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Major Article

Utility of urine lipoarabinomannan (LAM) in diagnosing mycobacteria infection among hospitalized HIV-positive patients

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ABSTRACT

Objectives: Cross-reactivity with nontuberculous mycobacteria (NTM) species might limit the use of urine lipoarabinomannan (LAM) test to diagnose tuberculosis (TB) in people living with HIV (PLWH). This study aimed to investigate the utility of the LAM test among hospitalized HIV-positive patients.
Methods: This prospective study enrolled HIV-positive inpatients with any TB symptom or seriously ill patients with advanced immunodeficiency. Urine samples were tested using the Alere Determine LAM Ag, and participants were categorized as confirmed TB, confirmed NTM infection, unclassified mycobacteria infection, and no mycobacteria infection based on microbiologic reference standards.
Results: A total of 382 participants were included. The prevalence of confirmed TB and NTM infection was 5.24% (20 of 382) and 4.45% (17 of 382), respectively. The sensitivity and specificity of the urine LAM for TB diagnosis were 65.00% (95% confidence interval [CI] 40.78-84.61) and 89.36% (95% CI 85.68-92.36), respectively. The LAM test for NTM yielded a sensitivity of 58.82% (95% CI 32.92-81.56) and specificity of 88.61% (95% CI 84.87-91.70). Notably, the negative predictive values of the urine LAM for TB and NTM were 97.85% (95% CI 95.63-99.13) and 97.85% (95% CI 95.63-99.13), respectively.
Conclusions: Cross-reactivity with NTM cause high false-positive LAM for TB diagnosis in PLWH. The correct identification of mycobacteria species is crucial for deciding treatment strategies.

Introduction

People living with HIV (PLWH) who are co-infected with nontuberculous mycobacteria (NTM) or *Mycobacterium tuberculosis* (MTB) may have a similar clinical presentation. Although tuberculosis (TB) remains the leading cause of morbidity and mortality among PLWH (World Health Organization, 2020), emerging evidence suggests that the prevalence of NTM diseases is increasing (Brode et al., 2014; Rachow et al., 2011). This may be attributed to the grossly underestimated prevalence in the past because both MTB and NTM show positivity to the conventional smear acid-fast staining method (Liu et al., 2018; Tran et al., 2019). A recent study of intensified screening in Ghana found a substantial prevalence of unrecognized pulmonary TB (60 of 473, 12.7%) and frequent NTM isolation (38 of 473, 8%) among PLWH before initiation of antiretroviral therapy (ART) (Bjerrum et al., 2016). Otherwise, our previous study showed that NTM infection accounted for 52.9% among hospitalized HIV-positive patients with a positive tuberculosis culture, whereas TB accounted for 47.1% (Cai et al., 2017). The diagnostic methods for TB are more advanced and possess a higher rate of sensitivity and specificity than the tools available for NTM infections. In general, the NTM species display significant heterogeneity in their susceptibility to standard anti-TB drugs. Thus, incorrect di-

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; FN, false negative; FP, false positive; LAM, lipoarabinomannan; LF, lateral flow; MRS, microbiological reference standard; MTB, Mycobacterium tuberculosis; NPV, negative predictive value; NTM, nontuberculous mycobacteria; PPV, positive predictive value; Sn, sensitivity; Sp, specificity; TN, true negatives; TP, true positive.

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agnosis of NTM diseases as TB may lead to unfavorable outcomes and unnecessary quarantine.

Lipoarabinomannan (LAM) is a glycolipid present in the outer cell wall of mycobacterial species, which is released from metabolically active or degenerating bacterial cells. The World Health Organization (WHO) recommends the use of the rapid, point-of-care Alere Determine TB-LAM Ag lateral flow assay (LF-LAM) for the diagnosis of TB in PLWH (World Health Organization, 2015). Clinical evaluation of the urine LAM test has consistently shown promising results for diagnosing TB among PLWH in resource-limited settings, although sensitivity and specificity vary greatly among studies and in relation to the degree of immunodeficiency (De et al., 2020). However, there have been disagreements on whether NTM infection causes false-positive LAM results (Gupta-Wright et al., 2018; Nel et al., 2017). Interestingly, urine LAM was repurposed for diagnosing NTM infection in individuals with cystic fibrosis, which showed high specificity but low sensitivity (Bjerrum et al., 2016; Qvist et al., 2014). However, the performance of urine LF-LAM for the diagnosis of TB and NTM infection in PLWH is still unknown. Therefore, we conducted this diagnostic study in Shanghai, China, where the prevalence of TB and NTM diseases was relatively high.

Materials and methods

Study design and participants

This study enrolled PLWH from the Department of Infectious Diseases and Immunology, Shanghai Public Health Clinical Center, Shanghai, China. Hospitalized HIV-positive patients who met 1 of the following 4 criteria were eligible to participate in this study: (1) presence of any TB symptom (fever, cough, weight loss, night sweating), (2) seriously ill HIV-positive patients, (3) advanced HIV disease (WHO clinical stage 3 or 4), and (4) CD4 count <200 cells/μL. Patients were excluded if they had received specific treatment for either TB or NTM infection and/or quinolone for more than 7 days within the past 3 months.

The study procedures complied with the ethical standards of the ethical guidelines of the 1975 Declaration of Helsinki and were approved by the ethics committee of the Shanghai Public Health Clinical Center. Written informed consent was obtained from all patients before the screening process. The study was registered on ClinicalTrial.gov with registration number NCT04600232.

Study procedures

Random urine specimens were collected and immediately stored at 4°C. The LF-LAM tests were performed on unprocessed urine samples within 3 days of collection. The Alere Determine TB-LAM Ag assay (Alere Scarborough, Inc. United States) was performed by a trained technician following the manufacturer's instructions. For each sample, 60 µL urine was applied to the sample pad, and the test was read between 25 and 35 minutes later. The laboratory technician reading the urine LAM results was blinded to the participants' medical history. A grade 1 band intensity or higher was interpreted as a positive result.

Reference standard testing was performed in the reference laboratory of Shanghai Public Health Clinical Center and included modified acid-fast smear, Xpert, solid culture on the modified Roche medium, and liquid culture. Specimens, such as sputum, pus, secretions, tissue fluid, urine, and feces, were subjected to acid-fast staining and mycobacterial culture (modified Roche culture medium). Blood and tissue fluid were collected in BACTEC Myco/F Lytic bottles and cultured for 42 days in a BACTEC 9120 blood culture system (Becton Dickinson Biosciences, Franklin Lakes, NJ). Biopsy tissues were acid-fast-stained as part of the pathological examination. Strains obtained from the solid and liquid cultures were examined for MPB-64 protein by enzyme-linked immunosorbent assay and identified as MTB or NTM for positive or negative strains, respectively (Qi et al., 2020). The GeneXpert test was performed on the basis of the manufacturer's protocol. For some cases with NTM infection, species were identified using the PCR-reverse dot kit (Yanengbio, Shenzhen, China). The following variables and participant characteristics were collected: age, sex, estimated glomerular filtration rate, liver enzymes, serum albumin, and complete blood count. Current CD4 cell count and plasma HIV RNA levels were also tested. Demographic information and clinical history were obtained from patients through interviews and medical record review.

Before data analysis, clinical investigators, who were masked to LF-LAM test results, categorized patients as "confirmed TB," "confirmed NTM," "unclassified mycobacteria," and "no mycobacteria" using a microbiological reference standard. Positive GeneXpert results or positive MPB64 antigen and positive Mycobacterial culture results were classified as confirmed TB, whereas negative MPB64 antigen and positive mycobacterial culture were classified as confirmed NTM infection. Patients with unclassified mycobacteria had only acid-fast bacilli (AFB)-smear positivity in the sputum, stool, or other specimens, but negative results of culture or Xpert. Patients with as no mycobacteria had negative AFB smear, cultures, and Xpert test results or some patients who did not have microbiological examination but were definitely ruled out as having a mycobacteria infection based on clinical diagnosis. Patients with mycobacteria infection included patients with confirmed TB, confirmed NTM, and unclassified mycobacteria infection.

Statistical analysis

The data analysis was performed using MedCalc Version 15.8 (MedCalc Software, Broekstraat, Mariakerke, Belgium). For the descriptive analysis, the median and interquartile range (IQR) were calculated, and the frequencies of categorical variables were calculated. The difference in variables was tested using the Mann-Whitney *U* test, chi-square test, and Fisher exact tests to compare medians and proportions as appropriate. We calculated the performance measurements, including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and associated 95% confidence intervals (CIs) for urine LF-LAM test by comparison with a microbiological reference standard. A post hoc analysis was conducted to estimate pooled sensitivity and specificity across CD4 strata. $P < .05$ was considered statistically significant.

Results

Demographic characteristics of the study population

Between August 20, 2020, and March 24, 2021, 626 potentially eligible participants were screened. A total of 389 HIV-positive inpatients were considered eligible for LAM testing, and 382 were included in the analyses (Figure 1). The participants had a median CD4 cell count of 86.5 (IQR, 27-228) cells/μL, and most (340 [89.01%]) were male, and 204 (53.40%) were ART-naive (Table 1). A total of 160 participants (41.88%) did not present any symptoms, 119 (31.15%) presented 1 symptom, and 103 (26.96%) presented 2 or more symptoms. Of those, 5.24% (20 of 382) were classified as confirmed TB, 4.45% (17 of 382) as confirmed NTM, 1.31% (5 of 382) as unclassified mycobacteria, and 89.01% (340 of 382) as no mycobacteria, and none as mixed TB-NTM infection. A total of 53 patients (13.9%) yielded positive results for TB-LAM tests.

Diagnostic accuracy of LF-LAM test for diagnosing TB

In participants with confirmed TB, 65% (13 of 20) had at least 1 positive fluorescence smear microscopy result. Nineteen patients were culture positive. Of the 16 patients who performed the

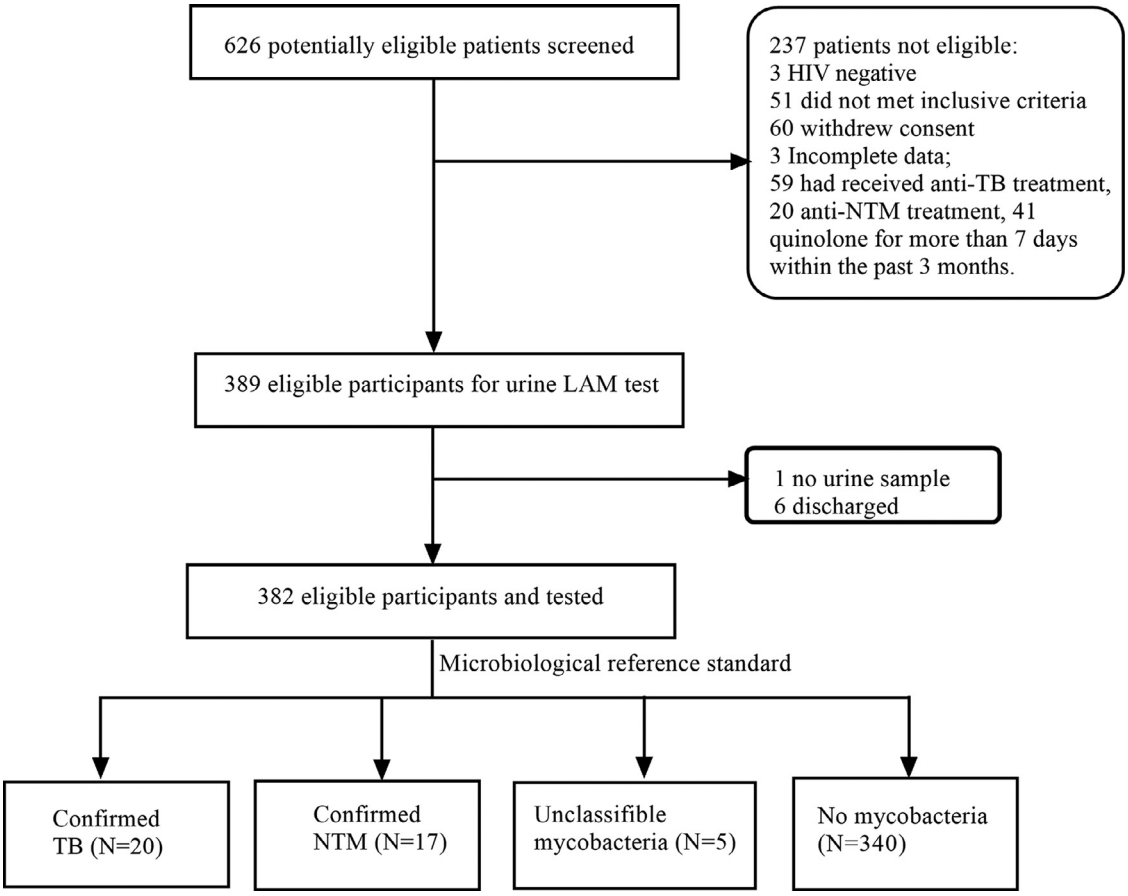

Fig. 1. Flow diagram of study participants.
Abbreviations: LAM = lipoarabinomannan; NTM = nontuberculous mycobacteria; TB = tuberculosis.

Table 1
Demographic and clinical characteristics.

Variables	Overall population (N = 382)	Confirmed TB (n = 20)	Confirmed NTM (n = 17)	Unclassified mycobacteria (n = 5)	No mycobacteria (n = 340)
Age (years)	45 (32-57)	41 (32-54.5)	49 (30.5-57.5)	48 (41-67.5)	44 (32-57.5)
Male, n (%)	340 (89.01)	17 (85.00)	15 (88.24)	5 (100)	304 (89.14)
ALT (U/L)	21 (13-37)	19.5 (13.5-28)	21.0 (11.5-29)	23 (18.75-86.25)	21 (13-39)
Albumin (g/L)	35.74 (30.70-40.23)	31.99 (25.91-34.01)	33.07 (29.15-37.48)	30.18 (23.91-35.66)	36.11 (31.09-40.76)
Glucose (mmol/L)	5.53 (4.70-6.58)	6.00 (4.66-7.54)	4.95 (4.50-6.05)	4.84 (4.63-7.84)	5.53 (4.71-6.46)
Hemoglobin (g/L)	116.5 (97-133)	94.5 (86.5-110.5)	103 (82.25-127)	103 (77.50-127.75)	119 (99-135)
White blood cell count ($\times 10^9$/L)	4.83 (3.67-6.81)	6.09 (4.61-7.27)	4.22 (2.89-7.08)	4.43 (3.60-8.07)	4.81 (3.60-6.57)
CD4 cell count (cells/μL)	87.5 (29-231)[a]	52 (29.5-146.5)	38 (11.75-102.75)	75 (31-178.25)	94.5 (30-253.5)[a]
On ART, n (%)	204 (53.40)	10 (50)	7 (41.18)	2 (40)	185 (54.41)
Positive TB symptom, n (%)	222 (58.1)	14 (70)	13 (76.47)	4 (80)	191 (56.18)
LAM positive, n (%)	53 (13.87)	13 (65)	10 (58.82)	2 (40)	28 (8.24)

Data are expressed as median (interquartile range), or n (%).
ART = antiretroviral therapy; IQR = interquartile range; LAM = lipoarabinomannan; NTM = nontuberculous mycobacteria; TB = tuberculosis.
[a] Four patients (no mycobacteria group) did not have CD4 cell counts available.

tests, GeneXpert test results were also positive among 13 patients (81.25%). The sensitivity and specificity of the LF-LAM test were 65.00% (95% CI 40.78-84.61) and 89.36% (95% CI 85.68-92.36), respectively (Table 2). In participants with CD4 $\leq$ 100 cells/μL, the sensitivity increased to 78.57% (95% CI 49.2-95.34) , but the specificity decreased to 87.17% (95% CI 81.51-91.60) (Table 3).

NTM infection and urine LF-LAM positivity

A total of 17 patients with confirmed NTM diseases were enrolled in this study. NTM species in 7 patients were identified, including *M. avium* (4 patients), *M. kansasii* (1 patient), *M. avium/M. kansasii* mixed (1 patient), and *M. intracellulare* (1 patient) (Table 4). Of the 17 patients with NTM diseases, LF-LAM test results were positive among 10 patients. Therefore, it yielded a sensitivity of 58.82% (95% CI 32.92-81.56) for diagnosing NTM diseases. The specificity was 88.61% (95% CI 84.87-91.70) (Table 2). The sensitivity and specificity in participants with CD4 $\leq$ 100 cells/μL were comparable to those in the total population (58.33% vs 58.82% and 85.19% vs 88.61% , respectively) (Table 3).

Diagnostic accuracy of LF-LAM test for diagnosing mycobacterial infection

After ascertaining that LF-LAM test results could be positive in both patients with TB and those with NTM diseases, we then

Table 2
Performance of the urine LAM test for diagnosis confirmed TB, confirmed NTM, and Mycobacteria infection.

	Confirmed TB	Confirmed NTM	Mycobacteria infection
N	377	377	382
TP	13	10	25
FP	38	41	28
FN	7	7	17
TN	319	319	312
Sensitivity (95% CI)	65.00% (40.78-84.61)	58.82% (32.92-81.56)	59.52% (43.28-74.37)
Specificity (95% CI)	89.36% (85.68-92.36)	88.61% (84.87-91.70)	91.76% (88.32-94.46)
PPV (95% CI)	25.49% (14.33-39.63)	19.61% (9.82-33.12)	47.17% (33.30-61.36)
NPV (95% CI)	97.85% (95.63-99.13)	97.85% (95.63-99.13)	94.83% (91.86-96.96)

CI = confidence interval; FN = false negative; FP = false positive; LAM = lipoarabinomannan; NPV = negative predictive value; NTM = nontuberculous mycobacteria; PPV = positive predictive value; TB = tuberculosis; TN = true negative; TP = true positive.

Table 3
Sensitivity and specificity of urine LAM against the MRS by CD4 strata.

CD4 strata	MRS	N	TP	FP	FN	TN	Sn (95% CI)	Sp (95% CI)	PPV (95% CI)	NPV (95% CI)
0-	Confirmed TB	201	11	24	3	163	78.57% (49.2-95.34)	87.17% (81.51-91.60)	31.43% (16.85%-49.29%)	98.19% (94.81-99.63)
100	Confirmed NTM	201	7	28	5	161	58.33% (27.67-84.83)	85.19% (79.31-89.92)	20.00% (8.44-36.94)	96.99% (93.11-99.01)
cells/μL	Mycobacteria infection	204[a]	20	17	9	158	68.97% (49.17-84.72)	90.29% (84.90-94.24)	54.05 (36.92-70.51)	94.61% (90.02-97.51)
>100	Confirmed TB	172	2	13	4	153	33.33% (4.33-77.72)	92.17% (86.98-95.76)	13.33% (1.66-40.46)	97.45% (93.61-99.30)
cells/μL	Confirmed NTM	172	3	12	2	155	60.00% (14.66-96.23)	92.81% (87.78-96.23)	20.00 (4.33-48.09)	98.73 (95.47-99.85)
	Mycobacteria infection	174[a]	5	10	8	151	38.46 (13.86-68.42)	93.79% (88.87-96.98)	33.33% (11.82-61.62)	94.97% (90.33-97.80)

CI = confidence interval; FN = false negatives; FP = false positives; LAM = lipoarabinomannan; MRS = microbiological reference standard; NPV = negative predictive value; NTM = nontuberculous mycobacteria; PPV = positive predictive value; Sn = sensitivity; Sp = specificity; TB = tuberculosis; TN = true negatives; TP = true positive.
[a] Two hundred four participants with CD4 $\leq$ 100 cells/μL, and 174 participants with CD4 > 100 cells/μL, and 4 participants lacked the data of CD4 cell counts.

Table 4
Further information on 7 patients with identified NTM species infection.

No.	Sex	Age (years)	CD4 (cells/μL)	On ART	AFB smear	Xpert	Culture	NTM species	LAM Results
1	Male	28	6	No	Positive	Not available	Positive, MPB64 negative	*M. avium complex*	Positive
2	Male	31	149	Yes	Positive	Not available	positive, MPB64 negative	*M. avium complex*	Positive
3	Male	49	25	Yes	Negative	Negative	positive, MPB64 negative	*M. avium complex*	Negative
4	Male	28	1	No	Positive	Negative	positive, MPB64 negative	*M. avium complex*	Positive
5	Male	59	11	No	Positive	Not available	positive, MPB64 negative	*M. avium complex/M. kansasii*	Positive
6	Male	65	77	Yes	Positive	Negative	positive, MPB64 negative	*M. kansasii*	Negative
7	Female	49	102	No	Negative	Negative	positive, MPB64 negative	*M. intracellulare*	positive

AFB = acid-fast bacilli; M. = mycobacterium; NTM = nontuberculous mycobacteria.

analyzed whether LAM tests could be used to rule out mycobacterial infections. Of the 329 patients who tested negative using urine LF-LAM tests, 17 patients were finally diagnosed as having mycobacterial infections (7 TB, 7 NTM diseases, and 3 unclassified mycobacteria), showing an NPV of 94.83% (91.86-96.96). LF-LAM tests presented a relatively low PPV in diagnosing TB and NTM diseases (25.49% 14.33-39.63] and 19.61% [9.82-33.12], respectively) (Table 2). However, when the LF-LAM test was used to predict mycobacterial infections, it showed a median PPV (47.17% [33.30-61.36]) (Table 2).

Discussion

To the best of our knowledge, this is the first study to investigate the utility of urine the LF-LAM test for the diagnosis of TB and NTM diseases in hospitalized PLWH. We found that the sensitivity of the urine LF-LAM test was comparable in diagnosing TB and NTM diseases. In our study, LF-LAM showed an overall sensitivity of 65.00% in hospitalized HIV-positive patients and a pooled sensitivity of 78.57%- in participants with CD4 $\leq$ 100 cells/μL for the dignosis of TB. However, the LF-LAM test is not recommended for the diagnosis of TB in PLWH with CD4 greater than 200 cells/µL because of a suboptimal sensitivity of 16% in this population (Bjerrum et al., 2019). The performance of the LF-LAM test in HIV-negative patients is very limited, with reported estimated sensitivities ranging from 4% to 31% (Kroidl et al., 2015; Sahle et al., 2017, Sigal et al., 2018; Suwanpimolkul et al., 2017). It is necessary to increase the diagnostic sensitivity of the assay to identify a pair of monoclonal antibodies with binding specificities to distinct LAM epitopes that are present in the urine samples of patients with TB. The novel Fujifilm SILVAMP TB-LAM (FujiLAM; Fujifilm, Tokyo, Japan) is substantially more sensitive for diagnosing TB in PLWH than LF-LAM (Broger et al., 2019). To improve sensitivity and maintain high specificity, FujiLAM uses a pair of high-affinity monoclonal antibodies directed toward largely *M. tuberculosis–specific* LAM epitopes and uses a silver-amplification step (Broger et al., 2019). A meta-analysis revealed that the overall sensitivity for TB detection was 70.7% (95% CI 59.0%-80.8%) for SILVAMP-LAM compared with 34.9% (95% CI 19.5%-50.9%) for LF-LAM applying a microbiological reference standard in 1,595 hospitalized and nonhospitalized PLWH (Broger et al., 2020). By screening a novel electrochemiluminescence assay using multiple monoclonal anti-LAM antibodies for TB case detection, George Sigal et al have shown that 93% sensitivity and 97% specificity can be achieved by the best pair of the capture and the detection antibodies (capture S4-20/detector A194-01) (Sigal et al., 2018). The gas chromatography/mass spectrometer (GC/MS) method is also an alternative technique for LAM detection (Amin et al., 2018; Flores et al., 2021). Further efforts to improve sensitivity are necessary to establish LAM as an optimal point-of-care test for TB diagnosis.

Cross-reactions between LAM tests and NTM antigens have been previously observed in patients with NTM diseases (Bjerrum et al., 2020; De et al., 2020; Huerga et al., 2020; Nel et al., 2017). LAM encompasses a large family of related

molecules that are expressed by all mycobacterial species. The Alere LAM assay does not differentiate between various species of Mycobacterium, such as *M. tuberculosis, M. leprae*, and *M. avium,* which may account for lowered specificity of LAM assays and might explain differences between studies in different countries as the prevalence of these organisms has a substantial geographical variation (Lawn, 2012). Lawn et al. reported 2 false positives in 8 patients with an NTM infection among HIV-positive patients with advanced immunodeficiency (Lawn et al., 2012). Jeremy Nel et al. also found an unexpectedly high false-positive rate in 19 of 21 HIV-positive patients with disseminated NTM infection (Nel et al., 2017). In our study, the LF-LAM specificity for diagnosing confirmed TB was 89.36%, and 10 of 38 false positives were associated with the presence of NTM infection when using a microbiological reference standard. Of 7 patients with identified NTM species, which were all slow-growing NTM, including *M. avium, M. intracellulare*, and *M. kansasii*, 5 had positive LAM results. The Alere LAM urine assay uses polyclonal anti-LAM antibodies to capture the urine LAM, which makes this assay nonspecific as LAM has multiple shared epitopes between TB and NTM (Sigal et al., 2018). Masanori Kawasaki et al. established a novel immunoassay to measure concentrations of LAM in sputum, which showed a higher sensitivity for slow-growing NTM than MTB, but it did not detect rapid-growing NTM (Kawasaki et al., 2019). Stephanie Bjerrum found that no cross-reactivity was observed for rapid-growing NTM, whereas 2 participants (2 of 27) were FujiLAM positive, both with slow-growing NTM isolated, which raises concerns that clinically important slow-growing NTMs among PLWH may affect FujiLAM test results (Bjerrum et al., 2020). In addition, it needs to be evaluated in studies with a higher quality of reference standards to discriminate between isolated TB disease, pulmonary/disseminated NTM diseases, and the possibility of mixed infections with TB and NTM (Bjerrum et al., 2020). Choudhary et al. produced and purified anti-LAM monoclonal antibodies, including CS35, A194, and MoAb1, and multiple reactivity patterns that differ in antibody specificity toward LAM have been observed in different host species (Choudhary et al., 2018). These results highlighted the complexity of the antigenic structure of LAM and the diversity of the natural antibody response against this target (Choudhary et al., 2018). A directional shift toward understanding the biology of mycobacterial organisms might be a solution to decrease the cross-reactivity of NTM in diagnostic procedures. Although a positive LAM result usually is presumed to be indicative of disseminated tuberculosis in high-burden TB settings, clinicians should remain vigilant to confirm the diagnosis among patients at high risk of NTM infections (Nel et al., 2018). The implications for clinical practice should be considered in the areas with a relatively high incidence of NTM disease, and further study on the implications of NTM infection in patients with positive LAM test result is needed.

Interestingly, our study showed that the sensitivity of LF-LAM in diagnosing NTM diseases is close to its application in TB. The diagnostic approaches to determinate NTM infection are very limited. Therefore, it could be used as an additional tool to diagnose NTM diseases, especially in areas where the prevalence of TB is relatively lower than that of NTM diseases. When using the LF-LAM test for diagnosing mycobacterial infection, it yielded mild sensitivity of 59.52% (95% CI 43.28%-74.37%) and high specificity of 91.76% (95% CI 88.32%-94.46%). These results showed urine LF-LAM had great potential for diagnosing mycobacterial infection in HIV-positive inpatients. HIV-associated mycobacterial infection is frequently extrapulmonary and is often challenging to either detect or exclude, especially in patients with advanced immunodeficiency (Lawn et al., 2015). The urine LAM test could be easily added into the diagnostic algorithm, and combining it with acid-fast smear, Xpert, and culture can help improve the diagnostic accuracy for mycobacterial infection. It is noteworthy that the LAM test had a high NPV regardless of CD4 strata or mycobacterial species, which indicates that patients with LF-LAM negativity are likely to be the absence of mycobacterial infection and would initiate ART earlier, especially in low prevalence of mycobacterial infection.

The strengths of this study were the consecutive enrollment in a setting where the prevalence of TB and NTM was comparable, whereas previous studies were mainly conducted in populations with a low incidence of NTM (Broger et al., 2020; Nel et al., 2017; Songkhla et al., 2019). In addition, the LF-LAM tests in our study were performed using fresh, unprocessed urine samples. Fresh urine samples contain more detectable LAM, which might improve the sensitivity of the LAM test. The use of fresh samples might be more crucial in patients expected to have low LAM concentrations, such as in those with a CD4 count higher than 200 cells/μL (Connelly et al., 2019; Gina et al., 2017).

We acknowledged several limitations of this study. First, we recruited hospitalized patients at a single center. Further diagnostic accuracy evaluation will need to be performed in a range of geographical settings, including inpatients and outpatients. Second, we did not compare results with the other urine LAM assay (eg, Fujifilm SILVAMP Urine LAM) at the same time. Third, some patients were unable to provide sputum or other body fluid samples, which may underestimate the potential of LAM to identify patients who are difficult to diagnose. In addition, the numbers of bacteriologically confirmed TB cases and NTM diseases are relatively small.

Conclusion

LAM has a comparable sensitivity in diagnosing TB and NTM diseases but could not distinguish TB from NTM diseases. The high NPV of LF-LAM suggests its application in excluding mycobacterial infection in hospitalized PLWH. Patients with NTM infection may result in positive urine LF-LAM results, which reduce the specificity of the LF-LAM assay in the diagnosis of TB. Additional microbiological tests or molecular tools were required to identify species of Mycobacterium. Meanwhile, the high NPVs indicated that the LF-LAM assay could still be used as a tool to rule out mycobacteria infection.

Declarations of Competing Interest

All authors declare that they have no competing interests.

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Disclaimer

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Authors' contributions

Jun Chen and Hongzhou Lu conceived and designed the study; Ling Gu, Yinzhong Shen, Renfang Zhang, Jiangrong Wang, Li Liu, Zhenyan Wang, Yang Tang, Wei Song, Jingna Xun, and Danping Liu participated in the conduct of the study and patient enrollment. Ling Gu carried out the LAM testing. Danping Liu and Ling Gu collected clinical data and performed data analysis. Danping Liu and Jun Chen wrote the manuscript, Hongzhou Lu, Jun Chen, and Yueming Shao performed critical revision of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the ethics committee of Shanghai Public Health Clinical Center. Written informed consent was obtained from all patients before the screening process. The study was registered on ClinicalTrial.gov with registration number NCT04600232.

Consent for publication

Not applicable.

Availability of data and materials

The full study protocol and the datasets, including all data fields reported in this study, are available, following manuscript publication, upon request from the corresponding author (Professor Jun Chen, qtchenjun@163.com), following the provision of ethics approval.

References

Amin AG, De P, Spencer JS, Brennan PJ, Daum J, Andre BG, et al. Detection of lipoarabinomannan in urine and serum of HIV-positive and HIV-negative TB suspects using an improved capture-enzyme linked immuno absorbent assay and gas chromatography/mass spectrometry. Tuberculosis (Edinb) 2018;111:178–87.

Bjerrum S, Broger T, Szekely R, Mitarai S, Opintan JA, Kenu E, et al. Diagnostic Accuracy of a Novel and Rapid Lipoarabinomannan Test for Diagnosing Tuberculosis Among People With Human Immunodeficiency Virus. Open Forum Infect Dis 2020;7(1):ofz530.

Bjerrum S, Oliver-Commey J, Kenu E, Lartey M, Newman MJ, Addo KK, et al. Tuberculosis and non-tuberculous mycobacteria among HIV-infected individuals in Ghana. Trop Med Int Health 2016;21(9):1181–90.

Bjerrum S, Schiller I, Dendukuri N, Kohli M, Nathavitharana RR, Zwerling AA, et al. Lateral flow urine lipoarabinomannan assay for detecting active tuberculosis in people living with HIV. Cochrane Database Syst Rev 2019;10(1469-493X (Electronic)).

Brode SK, Daley CL, Marras TK. The epidemiologic relationship between tuberculosis and non-tuberculous mycobacterial disease: a systematic review. Int J Tuberc Lung Dis 2014;18(11):1370–7.

Broger T, Nicol MP, Szekely R, Bjerrum S, Sossen B, Schutz C, et al. Diagnostic accuracy of a novel tuberculosis point-of-care urine lipoarabinomannan assay for people living with HIV: A meta-analysis of individual in- and outpatient data. PLoS Med 2020;17(5).

Broger T, Sossen B, du Toit E, Kerkhoff AD, Schutz C, Ivanova Reipold E, et al. Novel lipoarabinomannan point-of-care tuberculosis test for people with HIV: a diagnostic accuracy study. Lancet Infect Dis 2019;19(8):852–61.

Cai RT, Yu FX, Tao Z, Qian XQ, Chen J, Lu HZ. Routinely detected indicators in plasma have a predictive effect on the identification of HIV-infected patients with non-tuberculous mycobacterial and tuberculous infections. Infectious diseases of poverty 2017;6(1):132.

Choudhary A, Patel D, Honnen W, Lai Z, Prattipati RS, Zheng RB, et al. Characterization of the Antigenic Heterogeneity of Lipoarabinomannan, the Major Surface Glycolipid of Mycobacterium tuberculosis, and Complexity of Antibody Specificities toward This Antigen. J Immunol 2018;200(9):3053–66.

Connelly JT, Grant B, Munsamy V, Pym A, Somoskovi A. Lipoarabinomannan point-of-care tests: evaluation with fresh samples needed. Lancet Infect Dis 2019;19(10):1053.

De P, Amin AG, Graham B, Martiniano SL, Caceres SM, Poch KR, et al. Urine lipoarabinomannan as a marker for low-risk of NTM infection in the CF airway. J Cyst Fibros 2020;19(5):801–7.

Flores J, Cancino JC, Chavez-Galan L. Lipoarabinomannan as a Point-of-Care Assay for Diagnosis of Tuberculosis: How Far Are We to Use It? Frontiers in microbiology 2021;12.

Gina P, Randall PJ, Muchinga TE, Pooran A, Meldau R, Peter JG, et al. Early morning urine collection to improve urinary lateral flow LAM assay sensitivity in hospitalised patients with HIV-TB co-infection. BMC Infect Dis 2017;17(1):339.

Gupta-Wright A, Kerkhoff AD, Meintjes G, Corbett EL. Urinary Lipoarabinomannan Detection and Disseminated Nontuberculous Mycobacterial Disease. Clin Infect Dis 2018;66(1):158.

Huerga H, Cossa L, Manhica I, Bastard M, Telnov A, Molfino L, et al. Systematic, Point-of-Care Urine Lipoarabinomannan (Alere TB-LAM) Assay for Diagnosing Tuberculosis in Severely Immunocompromised HIV-Positive Ambulatory Patients. The American journal of tropical medicine and hygiene 2020;102(3):562–6.

Kawasaki M, Echiverri C, Raymond L, Cadena E, Reside E, Gler MT, et al. Lipoarabinomannan in sputum to detect bacterial load and treatment response in patients with pulmonary tuberculosis: Analytic validation and evaluation in two cohorts. PLoS Med 2019;16(4).

Kroidl I, Clowes P, Reither K, Mtafya B, Rojas-Ponce G, Ntinginya EN, et al. Performance of urine lipoarabinomannan assays for paediatric tuberculosis in Tanzania. Eur Respir J 2015;46(3):761–70.

Lawn SD. Point-of-care detection of lipoarabinomannan (LAM) in urine for diagnosis of HIV-associated tuberculosis: a state of the art review. BMC Infect Dis 2012;12(1471-2334 (Electronic)):103.

Lawn SD, Kerkhoff AD, Nicol MP, Meintjes G. Underestimation of the True Specificity of the Urine Lipoarabinomannan Point-of-Care Diagnostic Assay for HIV-Associated Tuberculosis. Journal of acquired immune deficiency syndromes 2015;69(4):e144–6 (1999).

Lawn SD, Kerkhoff AD, Vogt M, Wood R. Diagnostic accuracy of a low-cost, urine antigen, point-of-care screening assay for HIV-associated pulmonary tuberculosis before antiretroviral therapy: a descriptive study. Lancet Infect Dis 2012;12(3):201–9.

Liu L, Zhang R, Tang Y, Qi T, Song W, Wang Z, et al. The importance of non-tuberculous mycobacteria identification in Chinese patients infected with HIV. Bioscience trends 2018;12(5):515–16.

Nel JS, Ive P, Lippincott CK, et al. Reply to Gupta-Wright. Clin Infect Dis 2018;66(1):159.

Nel JS, Lippincott CK, Berhanu R, Spencer DC, Sanne IM, Ive P. Does Disseminated Nontuberculous Mycobacterial Disease Cause False-Positive Determine TB-LAM Lateral Flow Assay Results? A Retrospective Review. Clin Infect Dis 2017;65(7):1226–8.

World Health Organization. Global tuberculosis report; 2020. Available from: https://apps.who.int/iris/bitstream/handle/10665/336069/9789240013131-eng.pdf. (Accessed 29 June 2021).

World Helath Organization. WHO policy guidance: The use of lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis and screening of active TB in people living with HIV; 2015. Available from: http://apps.who.int/iris/bitstream/10665/193633/1/9789241509633_eng.pdf?ua=1. (Accessed 29 June 2021).

Qi TK, Chen J, Zhang RF, Liu L, Shen YZ, Wang ZY, et al. A retrospective cohort study of early mortality among patients with HIV/TB co-infection in Shanghai municipality. HIV medicine 2020;21(11):739–46.

Qvist T, Johansen IS, Pressler T, Hoiby N, Andersen AB, Katzenstein TL, et al. Urine lipoarabinomannan point-of-care testing in patients affected by pulmonary non-tuberculous mycobacteria–experiences from the Danish Cystic Fibrosis cohort study. BMC Infect Dis 2014;14:655.

Rachow A, Zumla A, Heinrich N, Rojas-Ponce G, Mtafya B, Reither K, et al. Rapid and accurate detection of Mycobacterium tuberculosis in sputum samples by Cepheid Xpert MTB/RIF assay–a clinical validation study. PLoS One 2011;6(6):e20458.

Sahle SN, Asress DT, Tullu KD, Weldemariam AG, Tola HH, Awas YA, et al. Performance of point-of-care urine test in diagnosing tuberculosis suspects with and without HIV infection in selected peripheral health settings of Addis Ababa, Ethiopia. BMC Res Notes 2017;10(1):74.

Sigal GB, Pinter A, Lowary TL, Kawasaki M, Li A, Mathew A, et al. A Novel Sensitive Immunoassay Targeting the 5-Methylthio-d-Xylofuranose-Lipoarabinomannan Epitope Meets the WHO's Performance Target for Tuberculosis Diagnosis. J Clin Microbiol 2018;56(12).

Songkhla MN, Tantipong H, Tongsai S, Angkasekwinai N. Lateral Flow Urine Lipoarabinomannan Assay for Diagnosis of Active Tuberculosis in Adults With Human Immunodeficiency Virus Infection: A Prospective Cohort Study. Open Forum Infect Dis 2019;6(4):ofz132.

Suwanpimolkul G, Kawkitinarong K, Manosuthi W, Sophonphan J, Gatechompol S, Ohata PJ, et al. Utility of urine lipoarabinomannan (LAM) in diagnosing tuberculosis and predicting mortality with and without HIV: prospective TB cohort from the Thailand Big City TB Research Network. International journal of infectious diseases: IJID: official publication of the International Society for Infectious Diseases 2017;59:96–102.

Tran T, Bonham AJ, Chan ED, Honda JR. A paucity of knowledge regarding nontuberculous mycobacterial lipids compared to the tubercle bacillus. Tuberculosis (Edinb) 2019;115:96–107.