

DIAGNOSTIC PERFORMANCE AND OPTIMAL CUT-OFF VALUES OF PLEURAL FLUID ADENOSINE DEAMINASE IN DIAGNOSING TB PLEURAL EFFUSION AMONG ADULT PATIENTS AT THE LUNG CENTER OF THE PHILIPPINES: A CROSS-SECTIONAL ANALYTICAL STUDY

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ABSTRACT

Background: Tuberculosis (TB) remains a major global health problem, with 10.6 million cases and 1.3 million deaths reported by the World Health Organization in 2023. In the Philippines, 591,000 cases and 31,000 deaths were recorded in 2020. Tuberculous pleural effusion (TPE) is common but diagnostically challenging due to the low sensitivity of conventional tests.

Pleural fluid adenosine deaminase (pfADA) is a simple, rapid, and cost-effective biomarker for TPE. Its performance may vary by population. While international studies support a 40 U/L cut-off, data suggest lower thresholds for settings with a high TB burden, such as the Philippines. This study aims to evaluate the diagnostic accuracy of pfADA and determine optimal cut-off values in adult Filipino patients.

Objectives: The general objective is to appraise pfADA's diagnostic effectiveness in TB pleural effusion compared to other standard references. Specific goals included determining pfADA's accuracy, sensitivity, specificity, positive and negative predictive values, and discriminative ability through ROC curves. Through subgroup analyses, we sought optimal cut-off points for differentiating TB from non-TB effusions in patients with TB, age >45, and a history of TB treatment at least two years prior.

Methods: This retrospective, analytical, cross-sectional study involved adults with exudative, lymphocytic-predominant pleural effusion at the Lung Center of the Philippines (April–November 2023). We used ROC curves and Youden's J index to ascertain pfADA's optimal cut-off, and compared pfADA against GeneXpert TB/MTB and TB culture.

Results: Out of 660 cases, we extensively reviewed 146. The mean age was 45 years, with pfADA exhibiting a notable difference in various diagnostic categories. sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) stood at 82.08%, 92.40%, 90.16%, and 85.66%, respectively. The optimal cut-off value was 47.2 U/L. Stratifying by age, the study affirmed that patients aged 45 and older require a lower cut-off value at 25.2 U/L (sensitivity 72.34% and specificity 80.95%, respectively). The study also showed that retreatment cases of tuberculosis had a higher cut-off value at 94 U/L (sensitivity 88.89 and 48.08, respectively).

Conclusion: The optimum cut-off value for pfADA among the Filipino population is 47.2U/L. The study justifies pfADA's diagnostic performance in TB pleural effusion. The findings align with existing literature, highlighting pfADA's potential as a dependable TPE marker. However, given the study's single-center nature and limited sample size, broader multi-center validations are advocated for enhanced generalizability.

Keywords: Tuberculosis, pleural effusion, adenosine deaminase, diagnostic performance, cut-off point

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Tuberculosis is still a global health problem, especially in low-income countries. In 2023, WHO reported that TB afflicted an estimated 10.6 million worldwide and caused 1.3 million deaths, making it the 13th leading cause of mortality from a single infection. The global incidence of people newly diagnosed with TB was 7.5 million, the highest number since WHO began global TB monitoring in 1995.¹ In 2020, 591,000 people in the Philippines developed TB, while 31,000 people died.² Extrapulmonary disease is the initial presentation in about 25% of patients, involving lymph nodes and pleura.³ TB is currently one of the most frequent causes of exudative pleural effusion worldwide.⁴

Tuberculous pleural effusion (TPE) is a paucibacillary manifestation of tuberculosis, diagnosed through examination of the pleural fluid, including bacteriological tests and biomarker detection.⁵ Despite TPE being a common clinical entity, it can be difficult to diagnose. Conventional smear microscopy of pleural fluid has low yield rates of less than 10%.⁶ Even rapid molecular tests such as Xpert Mycobacterium tuberculosis/Rifampicin resistance (Xpert MTB/RIF) report a wide range of sensitivity, from 28% to 81% in pleural fluid and 90% in pleural tissue.⁵ While medical thoracoscopy has a sensitivity of up to 100% for TPE and can improve culture and Xpert MTB/RIF yield rates, it is invasive and not always available in resource-limited settings. Pleural fluid adenosine deaminase (pfADA) is a simple, rapid, inexpensive surrogate marker for tuberculous pleural effusion (TPE) that is used particularly to rule out the disease. A cut-off value of 40 U/L is currently being used based on overseas data. However, the level of pleural pfADA is influenced by several factors, which include geography (high TB burden vs low TB burden countries), age, and comorbidities (malignancy, previous history of TB treatment, etc.). The use of pleural fluid ADA has been limited in our locality, as only a few centers can perform such tests. Furthermore, only one local study has evaluated its diagnostic value and importance.

To bridge these gaps, this study aimed to evaluate the diagnostic performance of pleural fluid ADA compared with the composite standard of care references in a Filipino population and determine the different cut-off values to diagnose TB effusion appropriately for patients with different age groups and comorbidities.

MATERIALS AND METHODS

Study Design

This was a retrospective, analytical, cross-sectional study of medical records of adult patients who presented with exudative, lymphocytic-predominant pleural effusion.

Study Setting

This was a single-center study of patients' medical records managed at the Lung Center of the Philippines from April 2023 to November 2023.

Study Population

The study included adult patients with pleural effusion (e.g., malignancy, pneumonia, heart failure, and chronic kidney disease) identified through non-probability sampling, who underwent pleural fluid drainage via thoracentesis, video-assisted thoracoscopic surgery, thoracoscopy, chest tube thoracostomy, pigtail catheter insertion, or pleural catheter placement. Eligible participants were Filipino patients aged ≥ 19 years with imaging-confirmed pleural effusion (chest radiograph, computed tomography, or ultrasound) and exudative, lymphocyte-predominant pleural fluid (lymphocytes $>50\%$).

Only cases with pleural fluid adenosine deaminase (pfADA) measured at the Lung Center of the Philippines and a complete composite reference standard work-up were included. The composite reference standard consisted of sputum and pleural fluid Gram stain and culture and sensitivity, GeneXpert, acid-fast bacilli smear, and tuberculosis culture; pleural fluid glucose, protein, and lactate dehydrogenase; corresponding serum studies; and pleural fluid cytology.

Patients were excluded if pfADA testing was performed in external laboratories, pfADA results were unavailable, the composite reference standard was incomplete, or duplicate specimens were identified. For patients undergoing multiple thoracenteses, only the first pleural fluid sample was included in the statistical analysis, although all samples were reviewed to establish the reference standard diagnosis. A positive culture, GeneXpert, or acid-fast bacilli smear in any specimen was considered diagnostic.

Among the 146 lymphocytic predominant pleural effusion cases, 61 were Bacteriologically confirmed tuberculosis, 39 were clinically diagnosed tuberculosis, 41 were Not Tuberculosis without malignancy, and 5 were not TB with malignancy.

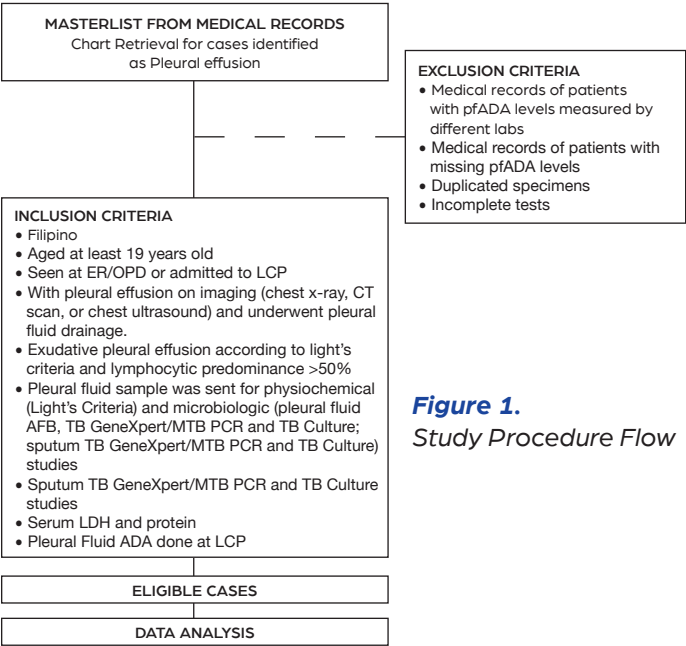


Figure 1.
Study Procedure Flow

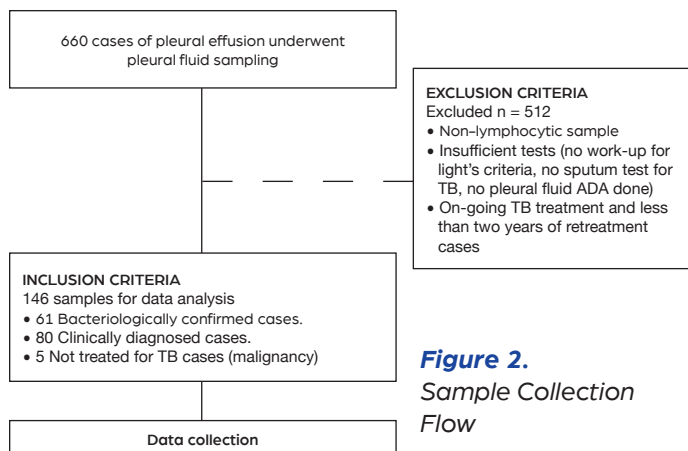


Figure 2.
Sample Collection Flow

Study Instrument/Intervention

Descriptive statistics were used to summarize participant characteristics, using frequency and proportion for categorical variables, mean/standard deviation for normally distributed interval/ratio variables, while median/interquartile range was used for non-normally distributed ones. The Shapiro–Wilk test was used to assess data normality. For determining the optimal cut-off of pleural fluid adenosine deaminase (pfADA) in tuberculosis pleural effusion detection, a receiver operating characteristic (ROC) curve was constructed. The AUC–ROC indicated accuracy, categorized as failing, poor, exemplary, or excellent. Youden's J index was used to calculate overall diagnostic effectiveness, with the maximum value determining the optimal cut-off. PfADA's diagnostic performance—using sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), negative likelihood ratio (LR–), and accuracy—was compared against a composite reference standard comprising GeneXpert MTB/Rif, TB Lamp, AFB smear, cytology, and TB culture, serving as the gold standard.

Study Procedure

The study was conducted from April to November 2023. Approval was obtained from the Institutional Review Board of the Lung Center of the Philippines before study initiation. To ensure confidentiality, all records were anonymized using coded identifiers.

Data were extracted from the medical charts, including baseline characteristics (age, sex, smoking history, comorbidities, tuberculosis exposure, and clinical features), imaging findings (chest radiograph, computed tomography, and ultrasound), and procedural details. Pleural fluid analyses collected included clinical chemistry (pleural fluid ADA) and microbiologic tests (pleural fluid TB culture, GeneXpert/PCR, AFB smear; sputum TB culture, GeneXpert/PCR, and AFB smear).

Outcome Measures

The diagnostic performance of pleural fluid adenosine deaminase (ADA) for tuberculous pleural effusion was analyzed for sensitivity, specificity, positive and negative predictive values, likelihood ratios, and receiver operating characteristic (ROC) curve analysis, with the optimal cut-off defined as the value maximizing sensitivity and specificity.

Primary outcomes included the accuracy and diagnostic indices of pleural fluid ADA compared with a composite reference standard across bacteriologically confirmed tuberculosis, clinically diagnosed tuberculosis, and malignant cases. The study also aimed to determine the optimal ADA cut-off values for differentiating tuberculosis from non-tuberculous exudative pleural effusions among patients aged ≥ 45 years and those with ≥ 2 years of tuberculosis treatment.

Pleural fluid ADA was the primary predictor. Potential confounders included age and prior tuberculosis treatment; effect modification was assessed by categorizing cases according to diagnostic category, age group, and treatment history.

Table 1. *Case classification among the study population*

DIAGNOSIS	NO OF PATIENTS (%)
Bacteriologically Confirmed	61 (42.1)
Clinically Diagnosed TB	39 (26.9)
Not Clinically Diagnosed TB Without Malignancy	41 (28.3)
Not Clinically Diagnosed TB With Malignancy	5 (3.4)
TOTAL	146

TB = Tuberculosis

Diagnostic Criteria

The ADA test is preferred over the IFN- γ test in countries with a high prevalence of TB because of its long history of success, simplicity, and cost-effectiveness.⁷ ADA was made available in the St. Luke's Medical Center in May 2019 (Danganan MD, Tan W. Diagnostic value of adenosine deaminase in tuberculous pleural effusion among adult patients in St. Luke's Medical Center

Quezon City. Unpublished data, 2021) The normal value of ADA in pleural fluid at SLMC–QC is 0–30 U/L, and as a highly sensitive test, a result of <30 U/L can exclude the diagnosis of tuberculous pleural effusion.

Tuberculous pleural effusion was diagnosed microbiologically when at least one pleural fluid microbiologic test (GeneXpert, TB culture, or AFB smear) yielded a positive result. Pleural fluid cytology

demonstrating granulomatous inflammation suggestive of tuberculosis was also considered diagnostic. Cases with positive direct microbiologic tests (pleural fluid GeneXpert, TB culture, or AFB smear) were classified as bacteriologically confirmed tuberculous pleural effusion. Clinically diagnosed tuberculous pleural effusion included cases with pleural fluid cytology showing chronic inflammatory patterns consistent with tuberculosis in the absence of microbiologic confirmation.

In this study, cases were classified based on the attending physician's diagnosis.

Statistical Analysis

For this study, prevalence referred to the number of cases of a given kind of pleural effusion divided by the total number of pleural effusions studied. Non-normal distributions were identified from their skew and kurtosis. The Student's T-test was used to estimate the statistical difference between means of normally distributed variables, and the Wilcoxon's T-test was used for non-normally distributed variables. The ADA levels were evaluated in terms of sensitivity, specificity, positive predictive value (PPV); and negative predictive value (NPV). Positive likelihood ratio (PLR), accuracy, and precision (Table 2) were computed as well.

The area under the ROC curve (AUROC) was used to measure the discriminative ability of pleural fluid ADA in

different clinical situations, particularly in differentiating tuberculosis from non-TB, identifying TB in patients 45 years old and older, and identifying TB in retreatment cases. The Youden Index was used to summarize the receiver operating curve and select the optimal cut-off point.

Ethical Considerations

The study was approved by the Lung Center of the Philippines Ethics Research Committee and the Lung Center of the Philippines Institutional Review Board before data collection commenced. We also secured permission to review charts. All data were kept confidential.

RESULTS

Study Participants

Out of 660 pleural effusion cases from April 2023 to November 2023, 146 cases with complete work-up were extensively reviewed, achieving a confidence interval of 95% (level of confidence of 5%).

Baseline Characteristics

The population's demographic and clinical profiles are summarized in Table 3, delineating individuals based on varying Adenosine Deaminase (ADA) levels using a 30U/L cut-off, with associated p-values signifying the statistical significance of inter-group disparities.

Table 2. Summary of Formula

PPV	{PPV = (prevalence x sensitivity)/[(prevalence x sensitivity) + (1 - prevalence) (1 - specificity)]}
NPV	{(1 -prevalence) x specificity}/[(1 - prevalence) x specificity + prevalence x (1 - sensitivity)]}
PLR	[sensitivity / (1 - specificity)], negative likelihood ratio [(1 - sensitivity) / specificity]
Accuracy	[true positive + true negative/(TP + FP + TN + FN)]
Precision	[true positive/(true positive + false positive)]



BIOFIRE FILMARRAY Respiratory Panel 2.1

SPECIMEN: Nasopharyngeal Swab

WHAT IT CAN DETECT:

VIRUSES		BACTERIA
Adenovirus	Influenza A, including subtypes H1, H3 and H1-2009	Bordetella parapertussis
Coronavirus 229E	Influenza B	Bordetella pertussis
Coronavirus HKU1	Parainfluenza Virus 1	Chlamydia pneumoniae
Coronavirus OC43	Parainfluenza Virus 2	Mycoplasma pneumoniae
Coronavirus NL63	Parainfluenza Virus 3	
MERS-CoV	Parainfluenza Virus 4	
SARS-CoV-2	Respiratory Syncytial Virus	
Human Metapneumovirus		
Human Rhinovirus/Enterovirus		



Table 3. Demographic and clinical profiles of the respondents according to pleural fluid adenosine deaminase (ADA)

DEMOGRAPHIC & CLINICAL PROFILES	TOTAL (n = 146)	ADA ≤ 30U/L (n = 62)	ADA > 30 U/L (n = 84)	p-VALUE
	Frequency (%); Mean ± SD; Median (IQR)			
Age, years	45 (IQR 35–55)	42 (IQR 33–50)	48 (IQR 39–58)	<0.001
Sex				
MALE	51 (35%)	21 (34%)	30 (36%)	0.701
FEMALE	95 (65%)	41 (66%)	54 (64%)	
Comorbidities				
Hypertension	77 (53%)	27 (44%)	50 (60%)	0.052
Diabetes mellitus	50 (34%)	19 (31%)	31 (37%)	0.338
History of PTB	34 (23%)	14 (23%)	20 (24%)	0.838
COPD	29 (20%)	11 (18%)	18 (21%)	0.529
Pneumonia	10 (7%)	4 (6%)	6 (7%)	0.763
Heart Failure	15 (10%)	6 (10%)	9 (11%)	0.791
Cancer	14 (10%)	5 (8%)	9 (11%)	0.398
Others	5 (3%)	1 (2%)	4 (5%)	0.348
None	32 (22%)	15 (24%)	17 (20%)	0.515
Sign and Symptoms				
Cough	72 (49%)	30 (48%)	42 (50%)	0.819
Dyspnea	89 (61%)	38 (61%)	51 (61%)	0.989
Fever	65 (45%)	28 (45%)	37 (44%)	0.903
Orthopnea	23 (16%)	10 (16%)	13 (16%)	0.975
Chest pain	31 (21%)	12 (19%)	19 (23%)	0.575
Weight loss	54 (37%)	21 (34%)	33 (39%)	0.408

Main Results

The population's mean age was 45 years (IQR 35–55), with those having ADA ≤30U/L and ADA >30U/L demonstrating mean ages of 42 years (IQR 33–50) and 48 years (IQR 39–58), respectively. The age distribution differed significantly between individuals with ADA levels ≤30U/L and those with ADA levels > 30U/L (*p*-value < 0.001). The population was 35% male and 65% female, with comparable proportions observed in both ADA groups (*p* = 0.701). There was a higher prevalence of hypertension and diabetes among patients with elevated ADA, though these differences did not reach statistical significance (*p* = 0.052 and *p* = 0.338, respectively).

No significant differences were observed in the history of previous tuberculosis (*p* = 0.838), chronic obstructive pulmonary disease (*p* = 0.529), pneumonia (*p* = 0.763), heart failure (*p* = 0.791), or cancer (*p* = 0.398) between the ADA groups. There was likewise no difference in the prevalence of signs and symptoms (including cough, dyspnea, fever, orthopnea, chest pain, and weight loss).

Among the 61 cases with bacteriologically confirmed Tuberculosis (TB), 55 (90.16%) exhibited ADA levels > 30 U/L, representing true positives (TP). However, 12 cases with ADA levels > 30 U/L did not have bacteriologically confirmed TB, constituting false positives (FP). Conversely, among the 85 cases without bacteriologically confirmed TB, 73 with ADA levels ≤ 30 U/L were correctly identified

as true negatives (TN), while 6 cases with ADA levels ≤ 30 U/L had bacteriologically confirmed TB, constituting false negatives (FN) (Table 4).

Sensitivity and specificity were calculated using a 2x2 table (Table 4). The sensitivity was 82.08%, indicating the proportion of true positives among bacteriologically confirmed TB cases. The specificity was 92.40%, signifying the accuracy of identifying cases without bacteriologically confirmed TB. The positive predictive value was at 90.16%, while the negative predictive value was at 85.66%, demonstrating the pfADA's ability to discriminate true positive and true negative results.

Likelihood ratios were computed to discriminate the likelihood of pfADA among patients with TB effusion compared to the likelihood that the same result would be expected in a patient without TB effusion. Likelihood ratios of 1 indicate that the test is uninformative, whereas the likelihood ratios of more than 1 or close to 0 indicate that the test is informative. Positive and negative likelihood ratios were at 10.18 and 0.09, respectively. Thus, a positive pfADA would be 10.18 times more likely among patients with TB effusion than those without the disease, with a 0.09 likelihood to miss out on the disease in those with negative pfADA. The accuracy rate was computed at 87%, and the precision rate was at 90.16%. These results highlight the diagnostic performance of ADA levels.

Table 4. Sensitivity and Specificity

	TB	NON-TB	TOTAL
ADA >30	A 55	B 6	61
ADA <30	C 12	D 73	85
TOTAL	67	71	146

The boxplot analysis indicates that the distribution of values for each diagnostic category is positively skewed, as evidenced by the elongated right tails in the plots (Figure 2). For the Bacteriologically Confirmed diagnosis, the median (Q2) is approximately 53.1, with the majority of the data falling between 43.1 (Q1) and 66.8 (Q3), and some higher values extending up to 144.6. Similarly, the Clinically Diagnosed TB category displays positive skewness, with a median (Q2) of around 18.6. Most data range from 10.5 (Q1) to 46.4 (Q3), with outliers reaching 92.5. The Not Clinically Diagnosed TB Without Malignancy category also exhibits positive skewness, with a median (Q2) of approximately 13.2. Most of the data range from 8.6 (Q1) to 41.5 (Q3), with some higher values extending up to 136.7. Lastly, the Not Clinically Diagnosed TB With Malignancy data is positively skewed, with a median (Q2) of around 20.2. Most of the data range from 5.08 (Q1) to 42.4 (Q3), with a few outliers reaching up to 48.

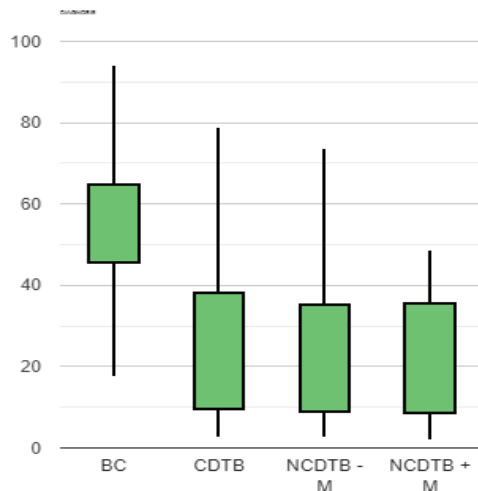


Figure 2. Boxplot analysis: Distribution of values for each diagnosis

The Receiver Operating Characteristic (ROC) curve (Figure 3) is a graphical representation of the performance of a binary classifier system. It plots the true positive rate (sensitivity) against the false positive rate (1 – specificity) for different threshold values. The area under the ROC curve (AUROC) quantifies the overall performance of the test. An AUROC closer to 1 indicates better discrimination ability, while 0.5 suggests the test performs no better than chance.

The AUROC was 0.867 (CI = 0.786 to 0.926, standard error = 0.039, p-value < 0.001), reaching statistical significance.

This suggests that the performance of pfADA in diagnosing tuberculosis is significantly better than chance (Figure 3, Table 5).

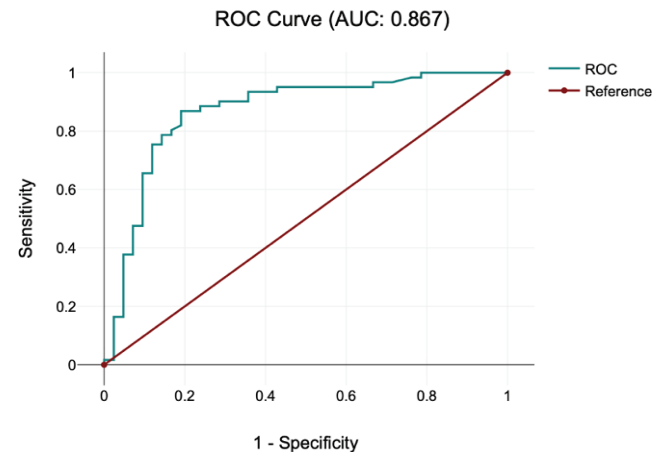


Figure 3. The Receiver Operating Characteristic (ROC) Curve of Pleural Fluid ADA in Diagnosing Tuberculosis

Table 5. The Area Under the ROC Curve (AUROC) of Pleural Fluid ADA in Diagnosing Tuberculosis

Test Result Variable	Area	Std. Error	P-Value	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Pleural Fluid ADA (pfADA)	0.867	0.039	<0.001 *	0.786	0.926

Note: * Significant at 0.05; Null hypothesis: true area = 0.50

The AUROC for patients 45 years old and older was 0.782 (CI = 0.665 to 0.873, standard error = 0.0618, p-value < 0.001), suggesting that the performance of pfADA in diagnosing tuberculosis in these patients was significantly better than chance (AUROC = 25.2) (Figure 4, Table 6).

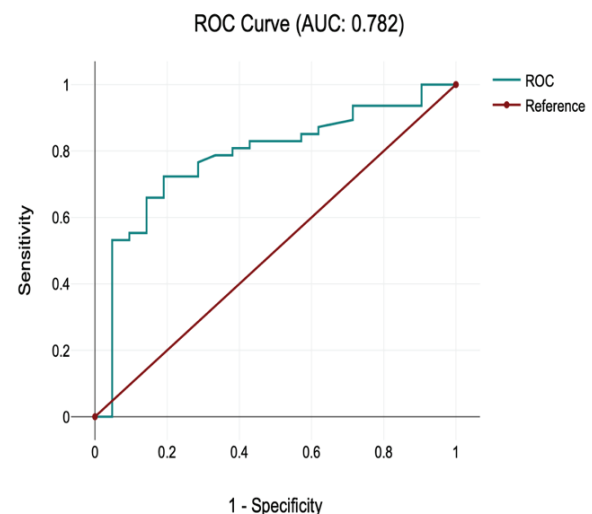


Figure 4. The Area Under the ROC Curve (AUROC) of Pleural Fluid ADA in Diagnosing Tuberculosis based on Age >45

Table 6. The Area Under the ROC Curve (AUROC) of Pleural Fluid ADA in Diagnosing Tuberculosis based on Age > 45

Test Result Variable	Area	Std. Error	P-Value	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Pleural Fluid ADA (pfADA)	0.782	0.0618	<0.001 *	0.665	0.873

Note: * Significant at 0.05; Null hypothesis: true area = 0.50

The AUROC for retreatment cases was 0.641 (CI = 0.508 to 0.760, standard error = 0.108, p-value = 0.1906) and did not reach statistical significance (Figure 5, Table 7).

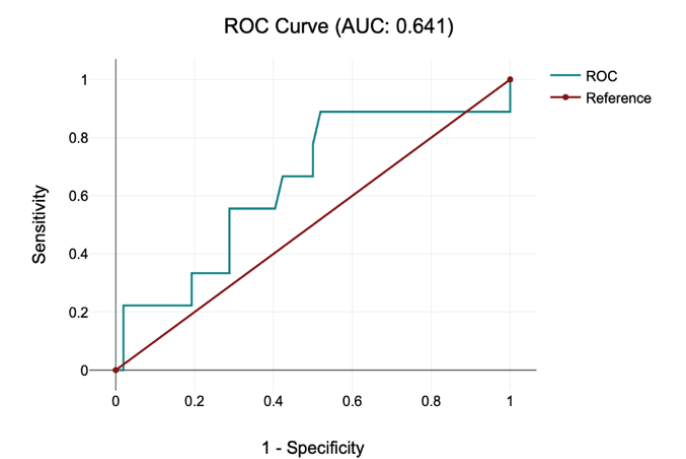


Figure 5. The Receiver Operating Characteristic (ROC) Curve of Pleural Fluid ADA in Diagnosing Tuberculosis based on Previous TB therapy, with at least 2 years of treatment

Table 7. The Area Under the ROC Curve (AUROC) of Pleural Fluid ADA in Diagnosing Tuberculosis based on retreatment.

Test Result Variable	Area	Std. Error	P-Value	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Pleural Fluid ADA (pfADA)	0.641	0.108	0.1906	0.508	0.760

Note: * Significant at 0.05; Null hypothesis: true area = 0.50

DISCUSSION

PfADA demonstrated good diagnostic accuracy, with a sensitivity of 82.08% and specificity of 92.40%.

A cut-off value of 47.2 U/L provided better diagnostic performance compared with the conventional 30 U/L threshold, supporting the use of higher cut-offs in settings with a high tuberculosis burden. Among patients aged ≥45 years, a lower cut-off (>25.2 U/L) was observed, consistent with prior studies, although the association between age and ADA was not statistically significant within the TPE subgroup-likely due to limited sample size. No significant difference in ADA levels was observed among retreatment cases based on AUROC analysis, although this finding may also be limited by small sample size.

These findings highlight the importance of population-specific cut-offs and reinforce the role of pfADA as a practical, rapid, and cost-effective diagnostic tool in exudative, lymphocytic pleural effusion. Subgroup analyses further support its utility across different clinical scenarios.

However, the study has limitations, including its single-center design, limited sample size, and potential selection and misclassification biases. Some cases of TPE may have been misclassified due to spontaneous resolution or uncertain etiology. In addition, the lack of physician blinding to ADA results may have introduced diagnostic bias.

CONCLUSION

In conclusion, pfADA is a reliable diagnostic marker for TPE. While the conventional cut-off of 30 U/L performs well, a higher threshold (~47.2 U/L) may be more appropriate in the Filipino population, with lower thresholds considered for older patients. Further multicenter studies are recommended to validate these findings and refine diagnostic thresholds.

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