Diagnostic utility of QuantiFERON-TB Gold and Xpert MTB/RIF combined with adenosine deaminase in tuberculous pleural effusion: a prospective study

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Objective Tuberculous pleural effusion remains difficult to diagnose despite numerous diagnostic tools. The aim of this study was to evaluate and compare the diagnostic efficiency of adenosine deaminase (ADA) analysis in the diagnosis of tuberculous pleural effusion when used alone or in combination with QuantiFERON-TB Gold (QFT-G) or Xpert MTB/RIF.

Patients and methods Seventy-one patients with unknown bilateral or unilateral pleural effusion were subjected to pleural tapping, and pleural fluid samples were subjected to total ADA, QFT-G, and Xpert MTB/RIF. Pleural biopsies (thoracoscopic or closed) were performed for 41 patients, sent for histopathology of tuberculosis culture and Xpert MTB/RIF, and they were differentiated into tuberculous (TB) and non-TB effusions by culture for *Myobacterium tuberculosis* and/or histopathology of pleural biopsy.
ADA results were further analyzed using receiver operating characteristic curve to determine the cutoff values to achieve the optimum sensitivity and specificity.

Results Forty-six patients had definite TB pleural effusion and 25 patients had non-TB pleural effusion. For ADA at a cutoff point of >40 IU/l, the sensitivity was 97.83% (*n*=45) [95% confidence interval (CI): 88.47–99.94%) and specificity was 76.00% (95% CI: 54.87–90.64%), but at a cutoff point of 70 U/l, the specificity was 96.00% (95% CI: 79.65–99.90%) and sensitivity was 84.78% (*n*=39) (95% CI: 71.13–93.66%). The sensitivity of QFT-G was 23.91% (95% CI: 12.59–38.77%) and specificity was 96.00% (95% CI: 79.65–99.90%). The sensitivity of Xpert MTB/RIF was 47.83% (95% CI: of 32.89–63.05%) and specificity was 100.00% (95% CI: 86.28–100.00%). Combined analysis of ADA at a cutoff value greater than 70 U/l with Xpert MTB/RIF showed a sensitivity of 85.64% (95% CI: 71.13–96.23%) and specificity of 98.50% (95% CI: 78.64–99.93%). ADA at a lower cutoff value greater than 40 U/l showed a sensitivity of 96.78% (95% CI: 77.13–99.66%) and specificity of 97.00% (95% CI: 79.65–99.90%).

Conclusion ADA is a useful marker in the diagnosis of TB pleural effusion. ADA specificity with low cutoff value can be improved by combining with Xpert MTB/RIF, resulting in an accurate test to identify patients with suspected TB pleural effusion.

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Keywords: adenosine deaminase, QuantiFERON-TB Gold, tuberculosis pleural effusion, Xpert MTB/RIF

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Introduction

Tuberculosis (TB) infection is a worldwide health problem. The most common form of infection is pulmonary TB, and the second common form is extrapulmonary TB, accounting for ~15% of cases. The most common extrapulmonary TB is tuberculous pleural effusion (TPE) as it constitutes about 5% of all TB cases [1] and its incidence is increasing [2], and it is a common cause of pleural effusion in prevalent regions [3].

TPEs may be encountered with early postprimary TB, associated with chronic pulmonary TB, or as a part of milliary TB. Despite this, it is a diagnostic challenge to physicians as obtaining pleural tissue biopsy and pleural fluid aspiration are two major samples obtained for the diagnosis of pleural TB. Diagnosis of TPE depends on the identification of *Mycobacterium tuberculosis* in pleural fluid, sputum samples, or from pleural biopsy staining, and can also be diagnosed through the identification of typical TB lesions (granuloma) in the parietal pleura biopsies. Culture-based diagnosis of the pleural fluid results in lower diagnostic yield (36%) [4]; the rate is higher with a result up to 70% if the culture was prepared using pleural biopsy [5]. Pleural biopsy is not readily available and is considered as an invasive, expensive, and time-consuming intervention; it is performed through either blind pleural biopsy, imaging-guided-pleural biopsy, and medical or surgical thoracoscopy with varying positive results in pleural TB diagnosis.

Markers that are commonly tested in clinical trials for the diagnosis of pleural TB include adenosine deaminase (ADA), TB PCR, γ-interferon, C-reactive protein, carcinoembryonic antigen, interleukin-6,

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lactate dehydrogenase (LDH), tumor necrosis factor-α, and vascular endothelial growth factor.

Measurement of ADA and interferon-γ using QuantiFERON-TB Gold (QFT-G; Cepheid Inc., Sunnyvale, California, USA) and PCR for *M. tuberculosis* (including Xpert MTB/RIF; Cepheid Inc., Sunnyvale, California, USA) in the pleural fluid has been used widely in the diagnosis of TPE. Although promising, most of these tests require more assessment and validation before their routine use as diagnostic biomarkers [6]. Being with highest sensitivity in endemic areas, ADA is the commonly used biomarker for the diagnosis and treatment decision of TB [7], where it is used to investigate undiagnosed pleural effusions, as it helps together with routine pleural fluid analysis when a tuberculous effusion is highly suspected. When ADA is used alone it carries the risk of high sensitivity but low specificity [8], especially if the population sample is heterogenous as ADA tends to be lower in old age giving false-negative results. Moreover, it may be affected by total lymphocytic count and pleural LDH [9]. Despite this, only few studies with small sample size have been carried out; most of these were from low-TB-burden countries, which is not applicable in endemic countries.

The objective of this study was to evaluate and compare the diagnostic efficiency of ADA analysis in the diagnosis of TPE when used alone or in combination with QuantiFERON-TB Gold (QFT-G) or Xpert MTB/RIF through different pleural sampling methods.

Patients and methods

Prospectively, 71 patients were enrolled during the 36 months' period of the study. Adult patients who visited our tertiary hospital with unknown cause of unilateral or bilateral pleural effusion were enrolled in the study after obtaining written consent. The Institutional Review Board of the Hospital approved this study.

Inclusion criteria were as follows: the presence of a large pleural effusion amenable to safe thoracentesis and, if needed, pleural biopsy, either closed using Abram's needle (Adroit Manufacturing CO., India) or thoracoscopic biopsies (amount >1/3 of the hemithorax in CXR), and age of 18 years or older.

All patients were asked about their past TB history and exposures, clinical symptoms and signs, and assessed for radiologic findings for pleural effusion.

Thoracentesis was performed for all patients. Pleural fluid samples were subjected to routine biochemical and microbiological examination including acid-fast bacilli (AFB) stain, analyzed for total and differential count including lymphocyte percentage, plasma proteins, glucose, LDH, pleural Gram stain, and bacterial culture, smear for AFB with Ziehl–Neelsen staining, culture on Lowenstein–Jensen medium, pleural ADA levels, and cytology. Additional tests including QuantiFERON-TB (QFT-G) and Xpert MTB/RIF were performed for all pleural fluid specimens.

Light's criteria were used for classifying transudative and exudative pleural effusion. If the criteria for an exudative effusion were borderline, the albumin gradient value (between serum and pleural fluid) greater than 1.2 g/dl was used for classification of borderline effusions as exudative.

Patients were diagnosed as having TB pleurisy if any of the following criteria were met: positive results for AFB or TB culture or from the respiratory specimens (sputum, pleural fluid, or pleural biopsy tissue analysis) or histolopathological analysis of the pleural tissue showing granulomatous lesions with multinucleated giant cells with or without caseous necrosis.

Patients who had exudative pleural effusion and inconclusive Gram stain, bacterial culture, AFB, and cytology were subjected either to thoracoscopy and biopsy of the parietal pleura or to closed pleural biopsy using Abram needle. Biopsies were performed for 41 patients, 33 of 46 patients in the TB group (17 patients were subjected to thoracoscopy and 16 patients were subjected to closed pleural biopsy, and it was not performed in 13 patients) and eight of 25 patients in the non-TB group (three were subjected to thoracoscopy and five to closed pleural biopsies).

Non-TPE was diagnosed by being negative for microbiological or histological evidence of *M. tuberculosis*, and/or for whom alternative diagnoses were available.

Patients were excluded if they had coagulation abnormalities (platelet count <75 000/μl; high prothrombin time, or activated partial thromboplastin time, 4 or 10 s, greater than control, respectively), acute coronary syndromes within the past 6 weeks, persistent cough, little amount of pleural effusion, or presence of adhesions on imaging (chest radiograph, ultrasonography, or computed tomography of the

chest) and failure to provide informed consent. Patients with renal failure were also excluded (high ammonia in pleural fluid invalidates ADA measurements) [10]. Patients previously diagnosed and previously known to have TB effusion and patients known to have transudative effusion secondary to systemic diseases on clinical evaluation or previous investigations were also excluded from the study.

Pleural fluid sample processing

A minimum of 150 ml of pleural fluid was collected for diagnostic testing by means of percutaneous pleurocentesis. The pleural fluid samples were divided into two parts, one part was sent for analysis of LDH, total proteins, pleural albumin, fluid ADA, total pleural leukocytic count, and differentiation including lymphocyte percentage, and the other part for AFB stain, QFT-G tests, LJ medium mycobacterial culture, and Xpert MTB/RIF analysis.

Pleural biopsy tissue processing

Pleural biopsies were also divided into two parts: one part was kept in normal saline and was sent for mycobacterial LJ culture and Xpert MTB/RIF, and the second part was kept in a sterile bottle containing 10% formalin solution and was sent for histopathologic examination. Upon receipt in the laboratory, the tissue specimens were cut and stained using Hematoxylin and Eosin staining technique. Presence of a granuloma and caseation was indicative of TB.

Pleural fluid adenosine deaminase analysis

ADA level was measured according to the manufacturing instructions (ADA; Diazyme Laboratories, San Diego, California, USA). Two ml of pleural fluid was collected in a sterile container and was either immediately analyzed or refrigerated at 4°C and analyzed within 2 days. An ADA value greater than 40 U/l was taken as the cutoff for calculating sensitivity and specificity according to receiver operating characteristic (ROC) curve. Another cutoff value was taken at greater than 70 U/l for increasing the specificity of the test.

Pleural fluid QuantiFERON-TB gold tests

The QFT-GIT was performed according to the manufacturer's instructions (QFT-GIT; Cellestis Ltd, Carnegie, Australia). A volume of 3 ml of sample was obtained from each patient and aliquots placed into three tubes (TB-specific antigens tube, mitogen tube, and nil tubes, respectively). The samples were placed in a humidified 5% CO_2 incubator at 37°C for 24 h, and then the tubes were centrifuged at 3000 rcf for 10 min and the fluid was collected after separation of cells; 500 µl of the supernatants was harvested and stored at −70°C until the IFN-γ was measured in an ELISA reader.

The results were reported as positive, negative, or indeterminate using QFT-G analysis software (QFT-G; Cellestis Ltd). On the basis of the IFN-γ secretion in response to TB antigen, after subtracting nil control IFN-γ, positive results were recorded if INF-γ was at least 0.35 IU/ml, and if the result was less than 0.35 IU/ml it was considered negative. If the negative result in the TB-specific antigen tube was associated with poor response in the mitogen tube (i.e. IFN-γ level in mitogen tube <0.5 IU/ml), it was considered as indeterminate result for QFT-G. Results with positive IFN-γ secretion more than 8.0 IU/ml in the nil tubes were also reported as indeterminate for QFT-G.

Pleural fluid and pleural tissue Xpert MTB/RIF analysis

According to the manufacturing recommendations, pleural fluid was centrifuged for 15 min and the concentrated sediment, resuspended in 1 ml of the original supernatant, was used for Xpert (Cepheid Inc., Sunnyvale, California, USA). Further, pleural tissue was finely ground and mixed in 1 ml of normal saline. After adding the Xpert reagent with 2 : 1 ratio for both pleural fluid and pleural tissue samples, 2 ml was transferred into a specific G4 cartridge and then inserted into the Xpert machine, and the results were read after 90 min. Xpert MTB/Rif test was reported as positive if MTB was detected. *M. tuberculosis* load is also determined as high, medium, low, or very low using semiquantitative analysis. Moreover, rifampicin resistance is detected using the same procedure and reported if identified; in our study none of the positive samples showed rifampicin resistance.

Statistical analysis

The results were analyzed for mean, SD, and *P* value using the SPSS software, version 17.0 (SPSS Inc., Chicago, Illinois, USA) and online MedCalc Statistical Software, version 15.6.1 (MedCalc Software bvba, Ostend, Belgium; *https://www.medcalc.org*). Numerical variables were reported as mean±SD and categorical variables as number with percentages. MedCalc software was used for assessing the diagnostic performance of each test focusing on sensitivity, specificity, positive predictive value, and negative predictive value. Two cutoff values were established using the ROC curve methodology. All tests of significance were two-tailed; *P* values up to 0.05 were considered as significant.

Results

A total of 71 patients with pleural effusion were enrolled. Participants were divided into two groups: a TB group (n=46) with a diagnosis of confirmed TPE, and a non-TB group (n=25) with a diagnosis of other non-TB diseases and were negative for TB (Table 1). The mean age in the non-TB group was significantly higher compared with those in the TB group (58 vs. 28, respectively, P<0.05). The mean levels of body temperature in TPE was significantly higher compared with those in non-TB effusion (38.2±1.03 vs. 37.2±0.86, P<0.05, respectively) and TST (18±9.6 vs. 7±5.6, respectively, P<0.05). Pleural fluid lymphocyte percentage was higher in TPE compared with non-TB pleural effusion (71±16 vs. 19±16, respectively, P<0.05).

In the TB group, patients were confirmed as having TPE (n=46), either using pleural AFB positive stain [four (8.6%)] or culture positive for *M. tuberculosis* in pleural fluid [17 (36.9%)], or histopathology [29 of 33 (89.1%) patients], regardless of whether diagnosed by means of thoracoscopy [17 (100%) patients were histopathologically positive) or closed pleural biopsy [12 out of 16 (75%) patients were positive], whereas it was not performed in 13 (28.26%) patients.

Sputum examination for AFB was carried out for all participants and positive results were recorded [three (6.5%)]; sputum examination for TB PCR was also performed as routine for all cases and positive results were recorded [nine (19.5%)].

The individuals with non-TB effusions (n=25) were either having exudative [11 (44%)] or transudative effusion [14 (56%)] according to Light's criteria. There was a spectrum of malignant and nonmalignant pleural effusions in this group. Malignancy diagnosed by means of cytology of the pleural fluid included primary and metastatic diseases [six (24%)]; five of them metastatic and one primary – lung adenocarcinoma, parapneumonic effusion [three (12%)], hepatic insufficiency [four (16%)], congestive heart failure [5 (20%)], chronic renal impairment [five (20%)], and one patient with systemic lupus erythematosus and another with rheumatoid arthritis. Thoracoscopy was performed [three (12%)], and closed pleural biopsy [five (25%)] whenever needed. The results were sent for histopathology and microbiology and all came negative for granuloma and TB.

ADA was measured in pleural fluid for all participants (71 patients), and median pleural ADA in the tuberculous effusion group was 91.35±21.98 (39–140 U/l). The cutoff values were determined using ROC curve (Fig. 1). It was higher with a statistically significant value (P<0.05) in the tuberculous than in the non-TB effusion group (25.6±16.15) (11.5–71.5 U/l) (Table 1 and Fig. 2). Some patients in the nontuberculous group [seven (28%)] showed high ADA of more than

Table 1 The demographic and clinical characteristics of study participants, procedures, and results, categorized as tuberculous (n=46) and nontuberculous (n=25) pleural effusion groups

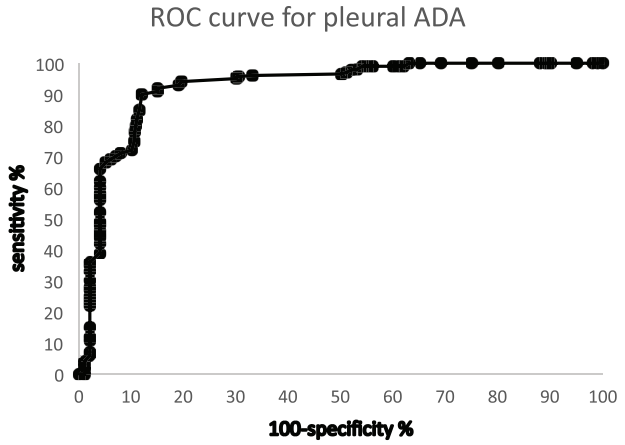
	Total (N)	Confirmed TPE	Nontuberculous effusion	P value
N	71	46	25	
Male/female	47/24	31/15	16/9	NS
Age [median (range) (SD)] (years)		28 (21–58) (12.7)	58 (37–84) (11.1)	<0.05
Comorbidities (DM) [n (%)]		10 (21.7)	See text	
Body temperature (°C)±SD		38.2±1.03	37.2±0.86	<0.05
TST (PPD)(mean±SD) (mm)		18±9.6	7±5.6	<0.05
ESR in (mm/1st hour)		47±31	37±34	0.7
Procedure				
Thoracentesis	71	46	25	
Thoracoscopy	24	17	3	
Closed pleural biopsy	21	16	5	
Exudative effusion	55	46	11	<0.05
Pleural lymphocytes (mean±SD%)		71±16	19±16	<0.05
Sputum AFB smear [n (%)]		3 (6.5)	0	<0.05
Pleural fluid AFB smear [n (%)]		4 (8.6)	0	<0.05
Sputum Xpert MTB/RIF [n (%)]		9 (19.5)	0	<0.05
Pleural TB culture [n (%)]		17 (36.9)	0	<0.05
Histopathology (granuloma) [n (%)]		33 (89.1)	See text	
Pleural ADA (U/l)		91.35±21.98	25.6±16.15	<0.05
QuantiFERON-TB Gold (positive test) [n (%)]		11 (23.9)	1 (4)	<0.05
Xpert MTB/RIF (positive test) [n (%)]		22 (47.8)	0	<0.05

ADA, adenosine deaminase; AFB, acid-fast bacilli; ESR, erythrocyte sedimentation rate; PPD, purified protein derivative; TPE, tuberculous pleural effusion; TST, tuberculin skin testing.

40 U/l and it was seen in patients with malignant effusion [three (12%)], parapneumonic effusion [three (12%)], and rheumatoid effusion [one (4%)].

None of the transudative effusions in the non-TB group showed a high ADA above the cutoff value of 40 U/l in patients with TB effusion. Forty-five (97.8%) patients showed pleural ADA of more than 40 U/l and one patient showed ADA level below 40 U/l. On comparing TB effusion with non-TB effusions using this cutoff point (>40 IU/l), the results showed an ADA sensitivity of 97.83% (n=45) (95% CI: 88.47–99.94%) but with less specificity (76.00%) (95% CI: 54.87–90.64%), but when using the higher cutoff point of 70 U/l (39 patients in the TB group showed pleural ADA >70 U/l). It resulted in an increase in the specificity of the test to 96.00% (95% CI: 79.65–99.90%), but with a decrease in the sensitivity to 84.78% (n=39) (95% CI: 71.13–93.66%).

Figure 1

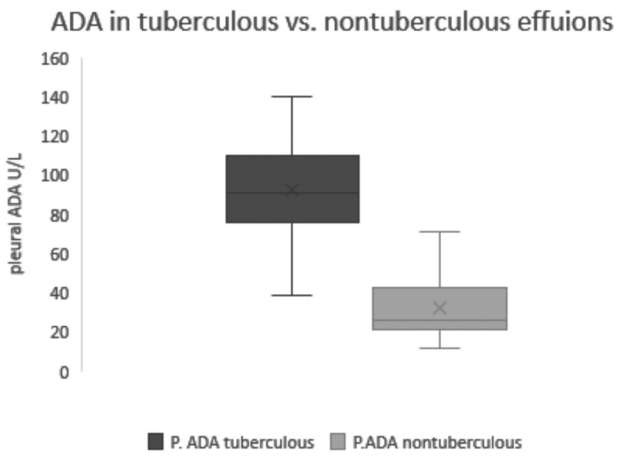


The receiver operator characteristic (ROC) curves for adenosine deaminase (ADA). The area under the curve (AUC) for cutoff 40 U/l was 0.91.

Only one patient in the nontuberculous group showed an ADA of more than 70 U/l (complicated parapneumonic effusion) with ADA of 71.5 U/l. The negative and positive predictive values and the positive and negative likelihood ratios are expressed in Table 2.

QFT-G was performed in all patients. In the TBs group, the results were as follows: positive [11 (23.9%)], indeterminate [eight (17.3%)], and negative [27 (58.6%)]. In the non-TB effusion group the test showed the following results: positive [one (4%)], indeterminate [seven (28%)], and negative [17 (68%), P<0.05] (Table 1). We added the indeterminate results to the negative results, and when comparing the two groups the sensitivity was 23.91% (95% CI: 12.59–38.77%) and specificity was 96.00% (95% CI: 79.65–99.90%). The negative and positive predictive

Figure 2



Data comparison graph of maximum and minimum values and median values for pleural adenosine deaminase (ADA) in tuberculous and nontuberculous pleural effusion, median, maximum and minimum were identified (P<0.05).

Table 2 Sensitivity and specificity of histopathology, adenosine deaminase with two cutoff values (40 and 70 U/l), QFT-G, and Xpert MTB-RIF

Test[a]	Sensitivity (95% CI)	Specificity (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
Histopathology	87.88% (71.80–96.60%)	100.00% (86.28–100.00%)	0NaN	0.12 (0.05–0.30)	100.00% (88.06–100.00%)	86.21% (68.34–96.11%)
ADA cutoff (40 U/l)	97.83% (88.47–99.94%)	76.00% (54.87–90.64%)	4.08 (2.03–8.20)	0.03 (0.00–0.20)	88.24% (76.13–95.56%)	95.00% (75.13–99.87%)
ADA cutoff (70 U/l)	84.78% (71.13–93.66%)	96.00% (79.65–99.90%)	21.20 (3.09–145.20)	0.16 (0.08–0.32)	97.50% (86.84–99.94%)	77.42% (58.90–90.41%)
QFT-G[b]	23.91% (12.59–38.77%)	96.00% (79.65–99.90%)	5.98 (0.82–43.66)	0.79 (0.66–0.95)	91.67% (61.52–99.79%)	40.68% (28.07–54.25%)
Xpert MTB/RIF	47.83% (32.89–63.05%)	100.00% (86.28–100.00%)	0NaN	0.52 (0.40–0.69)	100% (84.56–100.00%)	51.02% (36.34–65.58%)

'NaN' in any of the above cells means that the calculation contains the values entered include one or more instances of zero; ADA, adenosine deaminase; CI, confidence interval; QFT-G, QuantiFERON-TB Gold; [a]Sensitivity, specificity, and positive and negative predictive value are expressed as percentages for ease of interpretation. Their confidence intervals are 'exact' Clopper–Pearson confidence intervals; [b]QFT-G indeterminate results are counted as negative for ease of interpretation.

values and the positive and negative likelihood ratios are expressed in Table 2.

Pleural effusion and pleural biopsies of both groups were tested for Xpert MTB/RIF and the results were positive [22 (47.8%)] in the TB group, and none of the samples in the nontuberculous group was positive ($P<0.05$); sensitivity of the test was 47.83% (95% CI: 32.89–63.05%) and specificity was 100.00% (95% CI: 86.28–100.00%). The negative and positive predictive values and the positive and negative likelihood ratios are expressed in Table 2.

Correlations were made between positive results of ADA in pleural effusion with positive and negative results of Xpert MTB/RIF, QFT-G, and TB culture in TB and non-TB effusion using two cutoff points of ADA (>40 and >70 U/l) (Table 3). Combined analysis of ADA levels showed increased specificity of all other tests mentioned above, but remained of low sensitivity when pleural ADA cutoff level of more than 70 U/l was used (Xpert MTB/RIF 85.64%, QFT-G 56.68%, and TB culture 45.78%) but when the lower ADA cutoff point (>40 U/l) was used in the combined analysis, the sensitivity increased with Xpert MTB/RIF (96.78%) and remained low with QFT-G (56.68%) and TB culture (47.83%).

Discussion

The present study compared the diagnostic accuracy of conventional diagnostic tests with controversial biomarkers for TPEs. The most efficient and cost-effective tool for pleural TB diagnosis remains controversial [1], as the clinical presentation combined with systemic approach to analyze pleural effusions allows the clinician to diagnose the cause in 75% of patients [11]; TPE remains a diagnostic challenge [12].

Positive AFB stains, growth on TB culture, or histopathological demonstration of caseating granuloma are the gold standard for the diagnosis of TB [6]. However, negative bacteriological stains are common, and late culture results together with a low rate of positive cultures for *M. tuberculosis* in pleural effusion [13] render microbiological tests inadequate for the diagnosis of pleural TB; thus, there are still conflicting findings on the sensitivity and specificity of biopsy and mycobacterium culture of pleural fluid in the diagnosis. Thoracoscopy has a diagnostic yield of more than 99% for pleural TB [14]. However, it needs significant resources and expertise, making the blind closed pleural biopsy the only available option in most cases with a yield of less than 70% for TB [15,16].

In our study, pleural ADA was a sensitive test for the accurate diagnosis of tuberculous pleural effusion with a cutoff level of 40 U/l, reaching a sensitivity of 97.8% (95% CI: 88.47–99.94) but with low specificity (76%) (95% CI: 54.87–90.64%), and positive and negative predictive values were 88.2 and 95.0%, respectively. Compared with the studies conducted in 2016 [17] and 2015 [18] showing similar sensitivity (97.1 and 97%, respectively) but higher specificity (92.9 and 93%, respectively), an older study in 2007 [19] showed different results with lower sensitivity (76%) and higher specificity (100%) using a cutoff value of 45 U/l using pleural and serum

Table 3 Comparative analysis of positivity of different levels adenosine deaminase with positive and negative results of Xpert MTB/RIF, QFT-G, and tuberculosis culture

ADA	Xpert MTB/RIF		QFT-G		Tuberculosis culture	
	Positive	Negative	Positive	Negative	Positive	Negative
Positive ADA (cutoff >70 U/l) (*n*=39) [*n* (%)]	20 (51.28)	19 (48.72)	11 (28.20)	28 (71.80)	17 (43.59)	22 (56.51)
Combined positivity with cutoff >70 U/l						
Sensitivity	85.64% (71.13–96.23%)		56.68% (25.00–73.66%)		45.78% (34.13–64.66%)	
Specificity	98.50% 78.64–99.93%)		100.00% (86.28–100.00%)		100.00% (86.28–100.00%)	
Positive ADA (cutoff >40 U/l) (*n*=45) [*n* (%)]	22 (48.88)	23 (51.11)	11 (24.44)	34 (75.55)	17 (37.77)	28 (62.22)
Combined positivity with cutoff >40 U/l						
Sensitivity	96.78% (77.13–99.66%)		56.68% (25.00–73.66%)		47.83% (32.89–63.05%)	
Specificity	97.00% (79.65–99.90%)		100.00% (86.28–100.00%)		100.00% (86.28–100.00%)	
Negative ADA (cutoff < 40 U/l) (*n*=1) [*n* (%)]	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)

Sensitivity and specificity were calculated with addition of nontuberculous results in all groups; ADA, adenosine deaminase; QFT-G, QuantiFERON-TB Gold.

samples obtained from patients with pulmonary TB, pleural TB and non-TB pleural effusion, and normal controls, reflecting a wide variation in the sensitivity and specificity results with different cutoff values. However, a systematic review and meta-analysis conducted by Gui and Xiao [7] to compare 12 studies showed that the pooled estimates for ADA sensitivity was 86% (95% CI: 0.84–0.88%) and specificity was 88% (95% CI: 0.86–0.90%). The sensitivity was increased in subgroup analysis when the cutoff value of ADA increased to at least 50 U/l to be 89% (95% CI: 0.85–0.92%) and specificity was 87% (95% CI: 0.83–0.90%). The variations were due to heterogeneity present in the non-TB group, such as older age, which was associated with lower levels of ADA [20]. Malignant pleural effusion in the non-TB group showed high ADA and resulted in lower sensitivity of ADA, as two studies before showed high ADA levels in malignant pleural effusion [21,22], especially in areas where TB is of low or intermediate prevalence [23]. ADA values are high in malignant than in TB pleural effusion [24], parapneumonic effusions, and empyema. Therefore, assessment using ADA alone may be misleading [25]. Parapneumonic effusions can be differentiated from TB effusions by being rich in neutrophils, but to add to the difficulty, some TB effusions are being neutrophilic in early stages of disease and subsequent aspirations later showed a shift towards being lymphocytic.

In the present study, when a higher cutoff value of more than 70 U/l was used, the specificity increased to 96.00% (95% CI: 79.65–99.90%), but on the expense of the sensitivity, which decreased to 84.78% (95% CI: 71.13–93.66%) as seven cases fell below 70 U/l. This was supported by another study in Japan in 2009 [26] using ADA of less than 50 U/l to rule out the TB, but when they performed the thoracoscopic pleural biopsy on 50 patients with ADA level under 50 U/l, they defined TB in six (12%) of these patients. This indicates that using higher cutoff values for excluding TPE might result in missing cases.

IFN-γ release assay (IGRA) tests have been approved by FDA for use in the USA: the original QFT and QFT-G serum INF-γ and IGRA tests including QFT and QFT-G were used as confirmatory test for the diagnosis of latent TB infection, and QFT-G, in addition, was approved to detect active TB disease [27]. In our study, pleural QFT-G showed a sensitivity of 23.91% (95% CI: 12.59–38.77%), specificity of 96.00% (95% CI: 79.65–99.90%), and positive predictive value of 91%. Pleural IFN-γ was extensively studied in TPEs, and meta-analysis of these studies in 2007 [28] and earlier meta-analysis in 2003 [29] showed that the sensitivity ranged from 64 to 100% and specificity ranged from 86 to 100% in the first meta-analysis and in the second meta-analysis focusing on the effect of malignant effusions and paraneumonic effusions in the non-TB groups as factors that may reduce the specificity of the test. However, it was concluded that IFN-γ appears to be reasonably accurate at detecting TB pleurisy. Losi *et al*. [30], in their multicenter study on QFT-G in TB pleural effusion showed a sensitivity of 83.3% and specificity 53.3% (positive predictive value: 51.7% and negative positive value: 84.2%). Same results were reported in a study by Eldin *et al*. [31], on peripheral blood and pleural fluid INF-γ and QFT-G. They concluded that the sensitivity and specificity of INF-γ were higher compared with QFT-G, and its assay in peripheral blood or pleural fluid and/or washed pleural fluid cells is not superior to pleural fluid INF-γ in the diagnosis of pleural TB, although in the study by Eldin there was no mention of the indeterminate results. A systematic review and meta-analysis was performed by Aggarwal *et al*. [32], on 20 studies on blood IGRA and 14 studies on pleural IGRA involving 1085 and 727 individuals, respectively, and concluded that commercial INF-γ release assays performed either on whole-blood or pleural fluid samples have poor yield in confirming the diagnosis of TPE.

In the present study, we investigated the performance of Xpert MTB/RIF in TB and non-TB effusions; the sensitivity was 47.83% (95% CI: 32.89–63.05%) and specificity was 100.00% (95% CI: 86.28–100.00%), and this low sensitivity was observed even in culture-proven cases. Previous studies have reported much lower sensitivities between 15 and 48% [33–35], and recommended against the use of Xpert MTB/RIF as a tool to diagnose TPE [36]. The higher sensitivity in our study may be attributed to examining the pleural fluid and examining pleural biopsy tissues prepared for Xpert MTB/RIF. Sehgal *et al*. [37], performed a systematic review investigating the role of Xpert MTB/RIF in the diagnosis of TPE and showed pooled sensitivities and specificities of 51.4 and 98.6%, respectively, with culture used as a reference standard with a sensitivity of 22.7% and specificity of 99.8%, but most studies showed that positive Xpert MTB/RIF results were inferior to mycobacterial cultures in pleural fluid [36,38]. However, the added value of Xpert MTB/RIF is resistance detection, besides quick results rather than waiting for culture results to be obtained.

Single test studies for pleural fluid lack either the specificity or sensitivity to be a reliable diagnostic marker for TPE and for excluding other etiologies; thus, we investigated combinations of tests and compared their sensitivity and specificity.

Our results showed that a combination of positive pleural ADA (cutoff>40 U/l) with positive Xpert MTB/RIF resulted in a sensitivity of 96.7% and increased the specificity to 97%, compared with combined QFT-G and ADA (56 and 100%, respectively), and combined ADA (cutoff>70 U/l) and Xpert MTB/RIF (85.6 and 98.5%, respectively). It was superior to the combination of ADA (cutoff>70 U/l) and QFT-G (56 and 100%, respectively) indicating that the combination of ADA (cutoff>40 U/l) and Xpert MTB/RIF in pleural fluid provided the best predictive capacity. Liu *et al.* [39] showed that the combination of QFT-T in peripheral blood with ADA in pleural fluid increased the specificity to 100% and positive predictive value to 100% when used in the differential diagnosis between malignant pleural effusion and TPE, pointing to the superiority of blood QFT-G over pleural QFT-G in improving the diagnosis of TPE; the protocol of our study did not aim to assess peripheral blood QFT-G.

Limitations of the study

The study has limitations; the primary limitation is that we did not correlate pleural ADA, pleural QFT-G, and pleural Xpert MTB/RIF with pleural lymphocyte percentage to detect subsets of patient who might benefit from ADA, QFT-G, and Xpert MTB/RIF analyses. ADA works as a catalyst to facilitate the formation of inosine from adenosine, and it also plays a role in lymphoid cell differentiation; therefore, patients with low pleural lymphocyte count are expected to have lower values for ADA. However, Tay and Tee [40] in their study on factors affecting pleural ADA found a poor correlation with pleural lymphocytes, nullifying the effect of this correlation on pleural ADA levels but highlighting the age effect and the need for higher cutoff values for people older than 55 years, which we did not perform as well. Second limitation was the small sample size and the need for a large-scale study in the future to allow the differentiation of population sample into patients coming from high TB incidence and medium or low prevalence.

Conclusion

ADA is a useful marker in the diagnosis of TPE but its specificity is low with low cutoff value, and its sensitivity is low with high cutoff value. The specificity of ADA with low cutoff value can be improved by combining with Xpert MTB/RIF, resulting in an accurate test to identify patients with suspected TPE.

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The author was responsible for design and running the research, cases matching with inclusion criteria are examined and pleural samples were sent to laboratory, collection of results, sending to statistical analysis.

The work is conducted in Chest Diseases Department, Cairo University.

The author has been read and approved the author believes that the manuscript represents honest work.

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Conflicts of interest

There are no conflicts of interest.

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