Urinary Lipoarabinomannan as Predictor for the Tuberculosis Immune Reconstitution Inflammatory Syndrome

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Background: Upon initiation of antiretroviral therapy (ART), 15.7% [95% confidence interval (CI): 9.7% to 24.5%] of tuberculosis (TB)-HIV–coinfected individuals experience paradoxical worsening of their clinical status with exuberant inflammation consistent with immune reconstitution inflammatory syndrome (IRIS). We investigated whether a positive urinary TB lipoarabinomannan (LAM) antigen enzyme-linked immunosorbent assay test before ART initiation was associated with development of paradoxical TB-IRIS.

Methods: In a prospective observational cohort in Mulago Hospital, Kampala, Uganda, we measured pre-ART urinary LAM concentrations in HIV-infected patients on TB treatment. Patients who developed TB-IRIS (according to the International Network for the Study of HIV-associated IRIS case definition) were compared with patients who remained IRIS free for at least 3 months.

Results: Twenty-six individuals with TB-IRIS and 64 without IRIS were included in the analysis. The median time to TB-IRIS was 14 days (interquartile range: 11–14 days). Univariate analysis showed that a positive pre-ART urinary LAM test [OR: 4.6 (95% CI: 1.5 to 13.8), $P = 0.006$] and a CD4 count <50 cells/mL [OR: 21 (95% CI: 2.6 to 169.4), $P = 0.004$] were associated with an increased risk of TB-IRIS. In multivariate analysis, only a baseline CD4 T-cell count <50 cells/mL was predictive of IRIS ($P < 0.004$). Sensitivity and specificity of a positive pre-ART urinary LAM test to diagnose IRIS were 80.8% (95% CI: 60.6 to 93.4) and 52.4% (95% CI: 39.4 to 65.1), respectively.

Conclusions: If CD4 T-cell count testing is available, a pre–highly active antiretroviral therapy urinary LAM test has no added value to predict TB-IRIS. When CD4 T-cell count is not available, a positive LAM test could identify patients at increased risk of TB-IRIS.

Key Words: HIV, tuberculosis, HAART, immune reconstitution, lipoarabinomannan

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INTRODUCTION

According to the most recent World Health Organization (WHO) estimates,[1] the burden of tuberculosis (TB) worldwide was 9.4 million incident cases and 14 million prevalent cases in 2009.[2] Coinfection with HIV accounted for about 11%–13% of incident TB cases. Africa bears the largest burden of worldwide cases with 80% of coinfected individuals diagnosed on the continent. In resource-limited settings, smear microscopy is still commonly used for TB disease screening, despite its suboptimal performance in HIV patients with advanced immunosuppression and atypical disease presentation.[3]

Detection of circulating *Mycobacterium tuberculosis* (Mtb) lipoarabinomannan (LAM) antigen in TB suspects, a surrogate marker of infection, is among the portfolio of new point of care tests for TB diagnostics. A urinary-based commercial LAM–enzyme-linked immunosorbent assay (ELISA) assay, the Clearview TB ELISA (Inverness Medical Innovations, Waltham, MA) has been evaluated in various populations. Reported sensitivity was higher in HIV-positive individuals (21%–67%) but inversely correlated with CD4 T-cell counts,[4–9] indicating that LAM detection assays could

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TB IRIS Group members can be viewed in Appendix I.

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play a role as rapid rule-in test for Mtb in HIV-infected individuals with advanced immunosuppression.

Upon highly active antiretroviral therapy (HAART) initiation, 15.7% (9.7%–24.5%) of TB-HIV–infected individuals experience a paradoxical deterioration of their clinical status,[10] a condition known as TB immune reconstitution inflammatory syndrome (IRIS) or immune reconstitution disease. TB-IRIS can present as the sudden clinical inflammatory manifestation of occult subclinical TB disease (unmasking TB-IRIS) or as the worsening of a successfully treated TB infection (paradoxical TB-IRIS). IRIS is postulated to result from a rapid and unbalanced restoration of pathogen-specific immune responses after HAART initiation[11]. A rabbit model proposed that IRIS was dependent on antigen load at the time of immune reconstitution[12]. In humans, low CD4 T-cell counts at the start of HAART, a shorter delay between TB therapy and HAART, and the presence of disseminated TB were reported risk factors for TB-IRIS.[13] Based on data from a small study, it has been hypothesized that a higher LAM antigen load pre-HAART may also be predictive of TB-IRIS.[5]

We sought to evaluate the performance characteristics of pre-HAART urinary LAM as a predictor for TB-IRIS in a prospective observational cohort of TB-treated patients with HIV infection starting HAART in Kampala, Uganda.

METHODS

Study Population

This substudy was nested in a prospective cohort study of HIV-infected adults on treatment for active TB disease, HAART naive, and qualifying for HAART initiation. Patients were recruited at the National Tuberculosis and Leprosy Program Clinic, Mulago Hospital in Kampala, Uganda, between December 2007 and July 2010. Patients were followed up for 6 months to 2 years for the development of paradoxical TB-IRIS.

Eligibility criteria for the cohort study were as follows: (1) adult (>18 years) living within a 20-km radius of the hospital; (2) confirmed TB-HIV infection according to WHO guidelines; (3) having taken TB treatment for <2 months; and (4) eligible for HAART according to Uganda National Antiretroviral Treatment Guidelines (CD4 count ≤250 cells/μL). Individuals without a treatment supporter were excluded. The main findings of the cohort study have been published elsewhere.[14]

For inclusion in the LAM substudy, individuals needed (1) to have been followed up for at least 3 months and (2) not to have developed a non–TB-related type of IRIS.

Definitions

TB infection was defined according to the WHO TB/HIV guidelines[15] based on clinical examination, acid-fast bacilli stain microscopy, solid medium mycobacterial culture, and chest x-rays. Where indicated, histology and cytology of aspirates (lymph nodes, pleural fluid, abscesses) and ultrasound scan were additionally performed.

TB-IRIS was defined according to the International Network for the Study of HIV-associated IRIS (INSHI) case definition.[16] Study participants who developed signs and symptoms suggestive of another type of IRIS or study patients with late-onset IRIS (ie, fulfilling INSHI criteria but occurring >3 months after HAART initiation) were excluded from analysis.

Non-IRIS cases were patients with a follow-up of at least 3 months who did not develop signs and symptoms suggestive of IRIS.

Sample Collection

Urine collection started in July 2008. Urine samples were collected pre-HAART, after 1 and 3 months of HAART, and at IRIS. A midstream urine sample was collected in a sterile urine container, kept at 4°C while waiting for transportation on the same day. Samples were aliquoted in polypropylene tubes and kept frozen at —80°C until analysis. Urine gravity was measured by refractometry (URC/Nalpha, Atago, Japan).

LAM-ELISA Assay

LAM was measured using the Clearview TB ELISA (Inverness Medical Innovations), a direct antigen sandwich immunoassay, following manufacturer's instructions.

On the day of analysis, frozen urine aliquots were thawed, boiled for 30 minutes at 95°C, and centrifuged. Supernatant was collected for analysis. Each plate included in duplicate: a standard curve with purified LAM antigen (received from S. Svenson and B. Hamasur, Karolinska Institute, Sweden[17]), 100 μL of the positive and negative control provided with the Clearview TB ELISA kit, and 100 μL from each sample. The plate was read at 450 nm, and the ELISA test was considered valid if the mean of the positive controls minus the mean of the negative controls was between 0.3 and 0.5. Results were considered positive if the optical density was at least 0.1 above the signal of the negative control.

Ethical Considerations

The study was approved by the Makerere University Faculty Ethics committee, the Mulago Hospital Research Committee, and the Uganda National Council of Science and Technology. Informed consent was obtained from all study participants.

Statistical Analysis

All statistical analyses were performed using Stata statistics software (version 10.2; Stata Corp, College Station, TX). Data were summarized by count or median and interquartile range (IQR) for nonnormally distributed variables. Normality was assessed using D'Agostino and Pearson omnibus normality test.

Differences in LAM concentration medians were compared using the Wilcoxon rank sum test and the Wilcoxon signed rank test for paired data.

Test performance characteristics (sensitivity and specificity) of LAM were estimated in TB-IRIS and non-IRIS cases, using the "diagt" Stata component.

Logistic regression was used to study predictors of IRIS. A threshold of $P < 0.1$ was used for variable inclusion

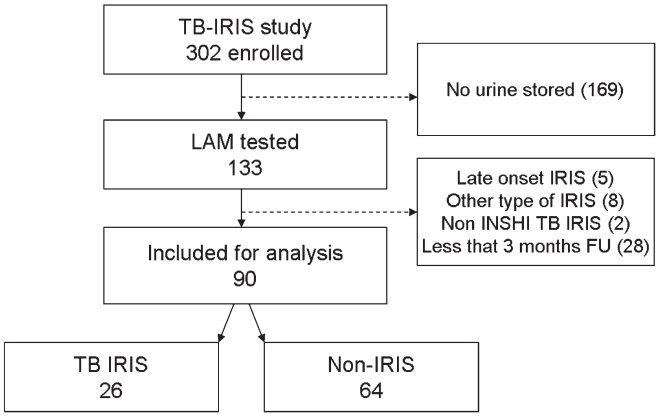

FIGURE 1. Flow chart.

in the multivariate model, which was then simplified by backward elimination. The following variables were analyzed: LAM concentration, age, sex, pre-HAART CD4 count, sputum smear (SS), smear Lowenstein–Jensen cultures, type of clinical TB presentation, and the time from TB treatment start to HAART initiation. Linear regression was used to assess the association between LAM positivity and degree of culture positivity, sputum grading (negative, scanty, or 1+, 2+, 3+), or the type of TB [SS-negative (–) pulmonary TB (PTB), SS-positive (+) PTB, extrapulmonary TB ± PTB]. All *P* values reported were 2-sided at alpha of 0.05.

RESULTS

Study Participants

Three hundred two patients were enrolled in the cohort study. Inclusion into the LAM substudy started 9.5 months after the start of the cohort study. Therefore, urine was obtained for only 133 individuals. Forty-three patients were excluded: 5 patients developed late-onset IRIS, 8 a non–TB-related form of IRIS [herpes zoster IRIS (5), herpes simplex IRIS (2), and toxoplasmosis IRIS (1)], 2 suspected TB-IRIS but not meeting the INSHI definition, and 28 did not complete the 3 months of follow-up; 5 patients started HAART, but died before month 3, the other 23 patients never started HAART (7 died, 4 declined to participate in the study, 1 moved out of the study area, and 11 resigned for "other reasons"). In total, 90 individuals were included in the analysis of which 26 were classified as TB-IRIS and 64 as non-IRIS (Fig. 1).

The median (IQR) time of follow-up for all patients in the substudy was of 332 days (168–353 days) and the median (IQR) time to IRIS was of 14 days (11–14 days). Non-IRIS individuals were followed up for a median (IQR) of 333 days (170–413.5 days). Baseline characteristics of substudy patients are presented in Table 1. All individuals had normal serum creatinine levels at study inclusion (reference range: 0.5–1.2 mg/dL). TB-IRIS and non-IRIS patients were comparable for all variables except pre-HAART CD4 T-cell count and delay in starting HAART after the onset of antituberculous therapy. Patients who developed TB-IRIS had a lower CD4 T-cell count [20.5 cells/mm^3 (11–40) vs. 71 (26–158), $P = 0.0001$] and a shorter time between TB treatment and the initiation of HAART [36 days (24–58 days) vs. 52 days (33–65.5 days), $P = 0.037$] than the non-IRIS group.

Pre-HAART LAM Positivity is Predictive of IRIS

Performance characteristics of the LAM assay to predict IRIS were as follows: sensitivity, 21/26 {80.8% [95% confidence interval (CI): 60.6% to 93.4%]} and specificity, 33/63 [52.4% (95% CI: 39.4% to 65.1%)].

In patients with positive pre-HAART urinary LAM, the odds ratio for developing TB-IRIS after the initiation of

TABLE 1. Characteristics of Study Patients

Characteristics	Total	All	TB-IRIS	Non-IRIS	*P*
Gender: male, n (%)	90	49 (54.4)	14 (53.8)	35 (54.7)	0.942
Age (years), median (IQR)	90	31.5 (27–37)	30 (27–35)	32 (26.5–37.5)	0.738
CD4 (cell/mm^3), median (IQR)	89	51 (19–118)	20.5 (11–40)	71 (26–158)	**<0.001**
Creatinine (mg/dL), median (IQR)	88	0.57 (0.51–0.72)	0.64 (0.53–0.67)	0.56 (0.51–0.72)	0.545
SS results, n (%)	70				0.524
Negative		31 (44.3)	9 (42.9)	22 (44.9)	
Scanty or 1+		22 (31.4)	5 (23.8)	17 (34.7)	
2+		9 (12.9)	3 (14.3)	6 (12.2)	
3+		8 (11.4)	4 (19.0)	4 (8.2)	
Culture positive, n (%)	46	33 (71.7)	12 (85.7)	21 (65.6)	0.286
Type of TB, n (%)	90				0.732
SS– PTB		28 (31.1)	7 (28.9)	21 (32.9)	
SS+ PTB		35 (38.9)	12 (46.1)	23 (35.9)	
Extrapulmonary TB ± PTB		27 (30.0)	7 (26.9)	20 (31.2)	
Urine specific gravity, median (IQR)	90	1.015 (1.010–1.017)	1.015 (1.012–1.017)	1.015 (1.010–1.018)	0.804
Delay between TB treatment and HAART initiation (days), median (IQR)	90	48 (31–63)	36 (24–58)	52 (33–65.5)	**0.037**

Bold *P* values indicate significant difference between groups.

HAART was 4.6 (95% CI: 1.5 to 13.8, $P = 0.006$). Moreover, the median pre-HAART LAM concentration was significantly higher in patients who developed TB-IRIS compared with those not developing IRIS [1.25 ng/mL (IQR: 0.2–7.1 ng/mL) vs. 0.1 ng/mL (IQR: 0–0.5 ng/mL), $P = 0.001$]. There was no significant difference in urinary LAM concentration between IRIS and non-IRIS patients at month 1 and month 3 of follow-up.

Variation in LAM Concentrations During HAART

Urinary LAM concentration decreased significantly during follow-up in IRIS and non-IRIS individuals (Fig. 2A). Urine from 8 patients was collected at IRIS time point. There was no increase in LAM concentration at the time of IRIS (Fig. 2B). In individuals who developed TB-IRIS, pre-HAART LAM concentration was not associated with the duration of TB treatment before HAART ($P = 0.567$).

LAM positivity was negatively correlated with CD4 T-cell counts [OR: 4.66 (95% CI: 2.43 to 8.93), $P < 0.0001$].

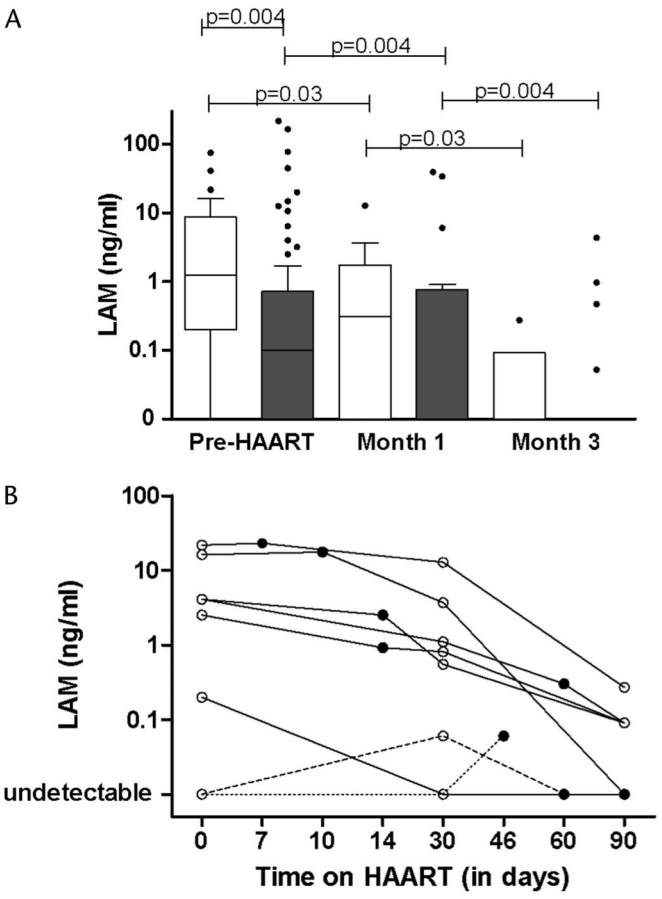

FIGURE 2. A, Variation in LAM concentration during HAART follow-up in TB-IRIS (□) and non-IRIS (■) individuals [IQR, median, and outliers (•)]. B, Dynamic of LAM concentration on HAART in patients developing TB-IRIS at different time points including TB-IRIS (•).

LAM and TB

Sputum culture positivity at time of enrollment was associated with LAM positivity (n = 45; OR: 6.75 (95% CI: 1.69 to 26.67), $P = 0.008$). However, no significant association was observed between LAM positivity and SS positivity grading (n = 69; $P = 0.29$), type of TB (n = 90; $P = 0.15$), duration of TB treatment (n = 88; $P = 0.506$), nor tuberculin skin test (TST) result (n = 89; $P = 0.110$). Patients with a positive baseline LAM result were TST negative and vice versa (Fig. 3).

Risk Factors for the Development of TB-IRIS

In the univariate model, a positive pre-HAART urinary LAM test result ($P = 0.006$) and a pre-HAART CD4 T-cell count <50 cell/mm^3 ($P = 0.004$) were associated with increased risk of TB-IRIS (Table 2). LAM concentration was inversely associated with CD4 T-cell count ($P = 0.043$). In multivariate analysis, a CD4 T-cell count <50 cells/mm^3 increased the risk of developing TB-IRIS by 21 (95% CI: 2.6 to 169.4, $P = 0.004$) compared with those with CD4 T-cell count >100/mm^3.

DISCUSSION

In this prospective observational cohort of HIV patients on TB treatment in Kampala, Uganda, we showed an association between LAM and TB-IRIS. Baseline urine LAM concentration was significantly higher among patients who subsequently developed paradoxical TB-IRIS. Moreover, the LAM levels decreased during HAART and TB treatment.

Our results corroborate data from a small study in South Africa comparing 5 TB-IRIS cases and 17 controls.[5] In this study, all 5 TB-IRIS cases (100%), but only 1 control patient tested positive for LAM at TB diagnosis before antiretroviral therapy initiation.

LAM is released by metabolically active bacteria.[18] This might explain the observed association between LAM positivity and sputum culture positivity, and the apparent lack of association observed between LAM positivity and SS grading.[19]

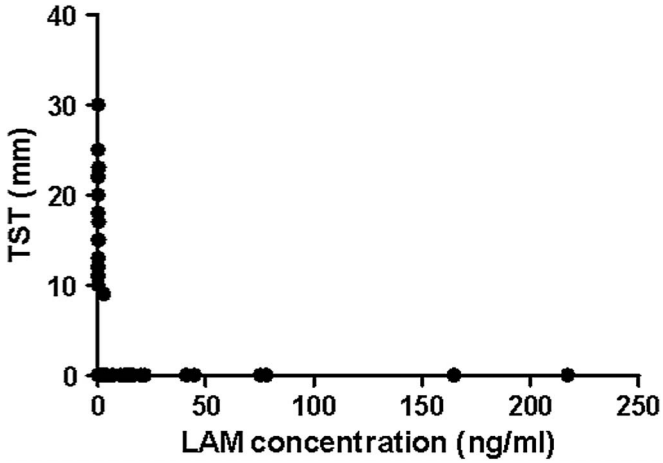

FIGURE 3. Relation between TST reaction (millimeters) and LAM concentration (nanograms per milliliter).

TABLE 2. Predictors of IRIS by Logistic Regression (Univariate Analysis)

	Total	TB-IRIS	OR	*P*
Pre-HAART LAM†				
Positive	51	21	4.6 (1.5–13.8)	**0.006**
Negative	38	5	1	
Gender				
Male	49	14	1.0 (0.4–2.6)	0.942
Female	41	12	1	
Age (years)				
<31.5	45	15	0.6 (0.3–1.6)	0.354
≥31.5	45	11	1	
CD4 (cells/mm^3)				<0.001
<50	44	21	21.0 (2.6–169.4)	**0.004***
50–100	21	4	5.4 (0.5–52.9)	0.146
≥100	24	1	1	
SS				0.564
Negative	31	9	1	
Scanty or 1+	22	5	0.7 (0.2–2.5)	0.609
2+	9	3	1.2 (0.2–6.0)	0.804
3+	8	4	2.4 (0.5–12.0)	0.270
Mtb culture				
Positive	33	12	3.1 (0.6–16.6)	0.178
Negative	13	2	1	
Type of TB				0.667
SS– PTB	28	7	1	
SS+ PTB	35	12	1.6 (0.5–4.7)	0.426
EPTB ± PTB	27	7	1.0 (0.3–3.5)	0.937
Delay of TB treatment (days)				0.382
<14	3	1	2 (0.7–5.5)	0.180
14–56	51	17	2 (0.2–25.3)	0.593
≥56	35	7	1	

*In the multivariate model (including pre-HAART LAM and CD4 T-cell count), only "CD4 T-cell count <50/mm^3" was significant: $P = 0.004$; OR: 21.0 (2.6–169.4).
†LAM measured at baseline for n=89.
Bold values indicate significant *P* value.

A lower CD4 T-cell count was predictive of IRIS, and LAM was inversely associated with CD4 T-cell count. Patients who developed IRIS were started on HAART sooner than non-IRIS patients. According to the Ugandan guidelines at the time of the study, patients with HIV TB coinfection with a CD4 count <200% cells/μL started HAART maximum 8 weeks after TB diagnosis. It may be, however, that clinicians started the "sicker" patients on HAART sooner.

In the absence of a CD4 count result, a positive LAM test could be used to identify patients who should be referred to a more specialized treatment center and who may require more frequent follow-up. A positive LAM test, however, should not be used as a reason to delay HAART to reduce the risk of IRIS because delaying HAART in patients with low CD4 count will increase mortality.[20]

No transitional increase was observed at TB-IRIS. This suggests that TB-IRIS may be a feature of immune response against Mtb rather than because of an increase in Mtb antigen load. This also might reflect that LAM is an imperfect measure of the antigen concentration at the site where TB-IRIS occurs.

Anergy to TST has been reported in severely immunosuppressed HIV-infected individuals.[21] In our cohort, all patients who tested TST negative had detectable LAM levels, whereas TST-positive subjects did not have detectable LAM Ag in their urine. Severely immunosuppressed individuals, anergic to TST, may present with higher LAM concentration as their disease is more likely to be more disseminated. Conversely, individuals with a strong response to TST are possibly more able to clear Mtb and LAM antigens faster during the anti-TB treatment and before initiation of HAART.

Our study was unable to evaluate the performance of urinary LAM for TB diagnosis as samples were collected a median of 44 days after beginning TB treatment.

In our study, urine samples were collected during study visit. Ideally, LAM should be measured on a 24-hour urine collection or on first morning urine. Urine specific gravity was similar in both groups indicating similarity in the TB-IRIS and non-IRIS patients in terms of urine concentration.

In conclusion, if CD4 T-cell count testing is available, a pre-HAART urinary LAM test has no added value to predict TB-IRIS. When CD4 T-cell count is not available, a positive LAM test could identify patients at increased risk of TB-IRIS. For the moment, performing a LAM test remains relatively complicated, but this may change if an easy to perform point of care test becomes available. The clinical utility of monitoring the LAM concentration during TB treatment needs to be investigated in larger cohorts.

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APPENDIX I: TB IRIS STUDY GROUP MEMBERS

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