Correlation of TB LAM Ag (*Tuberculosis Lipoarabinomannan Antigen*) Results from Urine with Adenosine Deaminase Levels from Pleural Fluid Patients with Pulmonary TB Accompanied by Pleural Effusion

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ABSTRACT

Background: One of the newest testing methods for active TB uses TB LAM Antigen, in which lipoarabinomannan (LAM) as the examined substance serves as the main component of the cell wall of M. tuberculosis (MTB) and the degradation product of MTB by macrophages in the human body. Patients with active TB with or without HIV infection may experience a decreased antibody response, which causes LAM not to bind to antibodies so that it can pass through the normal glomerular basement membrane and can be detected in the urine. Lateral flow urine lipoarabinomannan assay (LF-LAM) can detect this material with urine samples from active TB patients. Another TB screening method uses the measurement of adenosine deaminase (ADA) activity. ADA has a very important function for the proliferation and differentiation of lymphoid cells, especially T cells, and assists in the maturation of monocytes into macrophages. **Objective:** This study aims to qualitatively prove the correlation between the pleural fluid ADA levels of pulmonary TB patients with pleural effusion using the LF-LAM test results and examine the potential of TB LAM Ag, which correlates with increased pleural fluid ADA levels, as a diagnostic tool for diagnosing pulmonary TB. **Method:** It is an observational analytic study with a cross-sectional design. Materials for this study included temporary urine from active TB patients at Dr. Soetomo Regional Public Hospital collected in May-August 2022. The descriptive analysis was conducted using SPSS 25.0. The data were tested for normal distribution and with the homogeneity test of Shapiro-Wilk's data. The statistical analysis was performed using the Mann-Whitney test, while the kappa suitability test was carried out to determine the correlation and level of concordance between the ADA cut-off value of pleural fluid and the TB LAM Ag test results. **Results:** This study involved 50 subjects consisting of 22 (44%) subjects with ADA test results between 40-100 IU/L compared to 28 subjects (56%) with ADA test results below 40 IU/L. There were 14 (28%) subjects with positive TB LAM Ag test results, consisting of 10 (45%) active TB subjects with a high ADA test cut-off of 40-100 IU/L and 4 (14%) active TB subjects with a low ADA test cut-off below 40 IU/L, with a p-value = 0.012. **Conclusions:** There is a correlation between TB Lipoarabinomannan antigen test results of TB patients with pleural effusion. TB LAM Ag can be detected positive on ADA test results of < 40 IU/L and more positive in active TB patients with high ADA test results of > 40-100 IU/L. Therefore, the TB LAM Ag test is more sensitive than the ADA test. The cut-off value of the ADA test reached 76.4 U/L, and a positive TB LAM was found**.**

Key words: TB Lipoarabinomannan (LAM) Antigen, Adenosine deaminase (ADA) test, Active TB with pleural effusion.

INTRODUCTION

The WHO Global TB Report of 2018 estimated that worldwide TB incidence would reach 10 million new cases in 2017, with a mortality rate of 1.3 million cases. Indonesia was estimated to contribute 1 million cases of TB each year, and this number makes Indonesia the third highest country with TB cases after India and China.[1] This is undoubtedly a concerning condition because the Directly Observed Treatment Shortcourse (DOTS) strategy has been used to control this infection, but many TB patients around the world, including in Indonesia, are not diagnosed and not treated quickly and appropriately.[2] The diagnosis of TB has so far been performed through various tests such as smear (acid-resistant bacillus) staining, plain chest X-ray, and Mycobacterium tuberculosis culture as the gold standard. Mycobacterium tuberculosis culture using Lowenstein Jensen (LJ) medium is

the gold standard for diagnosing TB, but it takes eight weeks because Mycobacterium is a slow-growing organism. Other culture methods using liquid media, including BACTEC TM MGIT 960 and MGIT DST, which are approximately 7-12 days faster than the previous one, have been approved by WHO to be utilized to diagnose TB, but these methods require special expertise, are expensive, and tend to be contaminated with other bacteria.[3]

In recent years, Interferon-gamma (IFN-γ), geneXpert, and molecular Mycobacterium tuberculosis tests have been developed to support accurate and early diagnosis of TB. However, these tests are expensive and sometimes unavailable in areas with limited resources.[1] (WHO, 2020). The limitations of current tests make it increasingly difficult to detect and diagnose TB early, so a new, faster, more accurate, and inexpensive method that uses easy-to-obtain samples is required to diagnose

TB. The problem that many TB patients face is difficulty removing adequate sputum. Patients with extrapulmonary TB also often have difficulty getting samples to be examined, for example, pleural fluid, cerebrospinal fluid, etc. Thereby, they need other modalities, namely easy-to-obtain sample materials often used for diagnostics, such as urine.[4]

The TB LAM Ag test using the Lipoarabinomannan method can detect parts of the MTB cell wall which are the product of bacterial degradation after interacting with macrophages. Mannosylated caps lipoarabinomannan (ManLAM) molecule is a type of LAM for mycobacterial pathogenic species such as Mycobacterium tuberculosis, Mycobacterium leprae, and Mycobacterium bovis. This molecule plays an essential role in the defense of MTB cells.

This test is non-invasive, easy, cheap, and efficient and only takes ± 25 minutes of point-of-care testing. Patients with active TB with or without HIV infection may experience a decreased antibody response, which causes LAM not to bind to antibodies so that it can pass through the normal glomerular basement membrane and can be detected in the urine. Lateral flow urine lipoarabinomannan assay (LF-LAM) can detect this material with urine samples from active TB patients.[5]

Another TB testing method that can use the measurement of adenosine deaminase (ADA) activity is the biomedical method. ADA is an enzyme involved in purine metabolism. ADA has a very important function for the proliferation and differentiation of lymphoid cells, especially T cells, and assists in the maturation of monocytes into macrophages. ADA is an index for cellular immunity, and previous studies have proven its value in diagnosing TB, even for assessing TB pleural effusion. The activity of this enzyme increases in TB patients.[6]

The ADA cut-off value of the pleural fluid of TB patients in various studies varied between 40-100 IU/L with different and quite high sensitivity and specificity. Based on these data, an examination of the pleural fluid ADA levels needs to be utilized to diagnose pulmonary TB. This study qualitatively proves the correlation between the pleural fluid ADA levels of pulmonary TB patients with pleural effusion using the LF-LAM test results and examines the potential of TB LAM Ag, which correlates with increased pleural fluid ADA levels, as a diagnostic tool for diagnosing pulmonary TB.

METHOD

Study time and location

This observational analytic study applied a cross-sectional design, was performed from May to early August 2022, and received ethical approval from the ethics committee of Dr. Soetomo Regional Public Hospital, Surabaya, number: 0452/KEPK/VII/2022. The study was carried out in the pulmonary care room at Dr. Soetomo Regional Public Hospital, Surabaya, as a place for determining the diagnosis and sampling. Sample processing and examination of adenosine deaminase and TB LAM Ag were performed at the Clinical Pathology Laboratory of Dr. Soetomo Regional Public Hospital, Surabaya. The inclusion criteria included adult pulmonary TB patients (> 18 years), both drug-sensitive pulmonary TB and drug-resistant pulmonary TB, diagnosed clinically or bacteriologically, with or without HIV criteria, according to pulmonary specialists based on the International Standard of Tuberculosis Care (ISTC). The patients agreed to participate in this study by signing an informed consent form. The exclusion criteria included patients who had a history of autoimmune disease and malignancy, as well as those who were suffering from other diseases (infectious, chronic, etc.) besides the current pulmonary tuberculosis.

The descriptive statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 25.0. The data were tested for normal distribution, and then a data homogeneity test

was carried out using Shapiro-Wilk's test. The statistical analysis was conducted using the Mann-Whitney test because the data were not normally distributed. ROC is a statistical analysis used to determine the ADA cut-off value of the pleural fluid. The kappa suitability test was used to determine the correlation and level of concordance between the ADA cut-off value of pleural fluid and the TB LAM Ag test results.

The sample for the TB LAM Ag test is the middle portion of urine collected in a sterile urine pot. Then, as much as 60 μL urine was dripped into the TB LAM Ag Abbott POCT. The urine samples were immediately tested on the same day using TB LAM Ag as the pleural fluid samples were tested using the ADA test. The remaining samples were stored in the refrigerator at -80ºC. Pleural fluid sampling with a 10 mL syringe (contained in a syringe and labeled) was performed to check the pleural fluid ADA levels using the enzymatic calorimetric method with the Alinity automated device from Abbott and the ADA test reagent from Abbott. Pleural fluid sampling was conducted by a pulmonary specialist as the patient's clinician in charge.

The ADA test was performed with the Alinity automated tool manufactured by Abbott, USA, and using the enzymatic calorimetric refractometer method. The TB LAM Ag test was conducted using a point of care testing, namely an immunochromatography device with a nitrocellulose membrane from Abbott, USA.

RESULTS

In this study, the subjects amounted to 50 people, consisting of 22 people with ADA test results of 40-100 IU/L or more and 28 people with ADA test results of < 40 IU/L. The subjects were dominated by men, amounting to 25 people (50%). The mean age of each case and control group was 37.06 was 37.06 ± 15.170 (ADA test >40) and 28.81 ± 5.748 (ADA test<40), (Table 1).

A total of 14 subjects (28%) had positive TB LAM Ag test results consisting of 10 (45%) active TB subjects with high ADA test results of 40-100 IU/L or more and 4 (14%) active TB subjects with low ADA test results < 40 IU/L, presented in Table 4. The GeneXpert test was performed before the ADA test, and the results of the examination by a pulmonologist made a clinical and radiological diagnosis of TB before the ADA test. The two groups had statistically and significantly different test results, with a p-value = 0.012 (Table 2).

Table 1: Characteristics of the subject.

Variables	Groups			
	Case (ADA > 40)		Control (ADA < 40)	
	N	%	N	%
Sex				
Male	15	68.2	10	35.7
Female	7	31.8	18	64.3
Age (years)				
Mean ± Standard Deviation	37.06 ± 15.170		28.81 ± 5.748	
Median (min-max)	36 (18–77)		29 (22–41)	
Age Group				
18-40 years	12	54.5	25	89.3
41– 60 years	7	31.9	3	10.7
> 60 years	3	13.6	0	0

Table 2: Shapiro Wilk's test results - normal distribution test of TB LAM Ag levels.

Groups	N	P-value
Case (ADA > 40)	22	0.001
Control (ADA < 40)	28	0.012

Source: Processed from SPSS results

Table 3: Mann-Whitney Test – Comparison between the pleural fluid ADA levels in the case and control groups.

Groups	N	Median (min-max) P-value
Case (ADA > 40)	22	78 (56.6 – 100.3)
Control (ADA < 40)	28	34.7 (29.1 – 40.2)< 0.001

Table 4: Differences between the TB LAM Ag test results of active TB patients with ADA test results of < 40 IU/L and 40-100 IU/L or more.

Variables	Groups		Normality (p-Value)
	ADA Test results < 40 IU/L (%) n = 28	ADA Test results of 40-100 IU/L (%) n = 22	
TB LAM Ag :			p = 0.012
TB LAM (+)	4 (14)	10 (45)	
TB LAM (-)	24 (86)	12 (55)	

TB LAM Ag = tuberculosis lipoarabinomannan antigen; p < 0.05

Based on the normality assumption test, the statistical probability of Shapiro-Wilk's test is smaller than the significant alpha value of 5% (p < 0.05), meaning that the residuals in the data are declared not normally distributed. Thus, instead of the independent t-test, the Mann-Whitney test shall be utilized to test the analysis of differences in the pleural fluid ADA levels.

The median value of the pleural fluid ADA levels in the case group was 78 U/L, the lowest level was 56.6 U/L, and the highest level was 100.3 U/L. Meanwhile, the median value of the pleural fluid ADA levels in the control group was 34.7 U/L, the lowest level was 29.1 U/L, and the highest level was 40.2 U/L. Table 4 informs that the median value of pleural fluid ADA levels in the case group is higher than that in the control group. The median difference in the pleural fluid ADA levels in the case and control groups was 43.3 U/L. The testing for differences between the pleural fluid ADA levels in the case and control groups resulted in a significance value of < 0.001. For this reason, it was stated that there were differences between the pleural fluid ADA levels in the case and control groups. Because the median value of the pleural fluid ADA levels in the case group was higher than that in the control group, it can be concluded that there was an increase in the pleural fluid ADA levels of pulmonary TB patients.

The testing of the Cohen's Kappa coefficient of the positive LAM Ag TB group with ADA test results of > 40 -100 IU/L was performed using the SPSS 25.0 analysis procedure.

Cut-Off Value, Sensitivity, Specificity, PPV, NPV, and Accuracy of the Pleural Fluid ADA levels correlated with TB LAM Ag Results for Diagnosing Pulmonary TB.

The evaluation of prediction results is analyzed visually using the Receiver Operating Characteristic Curve (ROC Curve). The following are the criteria for the Area Under the Curve (AUC):

The results showed that the AUC value was 0.89. Based on these results, it can be concluded that serum ADA levels can classify pulmonary TB patients into the Good Classification criteria. Figure 1 depicts that the cut-off point of the sensitivity and specificity is at the 19th point, which results in a pleural fluid ADA cut-off value of 76.4 U/L when converted to serum ADA values. The test results using AUC are as follows:

Based on the ADA test cut-off value, it can be classified as in table 6.

DISCUSSION

The majority of the subjects in the case group were men, amounting to 15 people (55.9%). This data complies with the WHO report, which states that the incidence and prevalence of global TB patients are more

common in men than women.[1] The high cases of pulmonary TB in men are partly due to the habit of smoking and drinking alcohol, which is mostly done by men and can reduce the body's immunity so that they are more easily infected with TB.

The subjects in the case group were mostly in the age range of 18-40 years, amounting to 12 people (54.5%) who were still of economically productive age. The mean age is 37.06 years. These results indicate that more than half of the patients are in the productive age group. This is in line with previous WHO reports stating that two-thirds of TB cases occur in the economically productive age group (15-50 years).[2]

The latest study in Indonesia by Soedarsono et al. in 2019 indicated that ADA test activity increased in pulmonary TB patients (75.40 ± 9.619 IU/L) compared to pleural effusion patients other than pulmonary TB

Table 5: Criteria for the AUC of the pleura fluid ADA levels with TB LAM Ag.

Nilai AUC	Interpretasi
0.90 – 1.00	Exellent Clasification
0.80 – 0.90	Good Clasification
0,70 – 0.80	Fair Clasification
0.60 – 0.70	Poor Clasification
0.50 – 0.60	Failure

Table 6: Sensitivity, specificity, PPV, NPV, and accuracy of the testing.

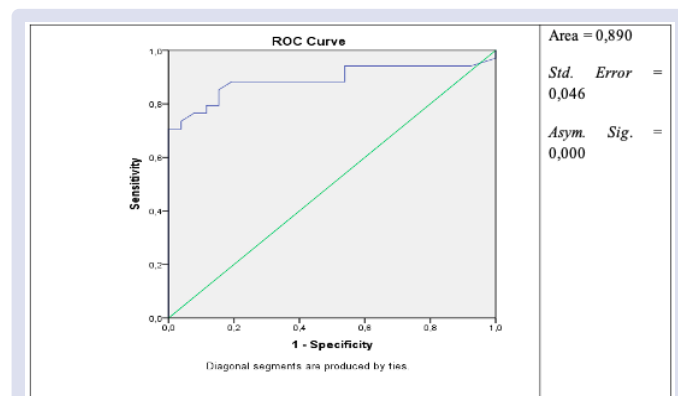| xxx	Results
Sensitivity	85.3%
Specificity	84.6%
PPV	87.9%
NPV	81.5%
Accuracy	85%

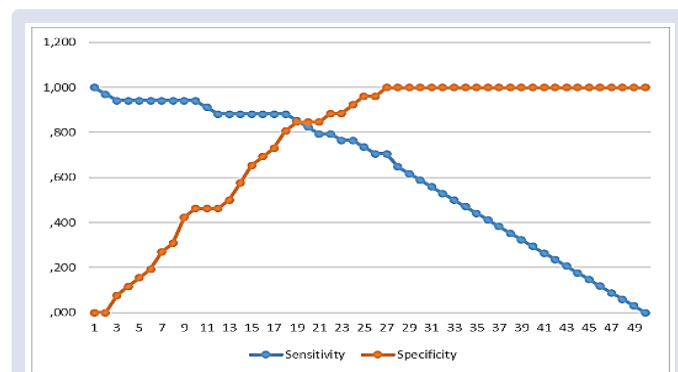**Figure 1:** ROC curve for pleural fluid ADA.

Figure 2: Sensitivity and specificity curves for finding the ADA cut-off value of pleural fluid.

(30.18 ± 2.39 IU/L. The difference between the proportions of the case and control groups was statistically significant (p = 0.012). It is similar to a previous study by Soedarsono et al. for increasing pleural fluid ADA levels in pulmonary TB patients and a study by Sara Puspita et al. regarding LF-LAM positivity in patients with active pulmonary TB.[7]

The correlation between positive TB Lipoarabinomannan antigen results from the urine of active TB patients and high levels of pleural fluid ADA test cut-off of > 40 IU/L is proportional and related to determining the therapeutic strategy and prognosis of active TB patients. MTB culture and molecular examination remain essential when diagnosing and determining the MDR status of rifampicin-resistant TB. TB LAM Ag test is a point-of-care testing method with high sensitivity and specificity developed to detect lateral flow-lipoarabinomannan degradation of urine in a single examination. Based on Singhroy's study in 2020, several countries with high prevalence rates of TB and HIV/AIDS, such as the Central African Republic, Malawi, Myanmar, Uganda, and Zimbabwe, can use the LAM urine test.[8] In Indonesia, this test has not been routinely performed but can help monitor and predict active TB patients due to limited infrastructure.

In this study, 10 (45%) subjects had positive TB LAM antigen test results with high ADA test results of 40-100 IU/L or more, and 4 (14%) subjects had positive TB LAM antigen test results with low ADA test results of < 40 IU/L. In total, there were 14 (54.5%) subjects with positive LAM TB, which complies with a study by Siddiqi et al., showing that 3 (19%) of 16 active pulmonary TB patients could concurrently have HIV infection where LF-LAM was positive and continued with Mtb culture where the results also showed positive TB.[4]

The results of pulmonary TB test with IFN-λ in a study by Yessy et al. to detect the compatibility of tuberculosis with chest X-ray examination and increased Th-1 immune response indicate the severity of pulmonary TB. Based on existing theory, many mechanisms underlie why LAM can be detected in the urine. First, MTB infects the kidney. Second, LAM molecules are small and do not bind to immune complexes so that they can penetrate the glomerular basement membrane (GBM). Third, MTB and LAM immune complexes can damage GBM so that they do not intake. On the other hand, it can be caused by the immunoglobulin composition that does not match the immune response, so the amount of LAM is greater than the antibodies that can bind to the LAM.[9]

Pleural fluid ADA levels as a tool for diagnosing active pulmonary TB had a cut-off value of 78, with p = 0.001, a sensitivity level of 85.3%, a specificity level of 84.6%, a positive predictive value (PPV) of 87.9%, a negative predictive value (NPV) of 81.5% and an accuracy of 85%.
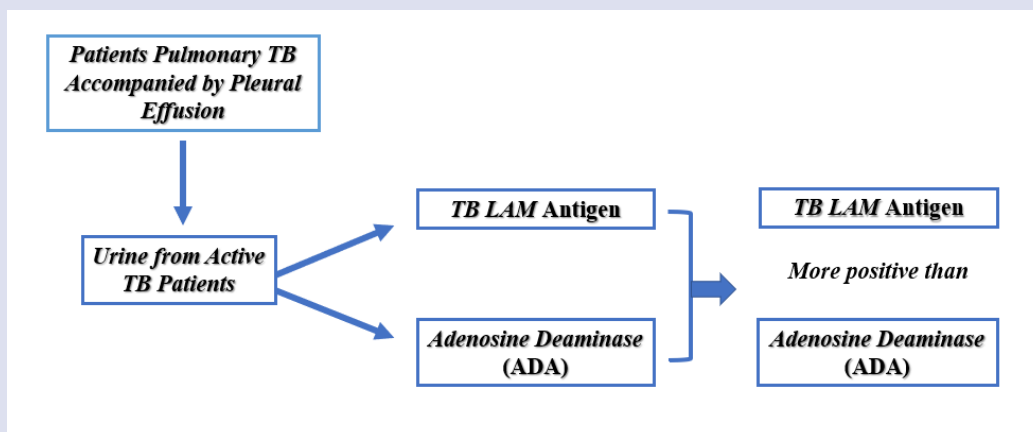
CONCLUSIONS

The TB Lipoarabinomannan antigen correlation test produced more positive results in active TB patients with high ADA test results with a cut-off value of 40-100 IU/L compared to that in active TB patients with ADA test results below 40 IU/L. In the future, a study with sample homogeneity related to comorbid diseases is required. This study has several limitations, namely other diseases, such as autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis, etc.), malignancy, especially lung cancer, HIV, thyroid disorders, and other diseases that can increase pleural fluid ADA levels, cannot be controlled because no tests were done to rule out these diseases.

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GRAPHICAL ABSTRACT