis test sensitivity (grade 2 cut-off) was 50% overall and 58% in the group with CD4 ≥ 200; specificity was 75% among participants whose cultures were negative.³² Specificity was higher (94%) when culture-negative participants who met clinical criteria for tuberculosis were omitted from the specificity calculation; it is worth pointing out that mycobacterial blood cultures typically were not performed and some culture-negative participants may have had tuberculosis.³² The second study reported by Peter et al. included 281 participants (median CD4 89 cells/mm³), and TB LAM sensitivity and specificity (grade 2 cut-off) were 46% and 96%.³³ An unresolved issue is that of the optimal band positivity threshold, and our findings differ from those of Lawn and colleagues who reported high specificity using a grade 1 positivity threshold. Possible explanations are batch-to-batch variation in TB LAM test strips, differences in visual interpretation of faint bands, use of frozen vs. fresh urine, and/or bacterial contamination of urine samples causing weak false-positive bands.¹⁴

Our study has important strengths, including adherence to the recommendations of the Standards for Reporting of Diagnostic Accuracy Studies (STARD) Initiative and the QUADAS tool for quality assessment of diagnostic accuracy studies.^{34,35} To accurately classify participants, we cultured blood and sputum for mycobacteria to provide a rigorous gold standard including ability to detect disseminated non-pulmonary disease. In contrast to existing published studies, we used fresh rather than frozen urine.^{31–33} Performance of our study in two African settings and in hospitalized as well as ambulatory patients helps to clarify the assay's generalizability and to underscore that its primary role is likely to be in hospitalized patients and/or those with advanced immunosuppression. For clinical, laboratory, and data management procedures we implemented rigorous internal quality management, performed the study according to Good Clinical Practice standards, and received periodic external quality monitoring. While these practices maximized the validity of our study results, some may be challenging to implement in routine clinical practice.

A study limitation was that repeat mycobacteriology assessments were performed at two months after enrollment only for a subset of participants, namely those who had a positive LAM test but no culture positive for *M. tuberculosis* at baseline. This approach could cause bias in favor of the TB LAM assay. However, only 8 participants (2.2% of participants with microbiologically-confirmed TB) with this pattern of baseline results had a follow-up culture positive for *M. tuberculosis*. Another limitation is that our testing algorithm, despite including sputum and blood cultures, may have failed to detect some participants with

tuberculosis (especially solely extrapulmonary, non-disseminated tuberculosis) and therefore overestimated the true sensitivity or underestimated the true specificity of the TB LAM test. Our study did not incorporate sputum Xpert MTB/RIF testing, which may have additive yield when combined with urine TB LAM testing and which therefore is a promising companion test for optimizing the diagnostic yield of a ‘same-day’ testing algorithm. We did not assess implementation feasibility or impact on clinical outcomes in the context of routine clinical care – important issues that warrant careful future assessment in light of the accuracy findings from our study and those of others and as a next step towards understanding whether and how the TB LAM test should be implemented.^{31–33} Cost will be an important consideration. A cost-utility analysis restricted to hospitalized, severely immunocompromised, HIV-infected African adults showed that the addition of lateral flow urine LAM testing to standard tuberculosis diagnostics detected additional TB cases at an incremental cost in US dollars per disability-adjusted life year averted of USD \$86 in Uganda and USD \$353 in South Africa, and was therefore likely to be cost-effective in those populations.³⁶ Finally, while we purposefully sought diversity within our study population, our findings may not apply to all other populations, especially those with less advanced immunosuppression and/or less severe tuberculosis.

In summary, our multicenter study showed that, in tuberculosis suspects with advanced HIV-related immunosuppression, the TB LAM urine test detected over half of tuberculosis patients, and the TB LAM test used in combination with sputum smear microscopy detected two-thirds of tuberculosis patients. TB LAM specificity was high in this study that rigorously sought a gold-standard tuberculosis microbiological diagnosis. These features in a truly point-of-care urine test might allow rapid tuberculosis diagnosis and treatment in resource-limited settings and thereby reduce mortality and morbidity in a vulnerable patient population. Our results provide strong rationale for studies to assess the impact of urine TB LAM testing on patient-centered outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Flow of study participants.

*Among these 14 participants, 4 (28.6%) had enrollment mycobacterial blood cultures that were not evaluable for *M. tuberculosis* (blood culture not performed in one participant, and blood cultures overgrown with a non-mycobacterial organism not subjected to species identification for 3 participants); all other enrollment respiratory and blood cultures were evaluable. Among these 14 participants, 7 (50.0%) died before the two-month follow-up visit, 3 (21.4%) were lost/not able to be contacted for the two-month follow-up visit, 3

(21.4%) completed study follow-up including one who had a blood culture positive for *M. tuberculosis* at follow-up, and one (7.1%) withdrew consent.

A.

B.

Figure 2.

(A) Receiver-operator characteristic curve for the Determine TB LAM Ag assay. The integers adjacent to points on the graph correspond to grade 1, grade 2, grade 3, grade 4, grade 5, and refer to minimum test band intensity required for characterization of a test result as positive.

(B) Determine TB LAM Ag test sensitivity among participants with culture-positive tuberculosis and specificity among participants with no evidence of tuberculosis, using a positivity threshold of grade 2 and stratified by CD4 count (in cells/mm³).

Table 1

Characteristics of the study population

	Uganda (N=501)	South Africa (N=496)	All (N=997)
<i>Demographic or clinical characteristic at enrollment, n (%) unless otherwise specified</i>			
Female	316 (63.1)	314 (63.3)	630 (63.2)
Median age in years (IQR)	32 (28, 49)	35 (28, 41)	33 (29, 37)
Age categories, in years			
< 30	209 (41.7)	173 (34.9)	382 (38.3)
30–39	208 (41.5)	185 (37.3)	393 (39.4)
40–49	71 (14.2)	99 (19.9)	170 (17.1)
50 or greater	13 (2.6)	39 (7.9)	52 (5.2)
Hospitalized	351 (70.1) ^a	201 (40.5) ^b	552 (55.4) ^c
Median CD4 (IQR) cells/mm ³	97 (21, 290)	217 (87, 389)	152 (41, 337)
CD4 counts (cells/mm ³)			
<50	194 (38.7)	84 (16.9)	278 (27.8)
50–99	55 (11.0)	51 (10.3)	106 (10.6)
100–199	86 (17.2)	97 (19.6)	183 (18.4)
200	161 (32.1)	262 (52.8)	423 (42.3)
missing	5 (1)	2 (0.4)	7 (0.7)
On antiretroviral therapy at enrollment	183 (36.5)	140 (28.2)	323 (32.4)
Ongoing cough	490 (97.8)	489 (98.6)	979 (98.2)
Fevers within past 4 weeks	464 (92.6)	366 (73.8)	830 (83.3)
Night sweats within past 4 weeks	377 (75.3)	430 (86.7)	807 (80.9)
Weight loss within past 4 weeks	449 (89.6)	475 (95.8)	924 (92.7)
History of prior TB treatment	84 (16.8)	105 (21.2)	189 (19.0)
<i>Distribution in final diagnostic category, n(%)</i>			
Culture-positive tuberculosis	182 (36.3)	185 (37.3)	367 (36.8)
Mtb cultured from respiratory specimens only	104 (57.1)	139 (75.1)	243 (66.2)
Mtb cultured from respiratory and blood specimens	66 (36.3)	43 (23.2)	108 (29.4)
Mtb cultured from blood specimen only	12 (6.6)	4 (2.2)	16 (4.4)
Possible tuberculosis	37 (7.4)	20 (4.0)	57 (5.7)
No evidence of tuberculosis	282 (56.3)	291 (58.7)	573 (57.5)

Footnotes

Median CD4 (IQR) cells/mm³ in hospitalized participants

^a₅₄ (17, 142);

^b₇₁ (23, 140);

^c₆₇ (20, 140)

Abbreviation: Mtb, *Mycobacterium tuberculosis*

Table 2

Accuracy of the Determine TB LAM Ag assay, overall and stratified by CD4 count (in cells/mm³)^a and enrollment site

	All participants	Uganda	South Africa	P-value for comparison of Uganda vs. South Africa
SENSITIVITY				
Overall	136/367 (37.1%) 32.1, 42.2	83/182 (45.6%) (38.2, 53.1)	53/185 (28.7%) (22.3, 35.7)	0.0008
CD4 100	116/196 (59.2%) (52.0, 66.1)	71/109 (65.1%) (55.4, 74.0)	45/87 (51.7%) (40.8, 62.6)	0.06
CD4 > 100	20/169 (11.8%) (7.4, 17.7)	12/71 (16.9%) (9.1, 27.7)	8/98 (8.2%) (3.6, 15.5)	0.08
SPECIFICITY				
Overall	559/573 (97.6%) (95.9, 98.7)	268/282 (95.0%) (91.8, 97.3)	291/291 (100%) (98.7, 100)	0.0001
CD4 100	156/165 (94.5%) (89.9, 97.5)	112/121 (92.6%) (86.4, 96.5)	44/44 (100%) (92.0, 100)	0.06
CD4 > 100	399/403 (99.0%) (97.5, 99.7)	154/158 (97.5%) (93.7, 99.3)	245/245 (100%) (98.5, 100)	0.01
PREDICTIVE VALUE OF A POSITIVE TEST				
Overall	136/150 (90.7%) (84.8, 94.8)	83/97 (85.6%) (77.0, 91.9)	53/53 (100%) (93.3, 100)	0.004
CD4 100	116/125 (92.8%) (86.8, 96.7)	71/80 (88.8%) (79.7, 94.7)	45/45 (100%) (92.1, 100)	0.02
CD4 > 100	20/24 (83.3%) (62.6, 95.3)	12/16 (75.0%) (47.6, 92.7)	8/8 (100%) (63.1, 100)	0.12
PREDICTIVE VALUE OF A NEGATIVE TEST				
Overall	559/790 (70.8%) (67.5, 73.9)	268/367 (73.0%) (68.2, 77.5)	291/423 (68.8%) (64.1, 73.2)	0.19
CD4 100	156/236 (66.1%) (59.7, 72.1)	112/150 (74.7%) (66.9, 81.4)	44/86 (51.2%) (40.1, 62.1)	0.0002
CD4 > 100	399/548 (72.8%) (68.9, 76.5)	154/213 (72.3%) (65.8, 78.2)	245/335 (73.1%) (68.0, 77.8)	0.83

^a CD4 results were missing for 7 participants, all of whom were included in the overall results but not in the results stratified by CD4

Table 3

Factors associated with a positive Determine TB LAM Ag assay among participants with culture-confirmed tuberculosis

Risk Factor	Total N=367	LAM positive N=136	Odds Ratio	Adjusted odds ratio [*]
Sex				
Female	210	75 (35.7%)	REF	
Male	157	61 (38.9%)	1.1 (0.8, 1.8)	1.0 (0.6, 1.8)
Age				
<30	146	56 (38.4%)	REF	
30–39	140	53 (37.9%)	0.98 (0.6, 1.6)	0.9 (0.5, 1.7)
40–49	66	24 (36.4%)	0.98 (0.5, 1.7)	0.8 (0.4, 1.7)
50 or greater	15	3 (20.0%)	0.4 (0.1, 1.5)	0.3 (0.05, 1.6)
Karnofsky score ^{**}				
80 or greater	157	37 (23.6%)	REF	
70 or less	209	99 (47.4%)	2.9 (1.8, 4.7) ^a	0.7 (0.3, 1.6)
Baseline cultures				
Only sputum culture(s) positive for <i>Mtb</i>	243	45 (18.5%)	REF	
Blood culture (+/- sputum culture) positive for <i>Mtb</i>	124	91 (73.4%)	12.1 (6.7, 22.3) ^a	6.1 (3.4, 11.1) ^a
CD4 ^{***}				
50	136	83 (61.0%)	15.7 (6.1, 40.2)	6.2 (2.5, 15.1) ^a
51–100	60	33 (55.0%)	12.2 (4.3, 34.2)	7.1 (2.7, 19.0) ^a
101–200	81	12 (14.8%)	1.7 (0.7, 4.5)	1.3 (0.4, 3.5)
>200	88	8 (9.1)	REF ^a	
Site of enrollment				
Cape Town	185	53 (28.7)	REF	
Uganda	182	83 (45.6)	2.1 (1.3, 3.2)	1.5 (0.7, 2.8)
Hospitalization status at enrollment				
Not hospitalized	121	22 (18.2)	REF	
Hospitalized	246	114 (46.3)	3.9 (2.2, 6.7) ^a	2.6 (1.1, 6.5) ^b
ART status at enrollment				
On ART	103	29 (28.2)	0.6 (0.4, 1.0)	0.6 (0.3, 1.1)
Not on ART	264	107 (40.5)	REF	
Cough				
Present at baseline	357	131 (36.7)	0.6 (0.2, 2.0)	0.6 (0.1, 3.6)
Not present at baseline	10	5 (50.0)	REF	

Risk Factor	Total N=367	LAM positive N=136	Odds Ratio	Adjusted odds ratio[*]
Fever				
Present at baseline	292	112 (38.4)	1.3 (0.8, 2.3)	1.2 (0.6, 2.6)
Not present at baseline	75	23 (32.0)	REF	
Night sweats				
Present at baseline	299	105 (35.1)	0.6 (0.4, 1.1)	0.8 (0.4, 1.6)
Not present at baseline	68	31 (45.6)	REF	
Weight loss				
Present at baseline	352	135 (38.4)	8.7 (1.1, 68.4) ^c	4.0 (0.4, 41.8)
Not present at baseline	15	1 (6.7)	REF	
Tuberculosis treatment history				
Prior tuberculosis treatment	64	23 (36.5)	0.9 (0.5, 1.7)	1.0 (0.4, 2.2)
No known prior tuberculosis treatment	303	113 (37.3)	REF	

Notes:

* adjusted for all other risk factors included in the table

** results missing for 1 participant;

*** results missing for 2 participant

^aP < 0.01;

^bP = 0.03;

^cP = 0.01

Abbreviation: Mtb, *Mycobacterium tuberculosis*