

Evaluation of lipoarabinomannan in the diagnosis of tuberculosis

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Background The detection of lipoarabinomannan (LAM) antigens in body fluids has several potential advantages compared with the diagnostic methods used currently.

Aim The aim of this study was to evaluate the possible role of the detection of LAM in the serum and the urine as a diagnostic aid in the diagnosis of different forms of tuberculosis (TB).

Patients and methods This study included 62 newly confirmed tuberculosis cases classified into two groups: group A included patients with pulmonary TB ($n=36$), and was further divided into two groups: group A₁ [the smear-positive pulmonary TB group ($n=24$)] and group A₂ [the smear-negative pulmonary TB group ($n=12$)]; group B included the extrapulmonary TB group ($n=26$); and 10 apparently healthy individuals served as the control group. The LAM level was measured in the serum and the urine by an enzyme-linked immunosorbant assay.

Results The mean level of quantitative serum LAM was higher in group A1 (0.55 ± 0.20 ng/ml) compared with group A2 (0.44 ± 0.30 ng/ml) or group B (0.41 ± 0.27 ng/ml). The mean level of quantitative urine LAM was higher in group A1 (0.81 ± 0.24 ng/ml) compared with group B (0.72 ± 0.35 ng/ml) and group A2 (0.65 ± 0.37 ng/ml; $P<0.001$). The quantitative urine LAM test correlated positively with the degree of bacillary burden ($P<0.05$). Quantitative serum LAM had a sensitivity of 88.7%, specificity 90%, accuracy 88.9%, positive predictive value 98.2%, and negative predictive value

56.3%. Quantitative urine LAM had a sensitivity of 85.5%, specificity 90%, accuracy 86.1%, positive predictive value 98.1%, and negative predictive value 50%. A combination of serum and urine LAM tests identified that 98.4% of the cases with a positive TB culture correlated with higher serum LAM levels. Advanced chest radiography involvement and TB culture correlated with higher urine LAM levels ($P<0.05$).

Conclusion The LAM test is a valuable addition in the diagnosis of TB and its different forms. A combination of quantitative serum and urine LAM increased the sensitivity of the test. The quantitative urine LAM test offers additional clinical insight into the degree of TB disease severity and has more applicability.

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Keywords: Antigen, different forms of TB, enzyme-linked immunosorbant assay, extrapulmonary tuberculosis, lipoarabinomannan, pulmonary tuberculosis, serum, tuberculosis, urine

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Introduction

Tuberculosis (TB) now ranks alongside HIV as a leading cause of death worldwide. Globally, in 2014, there were an estimated 9.6 million incident cases of TB: 5.4 million among men, 3.2 million among women, and 1.0 million among children [1].

Current tools and strategies for the diagnosis of TB are inadequate; they relies on the clinical presentation, supported by laboratory investigations, particularly the direct smear and culture method; therefore, there is a clear need for the development, the introduction, and the effective implementation of cost-effective new tools that contribute to an improvement in patient-centered outcomes and public health and that perform well for HIV-infected and HIV-uninfected individuals [2].

Lipoarabinomannan (LAM) is a structurally important 17.5 kD heat-stable glycolipid found in the cell wall of *Mycobacterium tuberculosis*. LAM can account for up to 15% of the total bacterial weight and serves as an immunogenic virulence factor that is released from

metabolically active or degrading bacterial cells during TB infection; the detection of LAM antigens in body fluids has several potential advantages compared with the diagnostic tests used currently [3]. Diagnostic tests based on the detection of LAM in the urine were among the first to move from the research to the commercial stage, due to their promising initial results [4].

LAM could be measured qualitatively and quantitatively, the former being used most widely. Qualitative LAM-ELISA (Chemogen Inc., Portland, Oregon, USA) was the first LAM targeting the assayed prototype [5–9]. Later, another commercial version named Clearview TB ELISA (Alere Inc., formerly Inverness Medical Innovations Inc., Waltham, Massachusetts, USA) was launched [10,11].

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A promising lateral-flow dipstick version of urinary LAM detection (Determine TB LAM Ag; Alere Inc.) has been developed. After its commercial launch in 2013, determining TB LAM remains the focus of ongoing clinical evaluation studies. This is a simple, low-cost, rapid assay that provides a qualitative (yes/no) readout of TB diagnosis within 30 min [12]. However, LAM enzyme-linked immunosorbent assay can also be used to provide a quantitative readout expressed as the optical density (OD) at 450 nm [13].

A meta-analysis study using qualitative commercial urine LAM assays in patients with microbiologically confirmed pulmonary TB reported that its sensitivity ranged from 13 to 93% and specificity ranged from 87 to 99% [3]. The sensitivity of the qualitative LAM test, although imperfect, appears to be higher than that of sputum smear microscopy, and the test performs with a high positive predictive value (PPV) in populations with high HIV and TB prevalence [7,14,15]. However, quantitative LAM test results have not been studied fully, and further research may allow a more complete understanding of its test performance and optimal test usage [11].

Aim of the work

The aim of this work was to evaluate the possible role of the detection of LAM in the serum and the urine to aid in the diagnosis of different forms of TB.

Patients and methods

This was a prospective case-control study that was conducted at Banha University Hospital, Chest Department, and Saudi German Hospital, Jeddah, from March 2014 to March 2016. The study included 62 newly confirmed TB patients as cases and 10 apparently healthy persons as the control group. Participants in the study were classified into the following groups according to National TB Program of Egypt [16]:

- (1) Group A: pulmonary TB patients (58%), who were further divided as follows:
 - (a) Group A₁: the smear-positive pulmonary TB group included 24 patients (66.67%).
 - (b) Group A₂: the Smear-negative pulmonary TB group included 12 patients (33.33%).
- (2) Group B: the extrapulmonary TB group included 26 patients (42%).
- (3) Group C: the control group included 10 age- and sex-matched apparently healthy persons.

All cases were identified on the basis of the National TB Program of Egypt guidelines [16] as follows:

- (1) Complete history taking and thorough general and local chest examination were performed.
- (2) Radiological investigations were performed (chest radiography posterior-anterior and lateral views). A computed tomography of the chest was performed for some patients when needed.
- (3) Routine laboratory investigations included a complete blood count, the erythrocyte sedimentation rate, liver and kidney functions tests, and fasting blood sugar.
- (4) The tuberculin skin test (Mantoux method) was performed.
- (5) Three successive morning-sputum samples were collected for acid-fast bacilli (AFB) examination by Ziehl-Neelsen (ZN) stain for all pulmonary cases, and some nonrespiratory samples (pleural effusion, sinus discharge, etc.) were collected for AFB examination by ZN stain for extrapulmonary cases. When acid-fast organisms are seen, the number of bacteria was reported semiquantitatively as follows: grade I, 10–99 AFB per 100 oil immersion field; grade II, 1–10 AFB per oil immersion field; and grade III, more than 10 AFB per oil immersion field [16].
- (6) Lowenstein-Jensen (LJ) tubes were inoculated for all respiratory samples and some nonrespiratory samples.
- (7) When the sputum could not be obtained, sputum induction was performed with 3% hypertonic saline. If the specimen still could not be obtained, patients were subjected to fiberoptic bronchoscopy, and bronchial lavage samples were sent to both ZN stain and LJ culture.
- (8) Molecular diagnosis: the Xpert MTB/RIF (Cepheid, Sunnyvale, USA) PCR technique was used for the diagnosis of some respiratory and nonrespiratory samples.
- (9) Histopathological examination: invasive procedures to obtain specimens from the lung, lymph nodes, the pleura, the brain, bones, testes, and the iliopsoas abscess were considered when noninvasive techniques did not provide a diagnosis, especially in extrapulmonary TB cases.
- (10) Patients already on anti-TB drugs and/or patients with renal impairment (nephrotic or nephritic syndrome, nephropathy, renal failure, etc.), patients with immune complex diseases, and immunocompromised patients were excluded from this study.

Lipoarabinomannan measurement

Collection and extraction of samples was carried out immediately in accordance with related documents.

After extraction, samples were kept at -20°C . Serum samples were allowed to clot for 10–20 min at room temperature and urine samples were collected in sterile tubes; then, both were centrifuged at 2000–3000 RPM for 20 min. Supernatants were collected carefully and stored at -20°C to be used later. All reagents and study samples were prepared and brought to room temperature before the assay procedure was started. The serum LAM test was processed first, followed by the urine LAM test. About 50 μl of standard was added to the standard well. Standard (S0→S5) concentrations were as follows: 0, 0.5, 1, 2, 4, and 8 ng/ml. A testing sample of 10 μl was added to sample wells, and then 40 μl of the sample diluent also added; the blank well between the standard wells and the sample wells was left without adding anything. About 100 μl of horseradish peroxidase-conjugate reagent was added to each well; they were covered with an adhesive strip, and incubated for 60 min at 37°C . Each well was aspirated, and then washed five times with a Wash Solution (Awareness Technology, Inc. Palm City, USA) (400 μl) in a squirt bottle using a manifold dispenser. About 50 μl of chromogen solution A and 50 μl of chromogen solution B were added to each well, mixed gently, and incubated for 15 min at 37°C . Stop Solution (50 μl) was added to each well. The color in the wells changed from blue to yellow. The intensity of the color change from blue to yellow was measured at 450 nm. A Stat Fax-2100 ELISA (USA) device was used to measure these changes within 15 min from adding the Stop Solution. A standard curve was used to determine the amount in an unknown sample. ODs of the final samples were determined by subtracting the OD of the negative control from the sample reading with the minimum value of 0 as the manufacture guide (Biotain Pharma, China).

Clinical data were recorded on a report form. These data were tabulated and analyzed using the computer program statistical package for social science, version 16 (SPSS Inc., Chicago, Illinois, USA). Two types of statistics were carried out.

Descriptive data

Descriptive statistics were calculated for the data in the form of:

- (1) mean and SD for quantitative data and
- (2) frequency and distribution for qualitative data.

Analytical statistics

In the statistical comparison between the different groups, the significance of difference was tested using one of the following tests:

- (1) The Mann–Whitney test was used to compare the mean of two groups of quantitative data.
- (2) The Kruskal–Wallis test was used to compare the mean of more than two groups of quantitative data.
- (3) An intergroup comparison of categorical data was performed using the χ^2 -test and the Fisher exact test.
- (4) The receiver operating characteristic curve was used to assess the sensitivity, the specificity, PPV and negative predictive value (NPV), and the diagnostic accuracy.

Significance

P value greater than 0.05 was considered statistically nonsignificant. *P* value less than 0.05 was considered statistically significant. *P* value less than 0.001 was considered statistically highly significant.

Results

This study included 42 male (68%) and 20 female (32%) patients with active TB. Their ages ranged from 4 to 56 years, with a mean of 32.79 ± 11.1 years. The control group included four male (40%) and six female (60%) patients; their ages ranged from 30 to 40 years, with a mean of 34.3 ± 3.3 years, with no clinically significant difference between the two groups with regard to their age and sex.

In this study, *Mycobacteria* spp. were identified in respiratory or nonrespiratory specimens by different methods: AFB smear microscopy was performed in 50/62 (80.6%) patients and was positive in 24 (38.7%) patients. LJ TB culture was performed in 44 (71%) patients and was positive in 39/62 (63%) patients. PCR TB by Xpert MTB/RIF (Cepheid Inc.) was performed in 22/62 (35.5%) patients and was positive in 17 (27.4%) patients. Histopathological analysis of tissue biopsies was performed in 30/62 (48.4%) patients and showed caseating granuloma. The tuberculin skin test was performed in 62 (100%) patients and was positive in 24 (38.7%) patients.

In this study, toxic symptoms (mainly fever) were the most common presenting symptoms in TB patients (85.3%), followed by cough (55%), expectoration (29%), dyspnea (22%), chest pain, and hemoptysis (17.6%).

Quantitative measurements of serum LAM in each group: serum LAM levels were significantly higher in group A₁, group A₂, and group B when compared with the control group C (*P*=0.001 for each). However, there

were no statistical significant differences on comparing different groups with each other [(A₁ and A₂), (A₁ and B), and (A₂ and B)] ($P>0.05$).

Quantitative measurements of urine LAM in each group: urine LAM levels were significantly higher in group A₁, group A₂ and group B when compared with the control group C ($P=0.001$ for each), and there were statistical significant differences on comparing different groups with each other [(A₁ and A₂), (A₁ and B)] ($P>0.05$); however, there was no significant difference between groups A₂ and B, as shown in Table 1.

There were increased quantitative serum LAM values with increasing grades of sputum and bronchoalveolar lavage (BAL) AFB smear positivity (bacillary burden), but with no clinically significant value ($P>0.05$). There were increased quantitative urine LAM values with increasing grades of sputum and BAL AFB smear positivity (bacillary burden), with a clinically significant value ($P<0.05$), as shown in Table 2.

The quantitative serum LAM test for the overall TB cases had a sensitivity of 88.7%, specificity 90%, accuracy 88.9%, PPV 98.2%, and NPV 56.3%. Group A₁ had a

Table 1 Mean±SD of quantitative serum and urine lipoarabinomannan (ng/ml) results in each group

| Groups | Serum LAM [mean±SD (range)] (ng/ml) | Urine LAM [mean±SD (range)] (ng/ml) | P value ^a |
|---|-------------------------------------|-------------------------------------|---|
| Group A ₁ : smear-positive pulmonary TB (24) | 0.55±0.20 (0.01–0.85) | 0.81±0.24 (0.046–0.995) | 0.001** Serum: $P_1=0.09$ $P_2=0.074$ $P_3=0.36$ Urine: $P_1=0.039^*$ $P_2=0.048^*$ $P_3=0.072$ |
| Group A ₂ : smear-negative pulmonary TB (12) | 0.44±0.30 (0.0368–0.76) | 0.65±0.37 (0.023–0.965) | 0.001** |
| Group B: extrapulmonary TB (26) | 0.41±0.27 (0.0169–0.88) | 0.72±0.35 (0.023–1.13) | 0.001** |
| Group C: control group (10) | 0.079±0.078 (0.0188–0.02) | 0.05±0.03 (0.007–0.081) | – |

LAM, lipoarabinomannan; TB, tuberculosis. ^a P values are a result from comparing each group with the control group. P_1 (A₁ and A₂), P_2 (A₁ and B), P_3 (A₂ and B). *Significant. **Highly significant.

Table 2 Grading of acid-fast bacilli smear positivity (bacillary burden) and quantitative serum and urine lipoarabinomannan values (ng/ml) for tuberculosis cases

| Positive ZN smears (sputum+BAL) | Grade I (N=5) (20.8%) | Grade II (N=8) (33.3%) | Grade III (N=11) (45.8%) | P value ^a |
|---------------------------------|-----------------------|------------------------|--------------------------|--|
| Serum LAM (mean±SD) (ng/ml) | 0.41±0.30 | 0.58±0.18 | 0.60±0.14 | 0.199 Serum: $P_1=0.15$ $P_2=0.083$ $P_3=0.782$ Urine: $P_1=0.002$ $P_2=0.001$ $P_3=0.998$ |
| Urine LAM (mean±SD) (ng/ml) | 0.52±0.41 | 0.89±0.07 | 0.89±0.08 | 0.003* |

BAL, bronchoalveolar lavage; LAM, lipoarabinomannan; ZN, Ziehl–Neelsen. ^a P_1 (grade I and grade II), P_2 (grade I and grade III) and P_3 (grade II and grade III). *Significant.

Table 3 The validity of the quantitative serum lipoarabinomannan test as a diagnostic tool for tuberculosis diagnosis in overall tuberculosis cases and its different forms

| Serum LAM | Overall tuberculosis cases (%) | Smear-positive PTB (group A ₁) (%) | Smear-negative PTB (group A ₂) (%) | EPTB (group B) (%) |
|---------------|--------------------------------|--|--|--------------------|
| AUC | 0.961 | | | |
| Cut-off point | 0.021 | | | |
| Sensitivity | 88.7 | 95.8 | 83.3 | 84.6 |
| Specificity | 90.0 | 90 | 90 | 90 |
| PPV | 98.2 | 95.8 | 91 | 95.7 |
| NPV | 56.3 | 56.3 | 82 | 69.2 |
| Accuracy | 88.9 | 88.9 | 86.4 | 86.1 |

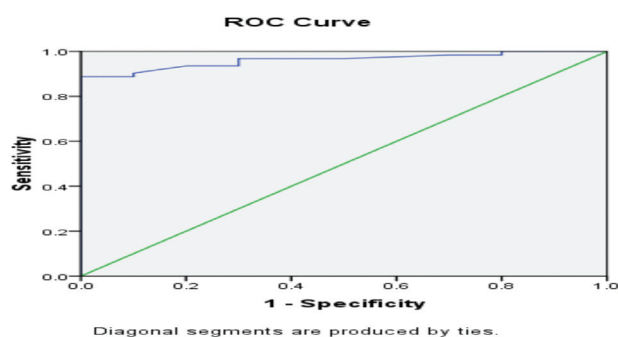
AUC, area under the curve; EXPTB, extrapulmonary tuberculosis; LAM, lipoarabinomannan; NPV, negative predictive value; PPV, positive predictive value; PTB, pulmonary tuberculosis.

sensitivity of 95.8%, specificity 90%, accuracy 94.12%, PPV 95.8%, and NPV 90.9%, which were higher than those of group A₂, with sensitivity 83.3%, specificity 90%, accuracy 86.4%, PPV 91%, and NPV 82%, and higher than those of group B, with sensitivity 84.6%, specificity 90%, accuracy 86.1%, PPV 95.7%, and NPV 69.2%, as shown in Table 3 and Fig. 1.

The quantitative urine LAM test for the overall TB cases had a sensitivity of 85.5%, specificity 90%, accuracy 86.1%, PPV 98.1%, and NPV 50%. Group A₁ had a sensitivity of 95.7%, specificity 90%, accuracy 91.2%, PPV 90%, and NPV 82%, which were higher than those of group A₂, with sensitivity 83.3%, specificity 90%, accuracy 86.4%, PPV 91%, and NPV 81.8%, and higher than those of group B, with sensitivity 80.8%, specificity 90%, accuracy 83.3%, PPV 95.5%, and NPV 64.3%, as shown in Table 4 and Fig. 2.

A combination of quantitative serum LAM testing and quantitative urine LAM testing increased the sensitivity to identify up to 98.4% of the tuberculosis patients, with a specificity of 81%, PPV 98.2%, NPV 56.3%, and accuracy 88.9%, as shown in Table 5.

Figure 1



The ROC curve of the quantitative serum LAM test in TB cases. LAM, lipoarabinomannan; ROC, receiver operating characteristic; TB, tuberculosis.

The serum LAM test was more sensitive than the ZN stain for AFB smear testing; the combination of both tests identified 94% of the confirmed TB cases, with specificity 90%, PPV 98.2%, NPV 56.3%, and accuracy 88.9%, as shown in Table 6.

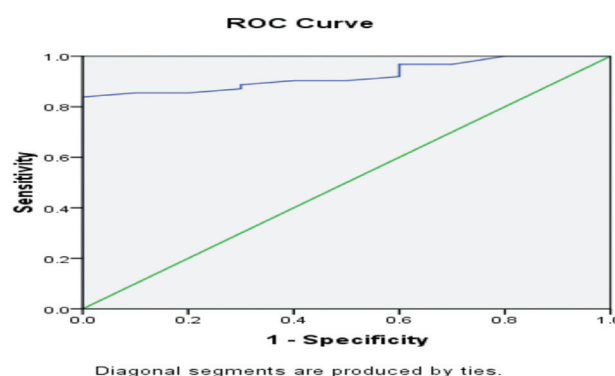
Urine LAM testing was more sensitive than the ZN stain for AFB smear testing; a combination of both tests identified 92.5% of the confirmed TB cases, with specificity 90%, PPV 98.1%, NPV 50%, and accuracy 86.1%, as shown in Table 7.

The TB culture was a significant predictor of high serum LAM levels. Patients with advanced chest radiography involvement, and a positive TB culture had significantly high urine LAM levels ($P < 0.05$), as shown in Table 8.

Discussion

In this study, quantitative measurements of serum LAM in group A₁ (smear-positive pulmonary TB, 0.55 ± 0.20 ng/ml) were higher than those of group A₂ (smear-negative pulmonary TB, 0.44 ± 0.30 ng/ml)

Figure 2



The ROC curve of the quantitative urine LAM test for tuberculosis cases. LAM, lipoarabinomannan; ROC, receiver operating characteristic.

Table 4 The validity of the quantitative urine lipoarabinomannan test as a diagnostic tool in overall tuberculosis cases and its different forms

| Urine LAM | Overall tuberculosis cases (%) | Smear-positive PTB (group A ₁) (%) | Smear-negative PTB (group A ₂) (%) | EPTB (group B) (%) |
|---------------|--------------------------------|--|--|--------------------|
| AUC | 0.923 | | | |
| Cut-off point | 0.081 | | | |
| Sensitivity | 85.5 | 95.7 | 83.3 | 80.8 |
| Specificity | 90.0 | 90 | 90 | 90 |
| PPV | 98.1 | 90 | 91 | 95.5 |
| NPV | 50.0 | 82 | 81.8 | 64.3 |
| Accuracy | 86.1 | 91.2 | 86.4 | 83.3 |

AUC, area under the curve; EPTB, extrapulmonary tuberculosis; LAM, lipoarabinomannan; NPV, negative predictive value; PPV, positive predictive value; PTB, pulmonary tuberculosis.

Table 5 The validity of the quantitative urine lipoarabinomannan test, the serum lipoarabinomannan test, and both methods in the diagnosis of tuberculosis cases

| Validity | Serum LAM (%) | Urine LAM (%) | Combined methods (%) |
|-------------|---------------|---------------|----------------------|
| Sensitivity | 88.7 | 85.5 | 98.4 |
| Specificity | 90 | 90 | 81 |
| PPV | 98.2 | 98.1 | 98.2 |
| NPP | 56.3 | 50 | 56.3 |
| Accuracy | 88.9 | 86.1 | 88.9 |

LAM, lipoarabinomannan; NPP, negative predictive value; PPV, positive predictive value.

Table 6 The validity of the quantitative serum lipoarabinomannan test, Ziehl–Neelsen for acid-fast bacilli smear, and both methods in the diagnosis of tuberculosis cases

| Validity | Serum LAM (%) | Positive ZN AFB smears (%) | Combined methods (%) |
|-------------|---------------|----------------------------|----------------------|
| Sensitivity | 88.7 | 48 | 94 |
| Specificity | 90 | 100 | 90 |
| PPV | 98.2 | 100 | 98.2 |
| NPP | 56.3 | 28 | 56.3 |
| Accuracy | 88.9 | 55 | 88.9 |

AFB, acid-fast bacilli; LAM, lipoarabinomannan; NPP, negative predictive value; PPV, positive predictive value; ZN, Ziehl–Neelsen stain.

and group B (extrapulmonary TB, 0.41 ± 0.27 ng/ml), with no clinically significant difference ($P > 0.05$). However, there was a highly significant difference on comparing each group with group C (control group) ($P < 0.001$). These results were in agreement with Abd el-Atty *et al.* [17], who found that smear-positive pulmonary TB patients had higher serum levels than smear-negative patients.

In this study, quantitative measurements of urine LAM in group A₁ (smear-positive pulmonary TB, 0.81 ± 0.24 ng/ml) were higher than those of group B (extrapulmonary TB, 0.72 ± 0.35 ng/ml) and group A₂ (smear-negative pulmonary TB, 0.65 ± 0.37 ng/ml), with a clinically significant difference ($P < 0.05$). Also, there was a highly significant difference on comparing each group with group C (control group) ($P < 0.001$). Similarly, Shah *et al.* [11] reported that patients with smear-positive pulmonary TB had an average OD that was 0.33 U higher than that of patients with smear-negative pulmonary TB ($P < 0.05$). Agha *et al.* [18] reported that patients with smear-positive specimens had a higher urine LAM level (0.63 ± 0.54 ng/ml) than smear-negative patients (0.040 ± 0.06 ng/ml) ($P < 0.001$). Also, Kerkhoff *et al.* [13] observed higher LAM OD units in patients with sputum smear-positive disease.

The detection of AFB in stained smears examined microscopically is the first bacteriologic evidence of

Table 7 The validity of the quantitative urine lipoarabinomannan test, Ziehl–Neelsen for acid-fast bacilli smear, and both methods in the diagnosis of tuberculosis patients

| Validity | Urine LAM (%) | Positive ZN AFB smears (%) | Combined methods (%) |
|-------------|---------------|----------------------------|----------------------|
| Sensitivity | 85.5 | 48 | 92.5 |
| Specificity | 90 | 100 | 90 |
| PPV | 98.1 | 100 | 98.1 |
| NPP | 50 | 28 | 50 |
| Accuracy | 86.1 | 55 | 86.1 |

AFB, acid-fast bacilli; LAM, lipoarabinomannan; NPP, negative predictive value; PPV, positive predictive value; ZN, Ziehl–Neelsen stain.

the presence of *Mycobacteria* spp. in a clinical specimen. A high bacterial load of 5000–10 000 AFB/ml is required for the detection of this bacteria in the stained smears. This fact reflects that smear-negative pulmonary TB is associated with a mycobacterial load lower than the threshold that can be detected by direct microscopy [19]. LAM is released from metabolically active or degrading bacterial cells during TB infection [3], and so it is not a surprise that LAM is directly proportional to a high mycobacterial load, which is more obvious in smear-positive pulmonary TB than in smear-negative pulmonary TB. The same concept is applied to extrapulmonary TB, which is characterized by the involvement of relatively inaccessible sites and fewer bacilli with large damage [19].

In this study, it was observed that quantitative urine LAM values (0.81 ± 0.24 , 0.65 ± 0.37 , and 0.72 ± 0.35 ng/ml for smear-positive pulmonary TB, smear-negative pulmonary TB, and extrapulmonary TB, respectively) were higher than quantitative serum LAM values (0.55 ± 0.20 , 0.44 ± 0.30 , and 0.41 ± 0.27 ng/ml for smear-positive pulmonary TB, smear-negative pulmonary TB, and extrapulmonary TB, respectively). This can be explained by the fact that LAM is released from the degrading mycobacterial cell wall, which leads to the development of high tissue concentrations of LAM at anatomic sites of the disease and favor the entry of LAM into the systemic circulation, but it is antigenic, and so largely exists in the form of circulating immune complexes, whereas small quantities of LAM exist as free LAM (target for assay). Both are filtered by the kidneys as free LAM, and hence, LAM can be detected at higher values in the urine than in the serum [3,20].

In this study, quantitative serum LAM measurements were found to have no significant correlation with the grading of the sputum and BAL AFB smear positivity ($P > 0.05$). This may be related to the presence of the large quantity of LAM in the form of circulating

Table 8 Predictors of quantitative high serum and urine lipoarabinomannan test in tuberculosis cases

| | Positive serum LAM (n=53) [n (%)] | Negative serum LAM (n=9) [n (%)] | Positive urine LAM (n=52) [n (%)] | Negative urine LAM (n=10) [n (%)] | Serum P value | Urine P value |
|---|--------------------------------------|-------------------------------------|--------------------------------------|--------------------------------------|------------------|------------------|
| Age | 32.13±11.93 | 35.0±11.42 | 32.37±11.92 | 33.5±11.77 | 0.505 | 0.783 |
| Sex | | | | | | |
| Male | 40 (75.5) | 6 (66.7) | 40 (76.9) | 6 (60.0) | 0.577 | 0.468 |
| Female | 13 (24.5) | 3 (33.3) | 12 (23.1) | 4 (40.0) | | |
| Type of TB | | | | | | |
| Smear-positive pulmonary (n=24) | 23 (43.4) | 1 (11.1) | 22 (42.3) | 2 (20.0) | 0.125 | 0.355 |
| Smear-negative pulmonary (n=12) | 9 (17.0) | 3 (33.3) | 9 (17.3) | 3 (30.0) | | |
| Extrapulmonary (n=26) | 21 (39.6) | 5 (55.6) | 21 (40.4) | 5 (50.0) | | |
| Toxic symptoms | | | | | | |
| Yes | 43 (81.1) | 9 (100) | 42 (80.8) | 10 (100) | 0.351 | 0.296 |
| No | 10 (18.9) | 0 (0.0) | 10 (19.2) | 0 (0.0) | | |
| Cough | | | | | | |
| Yes | 29 (54.7) | 4 (44.4) | 28 (53.8) | 5 (50.0) | | |
| No | 24 (45.3) | 5 (55.6) | 24 (46.2) | 5 (50.0) | 0.834 | 1.0 |
| Expectoration | | | | | | |
| Yes | 23 (20.5) | 1 (11.1) | 23 (44.2) | 1 (10.0) | 0.142 | 0.093 |
| No | 30 (56.6) | 8 (88.9) | 29 (55.8) | 9 (90.0) | | |
| Dyspnea | | | | | | |
| Yes | 21 (39.6) | 1 (11.1) | 21 (40.4) | 1 (10.0) | 0.202 | 0.139 |
| No | 32 (60.4) | 8 (88.9) | 31 (59.6) | 9 (90.0) | | |
| ESR | | | | | | |
| >50 | 32 (60.4) | 6 (66.7) | 32 (61.5) | 6 (60.0) | 1.0 | 1.0 |
| <50 | 21 (39.6) | 3 (33.3) | 20 (38.5) | 4 (40.0) | | |
| Tuberculin skin test | | | | | | |
| Positive (N=24) | 18 (34.0) | 6 (66.7) | 18 (34.6) | 6 (60.0) | 0.136 | 0.248 |
| Negative (N=38) | 35 (66.0) | 3 (33.3) | 34 (65.4) | 4 (40.0) | | |
| Chest radiography for pulmonary TB (N=36) | | | | | | |
| Mild (n=10) | 7 (70.0) | 3 (30.0) | 6 (11.5) | 4 (40.0) | 0.079 | 0.039* |
| Moderate advanced (n=22) | 21 (95.5) | 1 (4.5) | 21 (40.4) | 1 (10.0) | | |
| Far advanced (n=4) | 4 (100.0) | 0 (0.00) | 10 (19.2) | 2 (20.0) | | |
| AFB for pulmonary TB (N=36) | | | | | | |
| AFB smear-positive (n=24) | 23 (95.8) | 1 (4.2) | 22 (91.67) | 2 (8.33) | 0.19 | 0.39 |
| AFB smear-negative (n=12) | 9 (75.0) | 3 (25.0) | 9 (75.0) | 3 (25.0) | | |
| Xpert MTB/RIF PCR (N=22) | | | | | | |
| Positive (n=17) | 16 (73.0) | 1 (4.5) | 15 (68.0) | 2 (9.0) | 0.098 | 0.098 |
| Negative (n=5) | 2 (9.0) | 3 (13.5) | 2 (9.0) | 3 (14.0) | | |
| TB culture (N=44) | | | | | | |
| Positive (n=39) | 36 (92.5) | 3 (7.50) | 36 (90.0) | 3 (10.0) | 0.001** | 0.004* |
| Negative (n=5) | 2 (40.0) | 3 (60.0) | 2 (40.0) | 3 (60.0) | | |

AFB, acid-fast bacilli; ESR, erythrocyte sedimentation rate; LAM, lipoarabinomannan; TB, tuberculosis. *Significant. **Highly significant.

immune complexes compared with the small quantity of free LAM (target for assay) in the serum, causing this nonsignificant correlation of the serum LAM with the bacillary burden in the sputum. Infact, with the exception of the agglutination study for LAM antigen detection by Sada *et al.* [21], who did not study this correlation, there are no studies that measured the LAM concentration effectively in the patient serum [22]. However, in 2015, Abd el-Atty *et al.* [17] reported a positive correlation of the quantitative

serum LAM test with the degree of bacillary burden in microbiologically confirmed TB patients ($P<0.001$).

In this study, the quantitative urine LAM measurement was found to have a significant correlation with increasing grades of sputum and BAL AFB smear positivity. The mean urine LAM for grade 1+, grade 2++, and grade 3+++ were 0.52 ± 0.41 , 0.89 ± 0.07 , and 0.89 ± 0.08 ng/ml, respectively ($P<0.05$). Thus, the use of the quantitative urine

LAM test may offer additional value in grading the severity of TB disease. It may be related to the large quantities of free LAM in the urine, which actually reflect the mycobacterial load and correlate positively with the bacillary burden. This result was in agreement with Agha *et al.* [18], who reported that the quantitative urine LAM test results correlate positively with the degree of bacillary burden in patients with microbiologically confirmed TB; it was higher in high-inoculum specimens (0.84 ± 0.49 ng/ml). Shah *et al.* [11] also stated that there was a trend toward higher OD with increasing grades of smear positivity (median ODs of 0.13, 0.18, 0.26, and 0.38 for smear-negative, smear-positive grade 1+, smear-positive grade 2++, and smear-positive grade 3+++ cases, respectively).

In this study, data revealed that at a cut-off point of 0.021 (ng/ml), with an area under the curve of 0.961, the overall sensitivity of the quantitative serum LAM test was 88.7%, specificity 90%, accuracy 88.9%, PPV 98.2%, and NPV 56.3%. The validity of the quantitative serum LAM test in TB subgroups was as follows: in group A₁ (smear-positive pulmonary TB), the sensitivity was 95.8%, specificity 90%, accuracy 94.12%, PPV 95.8%, and NPV 90.9%, which were higher than those of group A₂ (smear-negative pulmonary TB), in which the sensitivity was 83.3%, specificity 90%, accuracy 86.4%, PPV 91%, and NPV 82%, and group B (extrapulmonary TB), in which the sensitivity was 84.6%, specificity 90%, accuracy 86.1%, PPV 95.7%, and NPV 69.2%. Thus, quantitative serum LAM tests represent a valuable addition in the diagnosis of TB and could help in the diagnosis of different forms of TB. This was in agreement with Sada *et al.* [21], who reported that the overall serum LAM test's sensitivity was 72% and specificity 91%; in smear-positive pulmonary TB, the sensitivity was 88% and the specificity was 97.6%, and in smear-negative pulmonary TB, the sensitivity was 67% and the specificity was 93%. Also, this was in accordance with Abd el-Atty *et al.* [17], who reported that the overall serum LAM test's sensitivity was 90% and the specificity was 100%.

The performance of the quantitative serum LAM test was better than that of the ZN AFB smear microscopy test: it was capable of detecting 88.7% of the overall TB cases, 83.3% of the smear-negative pulmonary TB patients, and 84.6% of the extrapulmonary cases compared with the 48% overall detection rate for ZN AFB smear microscopy test. The combination of quantitative serum LAM testing and ZN staining for AFB smear testing increased the sensitivity of

detection of TB cases up to 94%. This was in accordance with Sada *et al.* [21] and Abd el-Atty *et al.* [17], who reported a serum LAM sensitivity of 90% and a ZN AFB smear microscopy test sensitivity of 85%, and the combination of both tests identified 95% of the TB patients. The high validity of the serum LAM test in group A₂ (smear-negative TB) and group B (extrapulmonary TB) may provide additional benefits and an effective diagnostic method to overcome the difficulty in the diagnosis of such groups.

In this study, data revealed that at a cut-off point of 0.081 (ng/ml), with an area under the curve of 0.923 for the quantitative urine LAM test for overall TB patients, the sensitivity was 85.5%, specificity 90%, accuracy 86.1%, PPV 98.1%, and NPV 50%. This was in agreement with Agha *et al.* [18], who found that quantitative urine LAM test had sensitivity, specificity, accuracy, PPV, and NPV values of 81.2, 95.7, 86.4, 73.8, and 97.2%, respectively. Shah *et al.* [11] reported in their study that the overall LAM test sensitivity was 59% in participants with confirmed TB, especially among HIV-positive patients, and the specificity was 96%.

The validity of the quantitative urine LAM test in TB subgroups were as follows: group A₁ (smear-positive pulmonary TB) had sensitivity 95.7%, specificity 90%, accuracy 91.2%, PPV 90%, and NPV 82%, which were higher than those of group A₂ (smear-negative pulmonary TB), with sensitivity 83.3%, specificity 90%, accuracy 86.4%, PPV 91%, and NPV 81.8%, and those of group B (extrapulmonary TB), with sensitivity 80.8%, specificity 90%, accuracy 83.3%, PPV 95.5%, and NPV 64.3%. The high validity of the urine LAM test in group A₂ (smear-negative TB) and group B (extrapulmonary TB) may provide additional benefits and an effective diagnostic method to overcome the difficulty in the diagnosis of such groups. Similarly, Tessema and colleagues studied 200 TB patients and 800 non-TB patients recruited from the Ethiopian Health Center on the basis of an AFB smear and clinical follow-up, and reported a sensitivity of 74% and a specificity of 87%. This sensitivity was higher in smear-positive samples (81%) than in smear-negative samples (57%) [23]. Also, this was in agreement with Agha *et al.* [18], who found that the urine LAM test had sensitivity, specificity, PPV, NPV, and accuracy values of 81.2, 95.7, 97.2, 73.8, and 86.4%, respectively, and Shah *et al.* [11], who reported in their study that the overall LAM test sensitivity was 59% in participants with confirmed TB and the specificity was 96%.

The performance of the quantitative urine LAM test was better than the ZN AFB smear microscopy test: it was capable of detecting 85.5% of the overall TB cases, 83.3% of the smear-negative pulmonary TB patients, and 80.8% of the extrapulmonary TB patients compared with the 48% overall detection rate for the ZN AFB smear microscopy test. The combination of quantitative urine LAM testing and ZN AFB smear microscopy testing increased the sensitivity of detection of TB cases up to 92.5%. Similarly, Shah *et al.* [11], in their work, reported that the LAM test was more sensitive than sputum smear microscopy (42%), and it detected 56% of those who were sputum smear-negative, and the combination of urine LAM testing and ZN AFB smear microscopy testing identified 75% of the confirmed TB cases. Agha *et al.* [18], in their work, reported that the combination of ZN AFB smear microscopy and LAM testing identified 88.2% of the confirmed TB cases.

In this study, the quantitative urine LAM test was more applicable than the serum LAM test for the following reasons: urine samples were simple to collect, process, store, and there were much fewer infection-control concerns. Urine was a particularly useful specimen in young children. Urine LAM values were higher than mean serum values, with a significant increase in urine values in smear-positive TB compared with extrapulmonary TB or smear-negative TB ($P<0.05$); this could help in the diagnosis of different forms of TB. Urine LAM correlated positively with the bacillary burden in sputum smears ($P<0.05$), and so could offer additional clinical insight into the degree of TB disease severity. This was in accordance with Shah *et al.* [11], Agha *et al.* [18] and Kerkhoff *et al.* [13] who reported that the quantitative urine LAM analysis allows a more complete understanding of the test performance, offer grading of the disease severity, and allow the optimal use of the test in different forms of TB disease.

In the present work, a positive TB culture was a significant predictor of high serum LAM levels. This is in accordance with Abd el-Atty *et al.* [17], who reported that smear culture positivity was a significant predictor associated with positive qualitative serum LAM ($P<0.05$). Patients with advanced chest radiography involvement and a positive TB culture had significantly high urine LAM levels. TB culture is the gold standard for the diagnosis of TB. Advanced chest radiography involvements mirror the extensive damage by a high mycobacterial load in such patients. This is in

accordance with Lawn *et al.* [24], who explored pathogen and host factors potentially impacting LAM detection. They reported that 32/199 (16.1%) patients tested with the positive LAM enzyme-linked immunosorbant assay test were associated with a positive sputum smear and culture, and Agha *et al.* [18], who reported that smear and culture positivity and the extensive radiological lesions (regarding pulmonary TB) were significant predictors associated with positive qualitative urine LAM ($P<0.05$).

This study had some limitations such as the rather small sample and the use of stored specimens for LAM testing, which may not yield the same results as fresh specimens. Remote effects of TB progression and treatment on LAM testing could not be studied as it is not a follow-up study; also, the effect of immunocompromization on LAM testing was not studied.

Conclusion

The quantitative serum or urine LAM test represents a simple, rapid, and valuable addition in the diagnosis of TB and may help in the diagnosis of different forms of TB. The quantitative urine LAM test is more applicable than the serum LAM test. Urine LAM correlated positively with the bacillary burden in sputum smears ($P<0.05$) and can offer additional clinical insight into the degree of TB disease severity. The combination of quantitative serum and urine LAM testing identified 98.4% of the TB cases. The combination of quantitative serum LAM and ZN smear microscopy testing or urine LAM testing and ZN smear microscopy testing identified 94 and 92.5% of the TB cases, respectively. Advanced chest radiography involvement and positive TB culture can be used as significant predictors associated with high LAM values.

Recommendations

The assay needs further evaluation in the field to determine its sensitivity and specificity on a larger scale. It is better to use the quantitative urine LAM test than the serum LAM test as it is more applicable. The combination of serum or urine LAM with sputum smear microscopy increased the effectiveness of TB diagnosis and added benefits, especially in smear-negative pulmonary TB or extrapulmonary TB. Measuring LAM in other body fluids such as BAL, sputum, pleural fluid, and spinal fluid, need to be further investigated. Quantitative estimations of LAM test may have future utility as biomarkers reflecting the response to TB treatment.

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Conflicts of interest

There are no conflicts of interest.

References

- World Health Organization (WHO). *Global tuberculosis report (WHO/HTM/TB) 2015. 22*; 2015. Available at: http://www.who.int/tb/publications/global_report/gtbr15_main_text.pdf?ua=1. [Accessed 2016 Jan 10].
- Dorman SE. New diagnostic tests for tuberculosis: bench, bedside, and beyond. *Clin Infect Dis* 2010; **50**(Suppl 3):S173–S177.
- Minion J, Leung E, Talbot E, Dheda K, Pai M, Menzies D. Diagnosing tuberculosis with urine lipoarabinomannan: systematic review and meta-analysis. *Eur Respir J* 2011; **38**:1398–1405.
- Hamasur B, Bruchfeld J, Haile M, Pawlowski A, Bjorvatn B, Källenius G, Svenson SB. Rapid diagnosis of tuberculosis by detection of mycobacterial lipoarabinomannan in urine. *J Microbiol Methods* 2001; **45**:41–52.
- Boehme C, Molokova E, Minja F, Geis S, Loscher T, Maboko L, et al. Detection of mycobacterial lipoarabinomannan with an antigen-capture ELISA in unprocessed urine of Tanzanian patients with suspected tuberculosis. *Trans R Soc Trop Med Hyg* 2005; **99**:893–900.
- Daley P, Michael JS, Hmar P, Latha A, Chordia P, Mathai D, et al. Blinded evaluation of commercial urinary lipoarabinomannan for active tuberculosis: a pilot study. *Int J Tuberc Lung Dis* 2009; **13**:989–995.
- Lawn SD, Edwards DJ, Kranzer K, Vogt M, Bekker LG, Wood R. Urine lipoarabinomannan assay for tuberculosis screening before antiretroviral therapy diagnostic yield and association with immune reconstitution disease. *AIDS* 2009; **23**:1875–1880.
- Mutetwa R, Boehme C, Dimairo M, Bandason T, Munyati SS, Mangwanyana D, et al. Diagnostic accuracy of commercial urinary lipoarabinomannan detection in African tuberculosis suspects and patients. *Int J Tuberc Lung Dis* 2009; **13**:1253–1259.
- Reither K, Saathoff E, Jung J, Minja LT, Kroidl I, Saad E, et al. Low sensitivity of a urine LAM-ELISA in the diagnosis of pulmonary tuberculosis. *BMC Infect Dis* 2009; **9**:141.
- Dheda K, Davids V, Lenders L, Roberts T, Meldau R, Ling D, et al. Clinical utility of a commercial LAM-ELISA assay for TB diagnosis in HIV-infected patients using urine and sputum samples. *PLoS One* 2010; **5**:e9848.
- Shah M, Martinson NA, Chaisson RE, Martin DJ, Variava E, Dorman SE. Quantitative analysis of urine based assay for detection of lipoarabinomannan in patients with tuberculosis. *J Clin Microbiol* 2010; **48**:2972–2974.
- Lawn SD. Serological diagnostic assays for HIV-associated tuberculosis in sub-Saharan Africa? *Clin Vaccine Immunol* 2014; **21**:787–790.
- Kerkhoff AD, Wood R, Vogt M, Lawn SD. Prognostic value of a quantitative analysis of lipoarabinomannan in urine from patients with HIV-associated tuberculosis. *PLoS One* 2014; **9**:e103285.
- Shah M, Variava E, Holmes CB, Coppin A, Golub JE, McCallum J, et al. Diagnostic accuracy of a urine lipoarabinomannan test for tuberculosis in hospitalized patients in a high HIV prevalence setting. *J Acquir Immune Defic Syndr* 2009; **52**:145–151.
- Peter J, Green C, Hoelscher M, Mwaba P, Zumla A, Dheda K. Urine for the diagnosis of tuberculosis: current approaches, clinical applicability, and new developments. *Curr Opin Pulm Med* 2010; **16**:262–270.
- National Tuberculosis Program Egypt (NTP). *Programmatic management guidelines*; 2012. Available at: <http://www.ntp.mohealth.gov.eg/>. [Accessed 2015 Dec 14].
- Abd el-Atty HE, MohamadBakr R, El-Helbawy R, Fathyadass H, El-kalashy MM. The value of serum lipoarabinomannan in the diagnosis of pulmonary tuberculosis. *Menoufia Med J* 2015; **27**:733–739.
- Agha MA, El-Helbawy RH, El-Helbawy NG, El-Sheak NM. Utility of quantitative analysis of urine lipoarabinomannan in the diagnosis of tuberculosis. *Egypt J Chest Dis Tuberc* 2013; **62**:401–407.
- Diagnostic Standards and Classification of Tuberculosis in Adults and Children. This official statement of the American Thoracic Society and the Centers for Disease Control and Prevention was adopted by the ATS Board of Directors, July 1999. This statement was endorsed by the Council of the Infectious Disease Society of America, September 1999. *Am J Respir Crit Care Med* 2000; **161**(Pt 1):1376–1395.
- 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**:103.
- Sada E, Aguilar D, Torres M, Herrera T. Detection of lipoarabinomannan as a diagnostic test for tuberculosis. *J Clin Microbiol* 1992; **30**:2415–2418.
- Mukundan H, Kumar S, Price DN, Ray SM, Lee YJ, Min S, et al. Rapid detection of *Mycobacterium tuberculosis* biomarkers in a sandwich immunoassay format using a waveguide-based optical biosensor. *Tuberculosis (Edinb)* 2012; **92**:407–416. vcnb,smbvn,kdmb,m.
- Tessema TA, Hamasur B, Bjun G, Svenson S, Bjorvatn B. Diagnostic evaluation of urinary lipoarabinomannan at an Ethiopian tuberculosis centre. *Scand J Infect Dis* 2001; **33**:279–284.
- 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**:201–209.