Original Article

Combined evaluation of adenosine deaminase level and histopathological findings from pleural biopsy with Cope’s needle for the diagnosis of tuberculous pleurisy

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Abstract: Introduction: Closed needle pleural biopsy (CNPB) has historically been the gold standard procedure for the diagnosis of pleural tuberculosis. Adenosine deaminase (ADA) is an efficient biomarker for tuberculosis that is measurable in pleural fluids. Objective: We compared the diagnostic accuracy of the pleural ADA (P-ADA) level and histopathological findings of CNPB specimens in patients with pleural tuberculosis. Methods: This prospective study consisted of two groups of examinations with a proven diagnosis of pleural effusion. The P-ADA level was measured in 218 patients with pleural effusion due to a number of causes, and 157 CNPB specimens underwent histopathological analysis. Results: CNPBs were performed in patients with tuberculosis (n=122) and other diseases: adenocarcinoma (n=23), lymphoma (n=5), systemic lupus erythematosus (n=4), squamous cell carcinoma (n=2), and small cell lung cancer (n=1). According to the ROC curve, the optimal cut-off value of the P-ADA level (Giusti and Galanti colorimetric method) was equal to or greater than 40.0 U/L. The diagnostic accuracy of the P-ADA test was 83.0%, and that of histopathological examination of the CNPB tissue, was 78.8% (AUC=0.293, P=0.7695). The association between the P-ADA assay and pleural histopathology was 24.41 (P<0.0001). The tetrachoric correlation coefficient was 0.563 (high correlation). Conclusion: In Brazil and other countries with a high incidence of tuberculosis, P-ADA activity is an accurate test for the diagnosis of tuberculous pleural effusions, and its use should be encouraged. The high diagnostic performance of the P-ADA test could to aid the diagnosis of pleural tuberculosis and render CNPB unnecessary.

Keywords: Pleural effusion, tuberculosis, adenosine deaminase, pleural biopsy, diagnosis

Introduction

Tuberculosis (TB) continues to be a major global public health problem [1]. In Brazil, TB remains a predominant cause of infection in the population [2]. According to the World Health Organization, there was an incidence of 9.0 million cases of TB worldwide in 2014 [1]. TB was responsible for 1.5 million deaths [1]. Pleural extrapulmonary is the most frequent clinical presentation of human TB in Brazil, constituting 48% of the total number of cases [2]. Diagnosis of pleural TB is difficult to confirm because of the paucibacillary nature of the pleural fluid [3]. Symptoms, signs, and conventional tests such as microscopy and culture have relatively low sensitivity and a low negative predictive value for pleural TB [4]. Additionally, novel diagnostic modalities are necessary to simplify the analysis, reduce costs, and increase accuracy in developing countries. Gamma interferon is a TB pleural biomarker that is useful only for researchers [4, 5]. A new biomarker (interleukin-27) was less efficient than total ADA in the diagnosis of pleural TB [6].

Percutaneous or closed (blind) needle pleural biopsy (CNPB) has historically been the gold standard procedure for the diagnosis of pleural TB [4, 5]. However, this procedure can elicit several surgical complications and has limitations
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if performed in inexperienced hands. The British Thoracic Society guidelines report important hemorrhage related complications of pleural biopsies with an Abrams needle, including death [4]. Owing to these diagnostic difficulties, misdiagnosis of pleural TB is common [4, 5].

The adenosine deaminase (ADA) enzyme test is a diagnostic biomarker assay for TB using pleural fluids. ADA is a nonspecific biomarker released from monocytes/macrophages and neutrophils during the immune response to Mycobacterium tuberculosis by live phagocytosed micro-organisms [7]. The total ADA assay has a higher sensitivity, specificity, positive likelihood ratio, and post-test positive predictive value for differentiating tuberculous from malignant pleural effusions [7]. For this reason, this test should be recommended when thoracentesis without severe potential complications could be performed. In the present study, we compared the diagnostic accuracy of pleural ADA (P-ADA) measurements and histopathological findings from CNPB specimens in patients with pleural TB.

Methods

Study population and design

This was a prospectively designed study conducted from 2002 to 2011. The Ethics Committee of of the Antonio Pedro Hospital, a tertiary center of the Federal Fluminense University, located in the town of Niteroi, State of Rio de Janeiro, Brazil, approved this study in accordance with the recommendations found in the Declaration of Helsinki. Informed written consent was obtained from all patients.

Diagnostic criteria

The definitions used for the diagnosis of pleural effusion were based on previously published criteria [4, 5]. In cases where thoracentesis or CNPB were not sufficient to confirm the diagnosis, the patients were referred for more invasive procedures (thoracoscopy, pleuroscopy, and thoracotomy). The exclusion criteria were as follows: an absolute contraindication or refusal to undergo thoracentesis or CNPB, the use of corticosteroids, hemolysis in pleural fluids, renal failure, pleural effusion of unknown cause, HIV infection or other immunodeficiency disease, although ADA is not affected by the HIV status [8]. Pleural effusions were considered exudates according to classic and alternative biochemical criteria [5, 9]. Pleural TB was considered to be the cause of the pleural effusions upon positive Mycobacterium tuberculosis culture of pleural fluid and/or finding the presence of granulomas in the tissue from the pleural biopsy, in the absence of other pleural granulomatous diseases. Additionally, if clinical and radiological improvement was observed after 3 months of administration of anti-TB treatment, pleural TB was considered to be the cause [4, 5]. Pleural effusions were considered malignant with cytologic or histopathologic reports of cancer [4, 5]. Diagnosis of pulmonary embolization was considered in patients with risk factors, and confirmed using CT angiography, D-dimer levels in the peripheral blood, and duplex ultrasonography of leg veins [5]. Parapneumonic effusion was diagnosed in patients with bacterial pneumonia [5]. Empyema was considered for those pleural effusions with thick, purulent appearing pleural fluid [5]. Chylothorax was considered if the pleural fluid triglyceride concentration was greater than 110 mg/dL in patients with an etiologic factor [5]. The diagnosis of systemic lupus erythematosus was considered in any patient with an exudative pleural effusion with clinical manifestations, positive lupus erythematosus cell test result, and/or antinuclear antibody titers greater than 1:160 in the pleural fluid [5]. The diagnosis of traumatic hemor-thorax was suspected in a patient with nonpenetrating trauma to the chest [5].

Thoracentesis and CNPB were performed at the Ambulatory Surgical Center with a Cope’s needle using standard techniques after local anesthesia [10]. Neither transthoracic ultrasound nor computed tomography was utilized to orient the pleural procedure. Vital signs were obtained, and the patients were monitored continuously. For CNPB, at least three to five specimens containing parietal pleural tissue were obtained from the same site in the thorax [11].

ADA assay

The ADA assay is a colorimetric method described by Giusti and Galanti [12]. It is based on the measurement of ammonia in a Berthelot reaction [12]. Ammonia is generated when ADA reacts with adenosine (substrate). The chemical definition of one unit of total ADA is the
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Table 1. Demographic characteristics and P-ADA levels in pleural effusions of 218 patients with final diagnoses

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Patients (n)</th>
<th>Age (years) Mean ± SD</th>
<th>Sex (Male/Female)</th>
<th>P-ADA (IU/L) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculosis*</td>
<td>122</td>
<td>41.15±16.25</td>
<td>97/25</td>
<td>74.11±50.31</td>
</tr>
<tr>
<td>Transudative</td>
<td>27</td>
<td>62.74±12.13</td>
<td>16/11</td>
<td>11.55±8.36</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>23</td>
<td>58.52±14.40</td>
<td>12/11</td>
<td>13.52±7.66</td>
</tr>
<tr>
<td>Empyema</td>
<td>12</td>
<td>43.50±17.65</td>
<td>09/03</td>
<td>124.7±126.8</td>
</tr>
<tr>
<td>Parapneumonic</td>
<td>10</td>
<td>30.20±11.43</td>
<td>03/07</td>
<td>23.9±9.38</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>5</td>
<td>54.20±16.82</td>
<td>01/04</td>
<td>62.8±54.4</td>
</tr>
<tr>
<td>Other causes</td>
<td>19</td>
<td>45.52±20.73</td>
<td>09/10</td>
<td>13.0±9.14</td>
</tr>
</tbody>
</table>

*Tuberculous versus nontuberculous (Chi-square=104.26, P<0.0001), Kruskal-Wallis test of P-ADA; P<0.0001; Post-hoc Dunn test: TB vs. transudative, adenocarcinoma and parapneumonic: P<0.001; TB vs. empyema and lymphoma: P>0.05.

P-ADA: Pleural adenosine deaminase; SD: Standard deviation.

amount of ADA necessary to release 1 µmol of ammonia per minute from the substrate under standard experimental conditions. Sterile bidistilled water was used to prepare the solutions for the in-house kit to minimize residual amounts of ammonia. In the final chemical reaction, the formation of a blue color by indophenol was measured with a spectrophotometer at 628 nm. Both positive and negative control samples were included in all of the P-total ADA analyzed. Pleural fluid was collected in a sterile tube without anticoagulant for the total P-ADA assay. The enzyme is stable for at least 24 hours at 25°C, for 7 days at 4°C, and for 3 months at 20°C [12].

Histopathological evaluation

The pleural histopathology examination was performed according to the procedures of the Pathology Anatomy Department at Antonio Pedro Hospital. The pleural tissue was fixed in formalin (10%), sectioned at 5 µm, and stained with hematoxylin-eosin [13]. Ziehl-Neelsen staining, immunohistochemical staining, and pleural tissue culture were not performed. The finding of a pleural granuloma is accepted in Brazil as a histological diagnosis of pleural TB in patients without immunosuppression or lung disease suggestive of sarcoidosis, tularaemia, or rheumatoid pleuritis. TB is present in more than 95% of patients with granulomatous pleuritis. Caseous necrosis and acid-fast bacilli need not be found in pleural tissue [5, 14].

Statistical approach

In this study, we analyzed both descriptive and inferential statistics in all data using computer software (MedCalc Software, version 13.1.2, Ostend, Belgium). The laboratory data were analyzed using univariate analysis. A P value (two-tailed) less than 0.05 was considered to be statistically significant to reject the null hypothesis and define a type I error. The D’Agostino-Pearson test was used to assess the patients with pleural TB and in patients with pleural effusions of nontuberculous normality of the data. All data were expressed as mean and standard deviation (SD). P-ADA levels were measured origin as the controls. To compare the differences in the P-ADA levels between the two groups, we performed a Chi-squared test without Yates correction. The Chi-squared test was also used to measure the association between the P-ADA assay results and the pleural histopathology findings in a 2 × 2 contingency table. This is a test for statistical significance of categorical data. Correlation analysis between the P-ADA results and histopathological findings (dichotomous variables) with a tetrachoric coefficient was performed. Student’s t test was used to compare the differences in the arithmetic means of the patients’ ages. The Kruskal-Wallis test followed by the post hoc Dunn test was used to compare TB versus three or more unpaired (independent) samples in the nontuberculous groups. The optimal cut-off value of the P-ADA assay (value greater than or equal to 40.0 UI/L) was determined using the receiver operating characteristics (ROC) curve from another paper from our group [15]. The area under the ROC curve (AUC Z statistic) with the 95% confidence interval (95% CI) was calculated by the software using the method described by Hanley-McNeil with a nonparametric approach and trapezoidal rule [16]. This statistical test gives an estimated diagnostic efficiency. It is used to calculate the standard error of the AUC. It is important to describe and compare the performance of diagnostic tests. The results obtained from the pleural TB and control groups in the ROC curve for the P-ADA assay and histopathology from CNPB were the sensitivity, specificity, predictive values, accuracy, and likelihood ratios of the tests.
Results

Patient characteristics

The demographic characteristics of the patients are shown in Table 1. The mean age of the patients with tuberculous pleural effusion was 41.15±16.25 years. When the mean age of patients with pleural TB was compared with that of patients in the other groups, the results obtained from Student’s t test were as follows: adenocarcinoma (P<0.0001), transudate (P<0.0001), lymphoma (P=0.081), empyema (P=0.626), and parapneumonic (P=0.039). The male sex predominated in patients with pleural TB, transudate, adenocarcinoma, and empyema (Table 1).

Results of pleural effusion and CNPB examination

This prospective study consisted of two groups of examinations in patients with a proven diagnosis of pleural effusions. The P-ADA level was measured in 218 patients with various causes of pleural effusions. In this group of patients, 157 closed pleural biopsies were performed using Cope’s needle and histopathologically evaluated after analysis of various fragments of the parietal pleural tissue (Table 2).

Causes according to each group

The P-ADA group, (n=218) was segregated into seven subgroups of patients (Table 1): tuberculosis (n=122), transudative congestive heart failure (n=27), adenocarcinoma (n=23), nontuberculous empyema (n=12), simple and complicated parapneumonic effusion (n=10), lymphoma (n=5), and other causes (n=19), including pulmonary embolization (n=8), systemic lupus erythematosus (n=4), chylothorax (n=3), squamous cell carcinoma (n=2), small cell lung cancer (n=1), and traumatic hemothorax (n=1). The histopathological group was subdivided as follows: tuberculosis (n=122), adenocarcinoma (n=23), lymphoma (n=5), systemic lupus erythematosus (n=4), squamous cell carcinoma (n=2), and small cell lung cancer (n=1).

Table 2. Diagnostic performance of the P-ADA biomarker with an optimum cut-off value of 40.0 IU/L (218 patients) and conclusive histopathology from closed needle pleural biopsy for pleural tuberculosis (157 patients)

<table>
<thead>
<tr>
<th>Diagnostic Parameters</th>
<th>P-ADA (UI/L)*</th>
<th>Histopathology*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=218</td>
<td>n=157</td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>80.0 (71.5-85.7)</td>
<td>57.5 (40.9-73.0)</td>
</tr>
<tr>
<td>Specificity</td>
<td>86.4 (72.8-91.9)</td>
<td>100.0 (90.5-100.0)</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>88.2 (82.1-94.2)</td>
<td>100.0 (84.6-100.0)</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>77.0 (68.9-84.8)</td>
<td>68.5 (54.4-80.5)</td>
</tr>
<tr>
<td>Positive likelihood ratio</td>
<td>5.9 (3.5-9.8)</td>
<td>21.28 (3.02-149.78)</td>
</tr>
<tr>
<td>Negative likelihood ratio</td>
<td>0.23 (0.16-0.33)</td>
<td>0.44 (0.30-0.63)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>83.0 (77.0-88.0)</td>
<td>78.7 (67.9-87.3)</td>
</tr>
<tr>
<td>Diagnostic odds ratio</td>
<td>24.72 (11.9-51.4)</td>
<td>48.36 (6.06-391.26)</td>
</tr>
</tbody>
</table>

*The Chi-squared test was used to measure the association between the P-ADA assay and the pleural histopathology findings (Chi-square=24.41, P<0.0001). Tetrachoric correlation coefficient=0.563 (high correlation). CI: Confidential interval.

Diagnostic value of ADA

The comparison between the arithmetic means and standard deviations of the P-ADA levels between the TB and other groups was statistically significant (Kruskal-Wallis test, P<0.0001), as shown in Table 1. The P-ADA test had a non-significant p-value (Dunn’s test, P>0.05) in patients with lymphoma and empyema (Table 1). However, the P-ADA analysis helped to exclude adenocarcinoma, transudates, and parapneumonic effusions with a highly significant p-value (Dunn’s test, P<0.001), as shown in Table 1.

The AUC value calculated for the P-ADA assay was higher than that calculated for the pleural histopathology from CNPB (Figure 1). However, the difference was not statistically significant (AUC Z statistic=0.293, P=0.7695).

The diagnostic performance parameters obtained from the ROC curve for the P-ADA assay and histopathology from CNPB were the sensitivity, specificity, predictive values, likelihood ratios, and diagnostic odds ratio of the tests. These parameters are important to establish the potential utility of the compared tests for diagnostic investigation of patients with pleural TB. The performance of both the P-ADA assay, with an optimum cut-off (point above or equal to 40.0 IU/L, and histopathology from CNPB is shown in Table 2). The Chi-squared test (Chi-square value=24.41, P<0.0001) established the potential utility of these examinations for
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Discussion

The present study evaluated the diagnostic accuracy of a conventional diagnostic test and a controversial biomarker for tuberculous pleural effusions in Brazil, a developing country with areas of high prevalence of TB. A study of pleural fluid biomarkers could represent an interesting complementary approach to increasing the accuracy of a diagnostic test [17].

To prevent errors in this study, we used the methodological criteria recommended by the Standards for Reporting of Diagnostic Accuracy (STARD) and other expert authors [18, 19]. The standard measurements of validity are sensitivity and specificity, positive and negative predictive values, diagnostic odds ratios, likelihood ratios, and the AUC [18].

Accuracy is measured by the AUC. The authors defined diagnostic accuracy as the ability to discriminate between clinically relevant subclasses of subjects or the proportion of correctly identified subjects. Accuracy is a measure of the performance of a test. The AUC varies from 0.0 to 0.5 for a meaningless test and 0.5 to 1.0 for a test that differentiates between disease and nondisease [18, 19].

The ROC plots for the P-ADA levels and histopathology findings are shown in Figure 1. The AUC for the P-ADA assay was marginally better than that for the histopathology findings, but the difference was not statistically significant (AUC Z statistic=0.293, P=0.7695). A traditional statistical guide for classifying the AUC of a biomarker is as follows: excellent (0.90-1.00), good (0.80-0.90), fair (0.70-0.80), poor (0.60-0.70), and failed (0.50-0.60). According to this classification, the P-ADA assay and histopathological examination were classified as good and fair, respectively [19].

Table 1 shows higher levels of P-ADA activity in patients with tuberculous effusions than in a control group of patients with other causes of pleural effusion after rejecting the normality distribution with the D’Agostini-Pearson test for both groups. All of our data were non-parametric.

Several diagnostic studies have concluded that an elevated P-ADA level allows for a diagnosis of tuberculous pleuritic with a sensitivity of 80% to 100% and a specificity of 89% to 100% when measured by the Giusti and Galanti method [12]. The reported cut-off value for the P-ADA assay varies from 35.0 to 70.0 UI/L [20]. Our findings are similar to those reported in a number of studies (Table 2). The high accuracy of a biochemical analysis for P-ADA can contribute to a diagnosis of pleural TB.

The CNPB procedure for the examination of pleural surfaces is still considered the “gold standard” for a definitive diagnosis of pleural TB by many authors. However, its main complication, pneumothorax, may occur in 3% to 20% of patients not undergoing mechanical ventilation [21-23]. Approximately 20% of these patients require pleural drainage [21-23]. In experienced hands, the CNPB procedure has no fatal complications, but the literature reports several potential complications such as fatal hemothorax and tension pneumothorax. Other complications from CNPB include local pain, cough, obtaining no fluid, vasovagal reaction, re-expansion pulmonary edema, hypovolemia, subcutaneous hematoma, pleural infection, transient fever, and laceration of the diaphragm, heart, lung (bronchopleural fistula), liver, or spleen [14, 23]. CNPB has contraindications and an increased risk of certain complications when performed by inexperienced physicians without direct supervision, including

![Figure 1. ROC analysis with dichotomous variables. P-ADA levels (AUC=0.83, 95% CI=0.77-0.88) for distinguishing tuberculous pleuritis compared with histopathology from closed needle pleural biopsy (AUC=0.787, 95% CI=0.679-0.873). Comparison of the AUC was not statistically significant (AUC=0.293, P=0.7695).](image-url)
coagulopathy, the inability of the patient to cooperate, very small pleural effusions, chest wall infection, quick removal of large amounts of pleural fluid (more than 1.5 L), unstable medical conditions, use of anticoagulants, and patients with a platelet count below of 50,000/mm$^3$ [5, 23].

In the present study, the authors experienced two rare complications associated with these surgical procedures: tumor seeding along the needle tract, and breakage and failure to detach the hook from a new Cope’s needle in the pleural cavity. A similar complication was reported by Fité et al. with an Abram’s needle [24]. Chang et al. reported a complication involving the tip of a pleural biopsy needle breaking in the pleural cavity [25]. The complication of pneumothorax and diagnostic sensitivity are equal between Cope and Abrams needles [26]. Thoracoscopic pleural biopsy is indicated as a useful diagnostic procedure if prior thoracentesis is nondiagnostic [27].

In the medical literature, the sensitivity of the histopathology obtained by CNPB for the diagnosis of pleural TB ranges from 40.0% to 90.0% [4, 5, 28-31]. The original papers did not discuss the accuracy of this test. A study of nonspecific pleuritis by morphometric analysis showed that the presence of fibrin within the granulation tissue covering the submesothelial connective tissue had 100% specificity for the diagnosis of pleural TB [32]. This finding plus the presence of granuloma increases the yield of the histopathological diagnosis for pleural TB.

The nonparametric tetrachoric correlation was used to evaluate the correlation between the P-ADA test and pleural histopathology. This is a very useful statistical test for describing the relationship between dichotomous variables (positive or negative, conclusive or inconclusive). In this study, a 0.563 tetrachoric correlation coefficient was indicative of a high correlation (Table 2). Our results support the superiority of pleural fluid examination with P-ADA in the diagnosis of TB. Cytology has also been used to evaluate malignant pleural effusions, CNPB was of diagnostic utility in only 7% of the 414 patients studied by Prakash and Reiman when the cytology results were negative [33]. According to Arnold et al. [34] in a population with a low incidence of TB, a P-ADA value greater than 35.0 IU/L in lymphocytic pleural effusions makes pleural TB the most likely diagnosis. However, it does not replace pleural biopsy as the gold standard investigation.

A new biomarker (interleukin-33), has lower diagnostic accuracy than ADA for pleural TB [35]. In contrast to the ADA assay, the sensitivity and specificity of the Xpert for direct M. tuberculosis analysis in pleural fluid were 43.6% and 98.6%, respectively, in a high TB-endemic country [36].

In patients with large and massive pleural effusions, thoracentesis is of value in addition to anti-TB treatment, to relieve dyspnea and avoid residual pleural thickening [37].

In summary, what does this study add? This study concludes that in regions with a high incidence of TB, such as Brazil, the P-ADA activity assay is an accurate test for the diagnosis of tuberculous pleural effusions. The high diagnostic performance of the P-ADA could contribute to the diagnosis of pleural TB and render CNPB unnecessary. The P-ADA examination should be encouraged, but we agree with other authors that CNPB is not obsolete for the diagnosis of pleural TB. Invasive diagnostic procedures with potential complications should be indicated after the evaluation of specific biomarkers with high accuracy, such as P-ADA. However, a biomarker should be used in conjunction with clinical and imaging manifestations, and the epidemiological profile of a disease.

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Disclosure of conflict of interest

None.

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