SAS Journal of Medicine
Abbreviated Key Title: SAS J Med
ISSN 2454-5112
Journal homepage: https://saspublishers.com

OPEN ACCESS

Profile of Adenosine Deaminase and the Impact of Age and Female Sex on Its Level in 157 Patients with Pleural Effusion Syndrome

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DOI: 10.36347/sasjm.2021.v07i06.002 | **Received:** 23.04.2021 | **Accepted:** 03.06.2021 | **Published:** 06.06.2021

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Abstract | **Original Research Article**

Background: The profile of adenosine deaminase enzyme and the impact of demographic data on its level are important in patients with pleural effusion syndrome (PES). ***Objective*:** To evaluate the levels of adenosine deaminase (ADA) in the pleural fluids (P-ADA) of untreated and non-surgically manipulated female and male adult patients with several confirmed causes of PES. ***Methods*:** This observational, retrospective cohort study in the State of Rio de Janeiro, Brazil, involved 157 patients. The study variables were age, total P-ADA determined using a commercial kit, and male and female sex. *Results*: The causes, prevalence, and median P-ADA (n/%/U/L) were tuberculosis (44/28.0/42.0), adenocarcinoma (37/24.0/9.75), transudate (33/21.0/6.85), simple parapneumonic pleural effusions (PPE; 15/10.0/9.38), complicated PPE/empyema (8/5.0/32.9), lymphoma (7/4.0/401.2), squamous cell carcinoma (7/4.0/13.11), and others (6/4/15.2). For P-ADA, Dunn's post hoc test revealed significance for tuberculosis vs. transudates, vs. simple PPE, and vs. adenocarcinoma (all $P<0.05$), and not significant for CPPE/empyema, lymphoma, SCC, and others (all $P>0.05$). For age, Dunn's post hoc test revealed significance for tuberculosis vs. transudates, vs. simple PPE, and vs. adenocarcinomas (all $P<0.05$). Sex was not significant in the overall PES group (Chi=0.062, P=0.8028). Kendall's correlation of the relationship between P-ADA and age for pleural tuberculosis (n=41) was significant after 1000 iterations with bootstrap for 95% CI (Tau=-0.213, 95% CI - 0.449-0.0833, P=0.0490). A negative LOESS regression was evident between P-ADA and age >40 years. ***Conclusions*:** Evaluation of pleural ADA levels is useful for diagnosing pleural tuberculosis, while sex is not. A negative and significant relationship between P-ADA level and age >40 years was evident.

Keywords: Pleural effusion, Adenosine deaminase, Pleural tuberculosis, Malignancy, Demographic data, Sex.

INTRODUCTION

The causal diagnosis of pleural effusion syndrome (PES) depends on clinical manifestations, imaging findings, and pleural biomarkers in materials withdrawn via surgical procedures performed using different techniques [1].

The analysis of pleural biomarkers is important in clinical practice because the first approach in diagnosing the cause of PES is to correctly classify the pleural fluid as exudate or transudate, according to several published criteria [2-5].

The prevalent cause of PES in Brazil is tuberculosis (TB) [6]. The microbiological diagnosis of pleural TB is difficult to establish because of the paucibacillary nature of the pleural fluid. Therefore, conventional tests, such as bacilloscopy and culture of Mycobacterium sp. in pleural fluids, have low accuracy. Amplification of nucleic acids with a positive result is a presumptive case. However, a negative result cannot exclude a diagnosis. Although pleural gamma interferon is a highly accurate biomarker, it is only available in clinical research. Closed needle pleural biopsy (CNPB) with histopathological findings is an accurate procedure for pleural TB and adenocarcinoma. However, in

inexperienced hands, several fatal surgical complications may occur. Currently, it is desirable to refer a patient with PES without a causal diagnosis for video-assisted thoracoscopic surgery (VATS) after one or two inconclusive thoracentesis attempts with laboratory analysis of pleural fluid [7].

Total adenosine deaminase enzyme (ADA, E.C. 3.5.4.4) is an accurate biomarker for TB in pleural fluids obtained by thoracentesis. The test does not depend on a human immunodeficiency virus (HIV)-positive serostatus of the patient and tissue from CNPB [7-8].

In recent years in Brazil, 29% of the total notified cases and relapses of pulmonary TB involved female patients [6]. Evidence for differences between males and females is well documented for a variety of inflammatory conditions [9]. The aim of our research was to evaluate the levels of total ADA in pleural fluids (P-ADA) in female and male adult patients who were untreated and not surgically manipulated, with several confirmed causes of PES.

MATERIALS AND METHODS

Study design

The TRIPOD and STROBE guidelines were used to improve the transparency of our research [10, 11]. This was an observational study with a retrospective cohort conducted from March 2015 to December 2019 at two teaching hospitals in the State of Rio de Janeiro, Brazil. The Ethics Committee approved this study according to the guidelines of the Helsinki Declaration under number 80/02. Written consent was obtained from all patients.

Sample size

This study had a representative sample size of 157 pleural fluid samples from 157 patients with proven causes of PES. The sample size was appropriate according to the study design. It was precise enough to calculate descriptive and inferential statistics on type I and II errors, effect size, standard deviation, and was not influenced by administrative issues and costs. The sample size featured an acceptable level of significance, power, expected effect size, and underlying event rate in the population [12].

Inclusion criteria

The causal diagnosis of PES was confirmed after one or two thoracentesis procedures with laboratory evaluation of the pleural fluid and VATS, if necessary [7,8]. According to clinical manifestations and imaging findings, pleural TB was confirmed with a positive culture of pleural fluid or tissue. Additionally, confirmation involved the presence of granulomas in the pleural tissue by biopsy and the absence of other pleural granulomatous diseases. All patients had a favorable clinical course after 6–12 months of treatment [7, 8]. The Maranhão and Silva Junior criterium and clinical manifestations were used to diagnose a pleural transudate or exudate with total pleural proteins and lactate– dehydrogenase (LDH) activity only in pleural fluids [3]. Simple parapneumonic pleural effusions (PPEs) have been proven in patients with cough, fever, and lung image examinations with infiltrates or consolidations that disappeared with antibiotic treatment. Patients with empyema were diagnosed with pus in the pleural fluid after thoracentesis and septations on pleural ultrasound. Complicated parapneumonic pleural effusion (CPPE) was diagnosed based on the appearance and biochemical pleural biomarkers reported in the literature, specifically glucose, LDH, and pH [13, 14]. Malignancy in pleural effusions was confirmed if there was a cytologic or histologic diagnosis of cancer compatible with lymphoma, squamous cell carcinoma (SCC), leukemia, and adenocarcinoma in the pleural space [**1**]. Pseudo-Meigs syndrome was characterized by the presence of a benign pelvic tumor confirmed by histologic findings. Dressler syndrome was evident due to pericarditis and pleural effusion that evolved after myocardial infarction. Chylothorax was a rare diagnosis evaluated with the milky appearance of pleural fluid at thoracentesis, pleural biomarkers (cholesterol and triglyceride), clinical manifestations, and compatible image findings [1].

Exclusion criteria

The exclusion criteria included absolute contraindications, refusal to accept thoracentesis or other invasive procedures, use of immunosuppressive medications, hemolysis in pleural liquids, renal failure, HIV infection, pleural effusion of an unknown cause, and cases with missing values. According to the ADA dosage kit instructions, patients with biochemical and metabolic disturbances were excluded as these factors interfere with the enzyme level in pleural fluids [**15]**.

ADA assay

The ADA assay for clinical chemistry analyzers (Diazyme Laboratories, Inc., Poway, CA, USA) was performed with a commercial kit and a kinetic method that has more sensitivity than the classical colorimetric method of Giusti and Galanti [15]. One unit of ADA was defined as the amount of ADA that generated one μmol/min of inosine at 37 °C.

STATISTICAL ANALYSES

All quantitative and qualitative data from our patients were analyzed using Microsoft Excel, version 2010. Both descriptive and inferential statistics were performed using GraphPad Software version 6.0, and MedCalc for Windows version 15.0. The collected data were analyzed using univariate statistical tests. The Shapiro-Wilk test was used to assess the normality of the data and homogeneity of variances. Non-normal distributions were expressed as median and interquartile range (IQR). Qualitative or categorical variables were expressed as proportions. A chi-squared test was used

to compare the proportions. The Mann-Whitney (M-W) non-parametric test was used to compare medians of two independent group data when it was not normally distributed and because logarithmic transformation was not performed. The non-parametric Kruskal-Wallis (K-W) and Dunn's post hoc tests were used to compare medians of P-ADA among three or more unpaired groups of several causes of PES. Figures with box-and-whiskers in combination with dot plots are presented because they provide a statistical summary of the data from tables (median, range, and quartiles) without concealing the data. The degree of the relationship between the variables, P-ADA and age, for pleural TB was determined using Spearman's rho and Kendall's rank correlations with 95% confidence interval (CI) as the distribution of variables was not normal. The 95% CI for Kendall's tau was estimated using bootstrap with 1000 replications or iterations and 998 random number seeds [16]. A locally weighted scatterplot smoothing (LOESS or LOWESS) was performed to create a regression line through a scatter plot to determine the relationship between P-ADA and age in the pleural TB group in the correlation analysis.

RESULTS

Table 1 summarizes the prevalence of the causes of PES and the demographic characteristics of the 157 patients. TB was the most prevalent cause of PES (44/157, 28%). The Shapiro-Wilk test for age (W=0.87) revealed a P-value <0.05 (P=0.0017). The group of patients with older age was the transudate with a median age of 76 years (IQR, 63.0-86.25). The Kruskal-Wallis test for age (H=40.93, P<0.0001) and Dunn's post hoc test (P<0.05) revealed significance for TB vs. transudates, vs. simple PPE, and vs. adenocarcinomas. Male sex was the most prevalent in the lymphoma group (7/0, 100%). None of the seven patients with lymphoma were female. When the frequencies were compared for males and females, only adenocarcinoma and lymphoma were significant (chi-square tests, P=0.0021 and P=0.0003, respectively).

Table-1: Demographic characteristics and causes of pleural effusion syndrome in the 157 patients.

Cause	Patient (n)	Prevalence (%)	Age-years medians - IQR (25-75th)	Female (n/%)	Male (n/%)
Tuberculosis	44	28.0	39.0 (29.7-58.2)	22.0 (50.0)	22.0 (50.0)
Adenocarcinoma	37	24.0	61.0 (45.0-77.0)	25 (68.0)	12 (32.0)*
Transudate ǂ	33	21.0	76.0 (63.0-86.25)	17 (52.0)	16 (48.0)
Simple parapneumonic effusion	15	10.0	67.0 (56.0-85.0)	6 (40.0)	9 (60.0)
CPPE and empyema	8	5.0	52.5 (33.5-78.75)	2 (25.0)	6 (75.0)
Lymphoma	7	4.0	53.0 (47.0-63.0)	0 (0.0)	7 (100.0)*
Squamous cell carcinoma	7	4.0	66.0 (55.0-66.0)	4 (57.0)	3 (43.0)
Other exudates #	6	4.0	73.0 (53.0-79.0)	4 (67.0)	2 (33.0)
Total	157	100.0	58.0 (41.75-73.25)	80 (51.0)	77 (49.0)

Abbreviations: CPPE, complicated parapneumonic effusions; IQR, interquartile range. ǂTransudates: congestive heart failure (n = 26), chronic renal failure (n = 3), cirrhosis of liver with ascites (n=3), and serum low total protein levels (n=1). # -Other exudates: Pseudo-Meigs syndrome (n = 1), Dressler's syndrome (n = 3), chylothorax (n = 1), and leukemia (n = 1). *In male and female sexes, when the prevalence was compared by the Chi-square test, only adenocarcinoma and lymphoma were significant (P=0.0021 and P=0.0003, respectively). The sex differences in the overall pleural effusion syndrome group were not significant according to the chi-squared test (Chi=0.062, P=0.8028). The Shapiro-Wilk test for ages rejected normality (W=0.87) with a P-value <0.05 (P=0.0017).

Figure 1 shows the age-related statistical analysis of several causes of pleural effusion syndrome from Table 1 with Kruskal-Wallis and Dunn's post hoc tests.

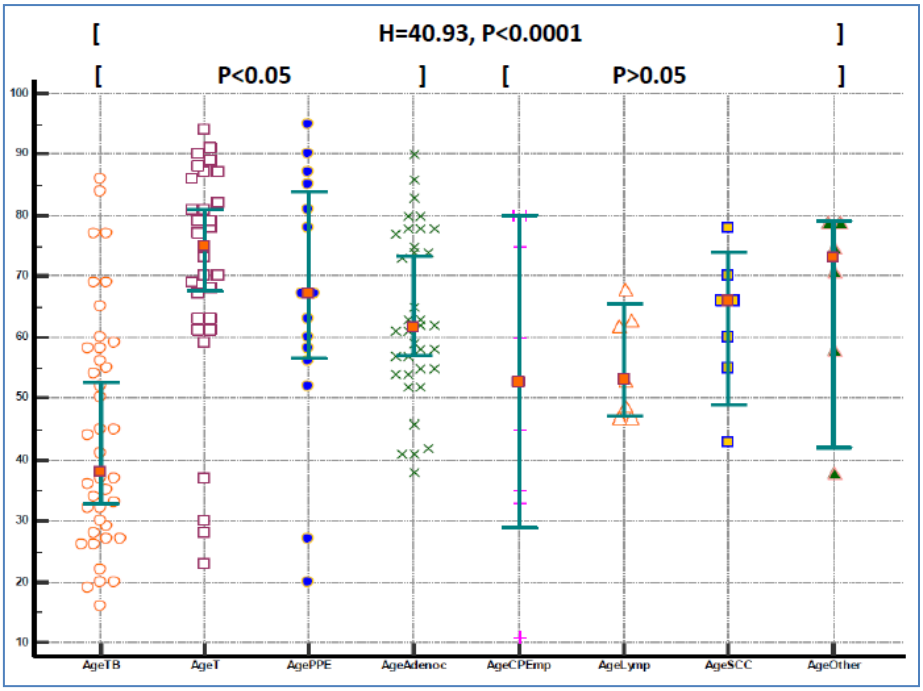

Fig-1: Age statistical analysis in several causes of pleural effusion syndrome from Table 1. Kruskal-Wallis test for ages was significant (H=40.93, P<0.0001). Dunn's post hoc test with P<0.05 was significant for tuberculosis vs. transudates, vs. simple PPE, and vs. adenocarcinomas. Abbreviations: CPPE, complicated parapneumonic effusions; TB, pleural tuberculosis; Lymp., lymphoma; SCC, squamous cells carcinoma; PPE, parapneumonic pleural effusion

The P-ADA levels of the 157 patients are shown in Table 2. The Shapiro-Wilk test for total P-ADA levels (W=0.347) revealed a P-value <0.05 (P=0.0001). The median values were statistically significant using the Kruskal-Wallis test (H=81.34, P<0.0001). When Dunn's post hoc test was used for pairwise comparison of subgroups, significant results (P<0.05) were obtained for tuberculosis vs. transudates, vs. simple PPE, and vs. adenocarcinoma. Results were not significant (P>0.05) for CPPE and empyema, lymphoma, SCC, and other exudates.

Table-2: Levels of pleural adenosine deaminase evaluated in a total of 157 causes of pleural effusion syndrome confirmed with reference standard diagnostic tests

Cause	Pleural fluid sample size (n)	P-ADA medians (U/L)	Median interquartile range (25th-75th percentile)
Tuberculosis	44	42.0	32.9-61.9
Adenocarcinoma	37	9.75	6.7-14.9
Transudate	33	6.85	2.67-11.26
Simple parapneumonic effusion	15	9.38	5.68-9.97
CPPE and empyema	8	32.9	16.0-61.7
Lymphoma	7	401.2	11.2-990.5
Squamous cell carcinoma	7	13.11	11.0-28.2
Other exudates	6	15.2	7.4-49.0

Abbreviations: CPPE (complicated parapneumonic effusions) and P-ADA (pleural adenosine deaminase). The Shapiro-Wilk test for total P-ADA levels (W=0.347) had a P=0.0001.

Figures 2 and 3 depict the statistical analysis from Table 2 (ADA levels) with Kruskal-Wallis and Mann-Whitney tests, respectively.

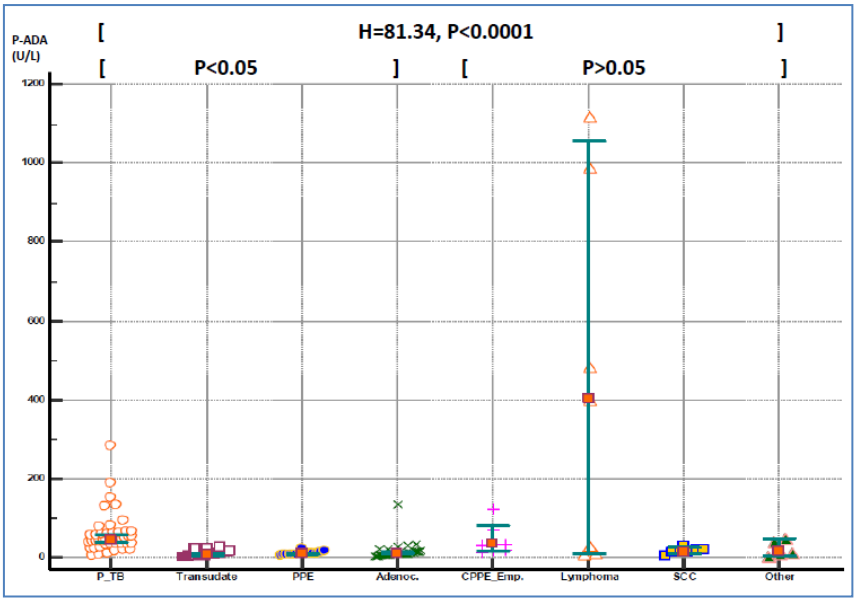

Fig-2: Pleural adenosine deaminase levels (P-ADA, U/L) in different diagnostic groups of pleural effusion syndrome. Kruskal-Wallis test, H=81.34, P<0.0001. Dunn's post -hoc test, P<0.05 for tuberculosis vs. transudates, vs. simple PPE, and vs. adenocarcinoma, and P>0.05 for CPPE and empyema, lymphomas, squamous cell carcinoma, and other exudates. Abbreviations: CPPE, complicated parapneumonic effusions; TB, pleural tuberculosis; Lymp., lymphoma; SCC, squamous cells carcinoma; PPE, parapneumonic pleural effusion

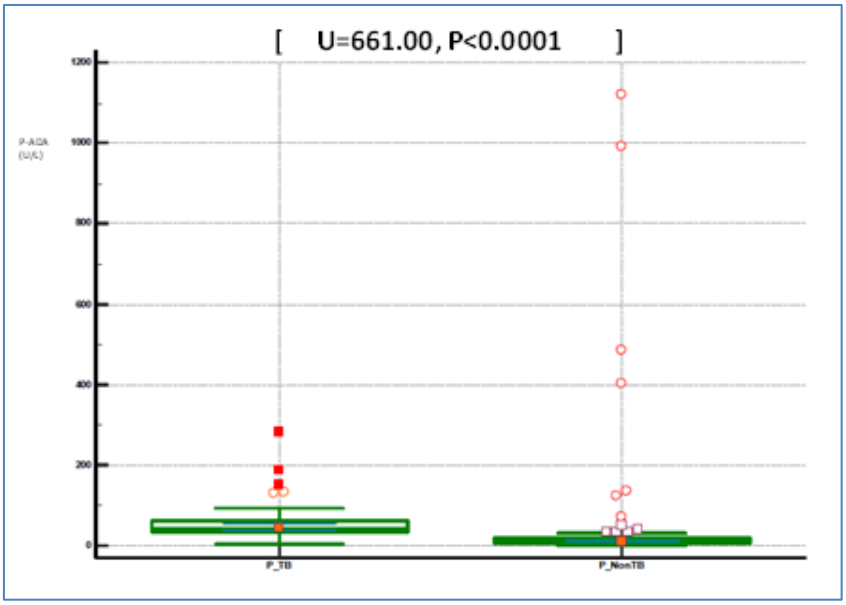

Fig-3: Median values of pleural adenosine deaminase levels (P-ADA, U/L) summarized in two groups of pleural effusion syndrome, tuberculosis (P-TB, median=42.03, IQR, 32.92-60.20) and non-tuberculosis (P-nonTB, median=9.87, IQR, 5.99-16.90). Mann-Whitney test (U=661.00; P<0.0001).

Figure 4 shows the degree of the relationship between the variables, P-ADA and age, for pleural TB in a sample size of 41 cases with LOESS regression line and statistical notes.

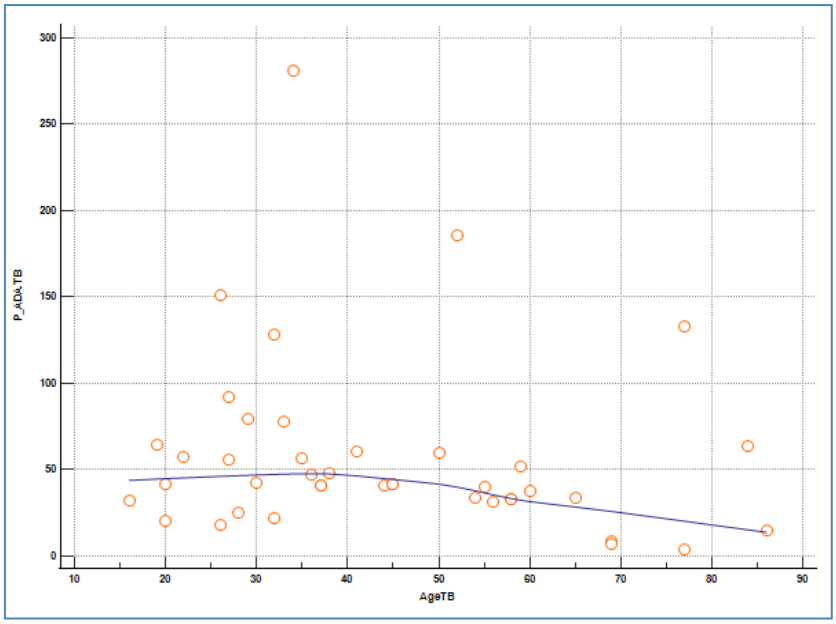

Fig- 4: Degree of the relationship between the variables, P-ADA and age, for pleural TB (n=41) with a LOESS regression line. Spearman coefficient of rank correlation (rho= -0.261, 95% CI, -0.526-0.0508, P=0.0994). The Kendall's correlation was negative and significant after 1000 iterations with bootstrap for 95% CI (Tau=-0.213, 95%CI, - 0.449-0.0833, P=0.0490).

DISCUSSION

The profiles of adenosine deaminase, age, and sex were investigated in 157 patients with PES. The study cohort excluded children and adolescents. The patients were from adult clinics at two tertiary hospitals.

Pleural TB is the most prevalent extrapulmonary TB in Brazil. In the state of São Paulo, 12,545 cases were reported (48%) between 1998 and 2005, with 25,773 cases of extrapulmonary TB (17%) [17]. TB was the most prevalent cause of exudative pleural effusion in our cohort (Table 1).

Female sex was most prevalent in adenocarcinoma, transudates, SCC, and other exudates (Table 1). However, when the prevalence rates were compared between males and females by the Chi-square test, only adenocarcinoma and lymphoma were significant (P=0.0021 and P=0.0003, respectively). In addition, the sex differences in the overall PES group were not significant according to the chi-squared test (Chi=0.062, P=0.8028).

TB was the cause of PES, with a minor median age. The Kruskal-Wallis test for age was significant (H=40.93, P<0.0001). Dunn's post hoc test (P<0.05) was significant for TB vs. transudates, vs. simple PPE, and vs. adenocarcinomas (Table 1 and Fig. 1). The sexes and median ages were similar to those reported in other studies published by our group [7, 8].

The Kruskal-Wallis test for P-ADA levels revealed significance (H=81.34, P<0.0001; Table 2). Dunn's post hoc test revealed significance (P<0.05) for TB vs. transudates, vs. simple PPE, and vs. adenocarcinoma, but was not significant (P>0.05) for CPPE and empyema, lymphoma, SCC, and other exudates (Fig. 2). The total ADA level is undoubtedly the most useful biomarker for pleural TB in Brazil.

Figure 3 shows the median levels of P-ADA evaluated in the two groups of PES, TB (P-TB) and non-TB (P-non-TB). When compared using the Mann-Whitney test, the level of P-ADA was significant (U=661.00, P<0.0001). A Spanish study with 4,147 patients concluded that total P-ADA had 93% sensitivity, 92% specificity, positive likelihood ratio of 12, negative likelihood ratio of 0.08, and an area under the curve of 0.968 for TB pleural effusion [18]. Developing countries, such as Brazil, India, Pakistan, and others, should implement ADA testing as the first option for pleural TB diagnosis [19].

P-ADA levels may be falsely low in elderly patients and falsely elevated in complicated parapneumonic effusions, empyema, rheumatoid arthritis, and lymphomas [7, 8, 18]. The degree of the relationship between P-ADA and age for pleural TB is shown in Figure 4. The Spearman's rank correlation coefficient was negative and not significant (rho= -0.261, 95% CI, -0.526-0.0508, P=0.0994). However, Kendall's correlation after 1000 iterations from bootstrap for 95% CI was negative and significant (Tau=-0.213, 95% CI, -0.449-0.0833, P=0.0490). The LOESS regression line clearly showed a negative relationship between P-ADA levels and age >40 years. This statistical finding is new and important for the pleural ADA study.

Finally, some considerations in this study must be mentioned. First, for clinical applications in PES, a profile of P-ADA, age, and sex was shown with

appropriate statistical approaches, such as the design of the study, distribution of the data, and nature of the observations (paired/unpaired). Second, as a future perspective for pleural TB diagnosis, different cut-off point values for ADA must be calculated in relation to the age of patients. Third, the limitations of this study were that diagnostic accuracy measures, specifically the rates of sensitivity, specificity, predictive values, likelihood ratios, and odds ratio from P-ADA levels were not calculate as a secondary objective.

CONCLUSION

In conclusion, evaluation of pleural ADA levels is useful for pleural TB. Sex was not found to be significant for differential diagnosis. However, a negative and significant relationship was evident between P-ADA level and age >40 years.

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