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ORIGINAL ARTICLE

Diagnostic and prognostic utility of urine LF-LAM assay for tuberculosis detection in advanced HIV

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ABSTRACT

Background: The diagnostic tests available for TB are very accurate but samples are yield dependent. WHO recommends the use of LF LAM for diagnosis of active TB in patients with advanced HIV disease.

Material and Methods: This cross-sectional validity study was conducted at the Infectious Diseases Department of the Pakistan Institute of Medical Sciences, a large public-sector tertiary care hospital in Pakistan, from October to December 2024. A total of 113 patients with advanced HIV disease were enrolled in this study. The patients underwent Xpert MTB Ultra and LF LAM. These patients were followed for 8 weeks to assess survival.

Results: There were 99(87.6%) males and 14(12.4%) females of total 113 patients. The number of patients who survived and those who did not survive at 8 weeks was 88(77.9%), and 25 (22.1%) respectively. The LF LAM was found to have sensitivity (76.1%), Specificity (86.1%), PPV (87.27%), NPV (74.13%), and LR^+ (5.42). The Discion tree analysis showed that positive LF LAM leads to the probability of 76.3% survival. The age (OR 0.946; 95%CI 0.905-0.978; p=0.012), Xpert MTB Ultra (OR 14.288; 95%CI 2.24-91.05; p=0.005), TB LAM (OR 0.416;95% CI 0.034-0.842; p=0.030) were found to be significant predictors of survival at 8 weeks.

Conclusion: LF LAM is a promising diagnostic strategy with good prognostic value in patients with HIV/TB coinfection.

Keywords: Diagnostic accuracy, Decision tree, LF LAM, Xpert MTB ultra

BACKGROUND

Human immunodeficiency virus is the most common cause of acquired immunodeficiency. Annually 36.7 million cases of HIV/AIDs are documented throughout the world, leading to mortality in 1.1 million cases. Till 2017, 97400 cases of HIV/AIDS have been recorded by the National AIDS Control Program (NACP).[1]

Tuberculosis (TB) is a major global public health problem with an estimated 10.4 million newly emerging active TB cases worldwide in 2016. It is the most common opportunistic infection occurring in patients with HIV. The improvement in diagnostic capability for tuberculosis has led to an increase in microbiologically diagnosed cases and fewer missed diagnoses.[2]

In the year 2010, about 34% or 2.1 million out of 39.5 million TB cases were recorded as TB patients who confirmed being HIV positive. Co-infection of TB in HIV-positive individuals is prevalent and ranges around 33%, and approximately 50% of the TB-infected individuals in the world are expected to have HIV. These people undergo faster HIV replication cycles and are prone to many other infections, including but not limited to TB. This quickened replication increases a patient's chances of becoming an AIDS victim at an alarming rate. According to further studies conducted, HIV was suspected to be within 1.1 million (13%) out of the 8.8 active TB patients. Estimates show that approximately 208,000 HIV-TB co-infected individuals died (between 177,000 and 242,000) after 2000.[3]

Lipoarabinomannan is a lipo-polysaccharide found in the cell walls of Mycobacterium. It is shed from metabolically active or dying bacterial cells.[4] Evaluation of urine LAM test has shown good results in

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This article can be cited as: Zafar A, Akhtar N, Virk ST, Virk KA, Umair MM, Saghir H. Diagnostic and prognostic utility of urine LF-LAM assay for tuberculosis detection in advanced HIV. Infect Dis J Pak. 2025; 34(1): 13-18.

DOI: https://doi.org/10.61529/idjp.v34i1.368

Receiving date: **08 Jan 2025** Acceptance Date: **12 Mar 2025**
Revision date: **27 Jan 2024** Publication Date: **30 Mar 2025**

detecting TB among PLWH in developing countries, although sensitivity and specificity vary to the degree of immunodeficiency.[4] Recently, urinary lipoarabinomannan (LAM) based tuberculosis diagnosis has been in discussions for a while. Urine collection is a less intrusive specimen, easily obtained from patients at home or in the hospital. Currently, few commercial urine LAM-based diagnostic tests are advised for patients with HIV and CD4 count less than 100 cells per microliter.[5] The World Health Organization (WHO) recommends the use of rapid point-of-care Alere Determine TB-LAM Ag lateral flow assay (LF-LAM) for the diagnosis of TB in PLWH and has kept the cut-off at ≤200.[6]

WHO published policy guidance about the use of lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis and screening of active tuberculosis in people living with HIV. It showed a sensitivity of 26% and specificity of 96% in all settings in patients with CD4 counts ≤200.[7]

Tuberculosis is the most prevalent co-infection in HIV. The diagnostic tests available for tuberculosis are very accurate, but samples are yield dependent. The patients with advanced HIV disease are mostly very sick and the invasive procedures to obtain samples for gene Xpert MTB testing are difficult. The LF-LAM can be used as an alternative for diagnosing TB in these complex situations. This study is designed to assess the diagnostic accuracy of LF-LAM in MTB detection in the local population. This test can be employed due to the ease of testing technique and cost-effectiveness. These benefits can have an impact on the prevalence of HIV-TB coinfection.

MATERIAL AND METHODS

This cross-sectional validity study was conducted at the Infectious Diseases Department of the Pakistan Institute of Medical Sciences, a large public sector tertiary care hospital in Pakistan from October to December 2024. This study is approved by the Institutional Review Board with Letter no.F.1-1/2015/ERB/SZAMBU/1310. The sample size was 102, calculated by the WHO calculator keeping the prevalence at 37%, sensitivity at 26%, specificity at 96%.[7] Adults with advanced HIV disease and signs and symptoms of TB (fever, cough, weight loss, night sweating, and patients CD4 count of ≤200 were included in the study. All patients who had signs and symptoms of tuberculosis and were HIV-negative were included. The patients who have signs of active TB but are already on antituberculosis drugs, and the patients who received ATT or quinolone for more than 7 days within the past 3 months were excluded.

The patient's data was collected in a predesigned and pretested Performa. Informed consent was obtained from the patient or attendant before gathering the information. They were assured that data would remain confidential, and that identity would not be revealed. The patient who is found to be HIV positive and has any symptom or sign that directs the workup of tuberculosis was identified. The Gene Xpert MTB ultra was obtained from the National TB reference lab. The Gene Xpert for MTB was performed on a machine (Model No Gene Xpert GXIV-4). All the patients who were presented to the ID clinic or ER, whether newly diagnosed with HIV or already on Anti-retroviral therapy (ART) and are hospitalized, were included in the study. The screening was done by HIV rapid test by UNI GOLD HIV kit by Trinity biotech and Abbott, and Gene Xpert for HIV by Cepheid S/N802318. The baseline CD4 count of every patient was recorded at the time of co-infection diagnosis. The CD4 count is performed by an analyzer by Alere Pima at the Islamabad center of NACP (National Aids Control Program). The Alere LAM was performed by using the TB LAM Ag (X25) kit by Abbott Laboratories. The Positive and negative tests were recorded.

The data was analyzed using IBM SPSS (Statistical Package for Social Sciences), version 29. The patient's demographic details, such as age, gender, CD4 count, Xpert MTB, and LF LAM results, were recorded and presented in percentages. The sensitivity, specificity, positive predictive value (PPV), negative predictive Value (NPV), Likelihood Ratio (LR), and diagnostic accuracy of LF LAM were calculated, keeping the Gene Xpert MTB as the gold standard for tuberculosis diagnosis. The probabilities were incorporated in the decision tree that was diagrammed based on the outcome of the patients at 8 weeks of diagnosis (Dead/Alive). The prognostic value of TB LAM was assessed by binary logistic regression.

RESULTS

A total of 113 patients were enrolled in the study. The demographic details of each patient were recorded, as

shown in Table 1. The normality of the variables was assessed by using the Kolmogorov-Smirnov test. All the variables were significantly non-normal for example, Age D (113) =0.10, p = 0.008, Xpert MTB D(113)=0.52 p=<0.001, and TB LAM D(113), The post-test odds were 6.81 calculated by a Likelihood ratio value of 5.42 obtained from sensitivity and specificity. The positive (PPV) and negative TB LAM assay (NPV) probability was also calculated.

The above calculations show that the TB LAM is a good assay to screen and even diagnose cases of TB/HIV Coinfections. These true positive (TPR) and true negative rates (TNR) can be depicted with the help of the decision tree in Figure 1. This simplified decision tree depicts the probability for positive and negative TB LAM compared to the disease probability. Further, it provides an insight into the probability of survival outcome at 8 weeks. This shows that the positive TB LAM assay correctly predicted patients' survival at 8 weeks in about 76.3% of the cases.

The prognostic value of TB LAM was assessed by its predictability of survival at 8 weeks follow-up. The binary logistic regression was employed for this purpose. All the variables in the model, notably Age, gender, CD4 Count, Xpert MTB, AFB smear and TB LAM positivity, Previous ART and ATT Experience, and WHO clinical Stage were significantly non-normal, D (113) =0.386-0.962, p<0.009.

The continuous variables (Age, CD4 Count) were tested for linearity of logit. The site of involvement and WHO clinical stage were found to be highly collinear, so the former was dropped from the final prognostic model. The Model overall predicted that 92% of those survived. The age of the patients and positivity of Xpert MTB ultra and TB LAM assay were found to predict the survival of patients at 8 weeks significantly, with Xpert MTB being followed most considerably by age and then TB LAM. The output of univariate and multivariate logistic regression analysis is given in Table 3 in terms of odd ratios (OR), and 95% Confidence Interval (CI) for these odd ratios.

The above results show that the TB LAM assay is a good predictor of patient survival at 8 weeks but is not better than Xpert MTB. This can be attributed to the early detection of TB but not to the start of anti-tuberculous (ATT) therapy in advanced HIV disease. The ATT was initiated for all patients when they were confirmed by Xpert MTB ultra, rather than on the positivity of TB LAM because this test was the gold standard in our study. This factor probably added to the significance of Xpert MTB ultra being a predictor of outcome.

Table-I: Characteristics of patients.

Demographic details		
Age		39.10±12.81
CD4 Count		96.66±57.08
Gender	Male	99 (87.6%)
	Female	14 (12.4%)
Site of involvement	Pulmonary	30(26.5%)
	Pleural	2(1.8%)
	CNS	10 (8.8%)
	TB Lymphadenitis	3 (2.8%)
	Disseminated	24 (21.2%)
ART Experience	Yes	18 (15.9%)
	No	95 (89.1%)
Survival of patients in 8 weeks	Dead	25 (22.1%)
	Alive	88(77.9%)
Previous ATT	Yes	21 (18.6%)
	No	92 (81.4%)
WHO Clinical stage	Stage 2	3 (2.7%)
	Stage 3	59 (52.2%)
	Stage 4	51 (45.1%)

Table-II: Parameters for diagnostic accuracy of TB LAM.

Parameters	Sensitivity	Specificity	PPV	NPV	LR^+
	76.1%	*86.1%*	*87.27%*	*74.13%*	*5.42*

*PPV; Positive Predictive Value, NPV; Negative Predictive Value; LR^+; Likelihood ratio for Positive test

Table-III: The prognostic model for TB LAM assay.

Factors	Study parameter		Univariate Logistic regression			Multivariate Logistic Regression		
			P value	Unadjusted OR	95% CI for UOR	P value	Adjusted OR	95% CI for AOR
Age								
Age	39.10±12.81		<0.001	1.027	0.178-1.090	0.012	0.946	0.905-0.938
Xpert MTB								
	Dead	Alive						
Positive	10 (8.8%)	53(46.9%)	0.706	1.187	0.488-2.887	0.005	14.288	2.242-91.05
Negative	15 (13.3%)	35 (31.0%						
TB LAM								
Positive	13 (11.5%)	42 (37.2%	0.07	0.44	0.178-1.090	0.030	0.416	0.034-0.842
Negative	12 (10.6%	46 (40.7%)						

**(R^2); 0.376 (Hosmer & Lemeshow), 0.221 (Cox & Snell), 0.338 (Nagelkerke), Model χ^2 (1) 28.153, p<0.002.*

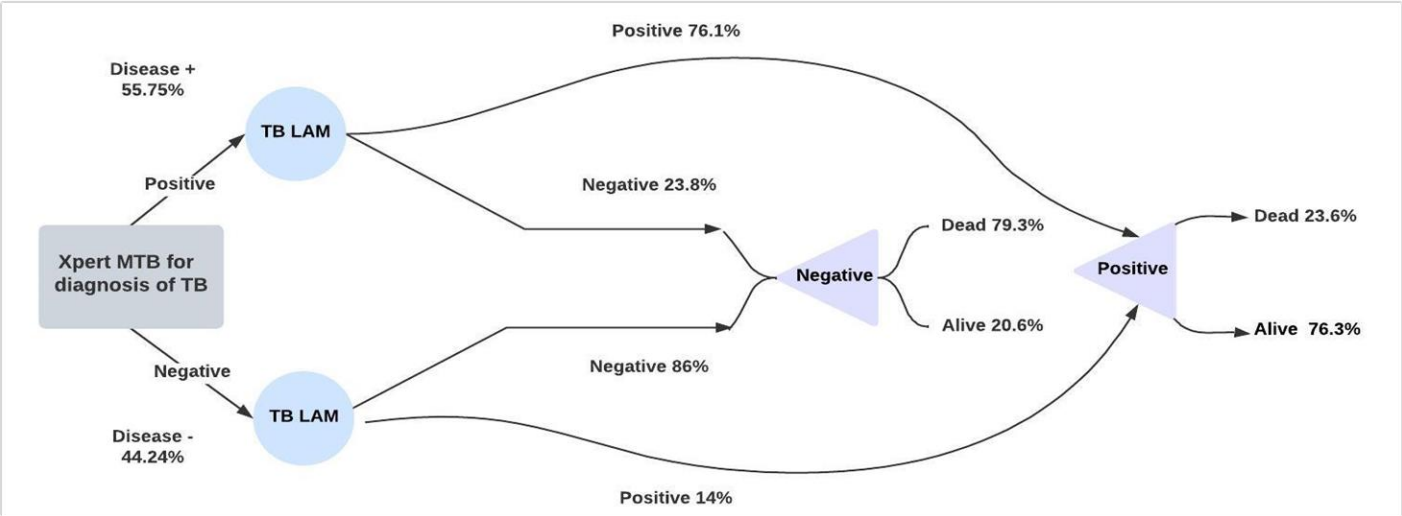

Figure-I: Simple decision tree for the TB LAM assay regarding patient survival.

DISCUSSION

The patients living with advanced HIV disease are usually hospitalized and sick, many of these are unable to produce diagnostic samples like sputum and cannot undergo surgery to obtain tissue or fluid samples for molecular and culture-based tests. The non-sputum samples (Novel TB Diagnostics) may have lower sensitivity than culture and molecular-based assays but have equivalent diagnostic yields when the ability to collect samples for testing is considered.[8]

Alere TB LAM has rapid turnaround time and simple techniques. These qualities proved the basis for the WHO's endorsement of TB LAM. It should be prioritized to guide the diagnosis and treatment of tuberculosis in adults, adolescents, and children with signs and symptoms of TB, advanced HIV disease, and a CD4 count of ≤ 200. WHO reports diagnostic accuracy of Alere TB LAM advanced HIV disease, irrespective of signs and symptoms as in inpatient settings, CD4<200, sensitivity 64% (35-87%), and specificity 82% (67-93%).[7] These values are approximately like the results of our study (sensitivity 76.1% and specificity 86.1%). Another study reported the comparative performance of Xpert MTB ultra and LF LAM. The sensitivity of LF LAM was 63% and specificity 88%. The sensitivity and specificity of Xpert MTB ultra were reported to be 76% and 98% respectively in the same study when the TB LAM <100. The diagnostic accuracy of TB LAM is not much different from Xpert MTB at CD4 count < 100.[9] One prospective cohort study in Africa stated that LF-LAM did not improve the diagnostic yield when used with Xpert Ultra.[10]

Alere TB LAM with acceptable diagnostic accuracy and good predictability of the outcome of patients with HIV/TB Co-infection as shown by the decision tree in our study. This can also be a cost-effective testing strategy for hospitalized patients with advanced HIV disease. The cost of Alere TB LAM is 7 USD/test and that of Xpert MTB ultra is 30 USD/test in Pakistan. The testing facility by Xpert MTB Ultra is provided by the

National TB Control Program (NTP) in Pakistan free of charge but requires proper sampling, trained staff, and at least a day of reporting time. These factors add to the strength of TB LAM. WHO recommends LF LAM as a cost-efficient strategy for hospitalized patients in terms of incremental cost-effectiveness ratio (ICERs), Willingness to Pay (WTP), and disability-adjusted life in years (DALYs). This recommendation is for Alere LAM because it is commercially available.[9]

Another study reported sensitivity of Alere LAM (34.40%), Fuji LAM (70.70%), Xpert MTB Ultra (87.60%), and Xpert MTB ultra +Fuji LAM (87.58%-98.05%). The Xpert MTB Ultra and Fuji LAM had the lowest ICER (0.635) and DALYs (676.9) and were the most cost-effective. Furthermore, Decision cost analysis for TB LAM and other testing strategies is required to incorporate it in the local guidance and clinical practices at major public and private hospitals.[11]

In our study's results, Xpert MTB Ultra and TB LAM significantly impact patients' outcomes at 8 weeks OR: 0.416 (95% CI 0.034-0.842) for TB LAM. A meta-analysis demonstrated a twofold greater mortality risk in patients with positive Urinary LAM than those who had negative test results. It reported adjusted odd ratios from more than five studies and found that urinary LAM is an independent predictor of mortality in HIV/ TB Coinfection[12]. One study derived a risk score containing TB LAM, and mortality at 2 months was lower in the validation cohort (22.8%) than in the derivation cohort (29.8%). Urinary LAM also predicted the mortality OR 2.8 (95% CI 1.0-3.2, p=0.040).[13]

The STAMP trial in Africa was a double-blind trial and presented its results in 2019. The urinary LAM was introduced as an intervention in TB care compared to the standard of care (routine testing for TB). Mortality at 56 days in the intervention and standard of care group was reported as adjusted OR -2.8 (95% CI -5.8-0.3 p=0.074) and adjusted OR (95% CI -0.69-1.01 p=0.068). The mortality rate was significantly lower with intervention in subgroups of anemia, CD 4 count <100, and suspected cases of TB on admission.

The EXULTANT trial is currently underway in two African countries. It’s a randomized double-blind trial that will compare Xpert MTB Ultra and TB LAMs efficacy regardless of TB symptoms versus standard of care (TB testing per WHO recommendations in symptomatic TB patients).[14]

The ease of technique, low cost, bedside availability, rapid turnaround time, and acceptable efficiency are the factors that prove TB LAM is a promising testing strategy for TB patients in advanced HIV disease in a resource-limited country like Pakistan. The spectrum of HIV disease in Pakistan is currently more towards advanced clinical stages (WHO stages 3 and 4) and TB is the most prevalent opportunistic infection in HIV-infected patients[15]. The inclusion of TB LAM testing in local guidance of HIV will provide survival benefits to patients with advanced HIV. The lack of research and proper decision-making cost analysis are the limitations to implementing TB LAM as routine TB testing in hospitals. Our study will prove to be a cornerstone for further studies and cost-benefit analysis in the future.

CONCLUSION

TB LAM has an acceptable diagnostic yield and promising prognostic ability in advanced HIV-diseased patients. Easy technique, low cost, bedside availability, rapid turnaround time, lack of expert requirements, and easy sampling are the additional factors that make Urinary LAM the most promising Novel Testing strategy in Low and Middle-Income countries (LMICs) like Pakistan.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Abeer Zafar: Main Conception of study, manuscript writing, final approval, accountable for every aspect of this research work

Nasim Akhtar: Critical revision, final approval, accountable for every aspect of this research work

Sana Tahir Virk: Data analysis, data interpretation, manuscript writing, final approval, accountable for every aspect of this research work

Kazim Abbas Virk: Study design, final approval, accountable for every aspect of this research work

Malik Muhammad Umair: Critical revision, final approval, accountable for every aspect of this research work

Hina Saghir: Interpretation of results, final approval, accountable for every aspect of this research work

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