

Diagnostic value of pleural fluid interferon-gamma and adenosine deaminase in patients with pleural tuberculosis in Qatar

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Objective: To investigate the diagnostic utility of interferon-gamma (IFN- γ) and adenosine deaminase (ADA) in tuberculous pleural effusions by determining the best cutoff levels of these two markers for pleural tuberculosis, in the context of the local epidemiological settings in Qatar.

Methods: We prospectively studied IFN- γ and ADA levels in the pleural fluid of patients presenting to Hamad General Hospital between June 1, 2009 and May 31, 2010.

Results: We studied 103 patients with pleural effusions, 72 (69.9%) with pleural tuberculosis, and 31 (30.1%) with nontuberculous etiologies. The mean IFN- γ concentration for the group with tuberculous effusions was significantly higher than that in the group with nontuberculous effusions (1.98 ± 81 vs 0.26 ± 10 pg/mL [$P < 0.0001$]). The mean ADA activity for the tuberculous effusions group was significantly higher than that in group with nontuberculous effusions (41.30 ± 20.09 vs 14.93 ± 14.87 U/L [$P < 0.0001$]). By analysis of receiver operating characteristic (ROC) curves, the best cutoff values for IFN- γ and ADA were 0.5 pg/mL and 16.65 U/L, respectively. The results for IFN- γ vs ADA were: for sensitivity, 100% vs 86%, respectively; for specificity, 100% vs 74%, respectively; for positive predictive value, 100% vs 88.5%, respectively; and for negative predictive value, 100% vs 69.7%, respectively.

Conclusion: IFN- γ and ADA could be used as valuable parameters for the differentiation of tuberculous from nontuberculous effusion, and IFN- γ was more sensitive and specific for tuberculous effusion than ADA.

Keywords: pleural effusion, parapneumonic effusion, malignant effusion

Introduction

Tuberculosis (TB) is the most common cause of pleural effusion in areas with a high incidence of TB. The sensitivity of both direct microscopy and pleural fluid cultures is relatively low, and the diagnosis is mainly based on the presence of caseating granulomas in a pleural biopsy specimen. However, the procedures for pleural tissue collection, such as thorascopic biopsy, have an invasive nature and are not without risk. Thus, efforts to find alternative, rapid, noninvasive, and safe tests, which maintain high sensitivities and specificities for the diagnosis of pleural TB, have been attempted. The measurement of adenosine deaminase (ADA) and interferon-gamma (IFN- γ) in the supernatant of pleural fluid specimens may serve as one example.^{1,2} Although many studies have shown an excellent diagnostic yield of these two markers (see "Discussion"), the cutoff points widely vary.

Tuberculosis is a common disease in Qatar, with an incidence rate of 37/100,000 and 553 new cases diagnosed in 2011.³ It is the most common cause of pleural effusion in

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this country.⁴ Pleural biopsy via video-assisted thoracoscopy (VAT) is the main procedure used in our hospital to diagnose this disease. Variation in the cutoff points for ADA and IFN- γ levels in different studies has created difficulties in the clinical application of these results, in our hospital. This prompted us to conduct this study to assess the role of ADA and IFN- γ in the differential diagnosis of pleural effusion, by determining the best cutoff levels of pleural fluid IFN- γ and ADA for the diagnosis of pleural TB in our epidemiological settings.

Methods and patients

Design and setting

This observational, prospective, case-control study was conducted at Hamad General Hospital, a tertiary care hospital that covers the whole of the State of Qatar.

The study was designed to include all sequentially encountered episodes of pleural effusion during the period from June 1, 2009 to May 31, 2010.

Case ascertainment

From June 1, 2009 to May 31, 2010, all patients with pleural effusion were identified prospectively by daily checks of the hospital admission records. All patients with pleural effusion (or their relatives) were interviewed, and all patients were examined by one of the authors. All patients with TB pleurisy were treated with a standard 6-month, directly observed anti-TB treatment regimen and followed up until treatment completion (6–9 months), while patients with nontuberculous effusion were observed for 3 months and monitored for signs or symptoms of tuberculosis.

Case definitions

Pleural effusions were diagnosed as tuberculous if at least one of the following criteria were fulfilled: (1) caseous necrotic granulomas were found in pleural biopsy tissue samples; (2) Ziehl–Neelsen stains or Lowenstein cultures of pleural fluid or biopsy tissue samples were positive; or (3) a sputum culture finding was positive for TB, in the presence of pleural effusion.

Patients were defined as having nontuberculous pleural effusion if: (1) an alternative diagnosis of pleurisy, different from TB, was established by cytologic or histologic testing; or (2) if patients did not fulfill the criteria of pleural tuberculosis and did not receive anti-TB treatment, and did not develop signs or symptoms of TB after 3 months. Nontuberculous cases that were submitted for histopathological examination but that did not fulfill the diagnostic criteria for tuberculous pleuritis were selected to form the control group.

Diagnostic workups

Thoracentesis and pleural biopsy by VAT

Patients underwent thoracentesis in the first 24 hours after the admission. Pleural fluid was analyzed for biochemical markers, Gram stain, and bacterial and TB culture, as well as for cytology and differential white blood cell count using standard cytopspin procedures and hematoxylin-eosin or Papanicolaou stains. At the same time, blood samples were taken for additional simultaneous pleural fluid and blood determination of the levels of total protein, albumin, and glucose. Effusions were classified as transudates or exudates, using Light's criteria.

Pleural biopsy (via VAT) was sent to the microbiology and histopathology laboratories for the diagnosis of tuberculous and malignant pleural effusions.

Pleural fluid samples (mean volume 50 mL) for ADA and IFN- γ measurements were centrifuged at 2000 revolutions per minute for 10 minutes, and the supernatant was frozen at -80°C until assayed for markers.

Enzyme-linked immunosorbent assay for IFN- γ

We used Quantikine[®] Human IFN- γ Immunoassay kits, manufactured and distributed by R&D Systems, Inc (Minneapolis, MN, USA). This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for IFN- γ was precoated onto a microplate; controls and samples were then pipetted into the wells, and any IFN- γ present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IFN- γ was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells, and color developed in proportion to the amount of IFN- γ bound in the initial step. The color development was then stopped, and the intensity of the color was measured.

Measurement of ADA activity

We used an ADA assay kit produced by BQKITS Diagnostics (San Diego, CA, USA), and biochemical measurements on pleural fluid samples were carried out using a Hitachi 917 analyzer (Hitachi Ltd, Tokyo, Japan). The ADA assay is based on the enzymatic deamination of adenosine to inosine, which is then converted to hypoxanthine by purine nucleoside phosphorylase. Hypoxanthine is converted to uric acid and hydrogen peroxide by xanthine oxidase. Hydrogen peroxide is further reacted with N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline and 4-aminoantipyrine in the presence of peroxidase to generate quinone dye, which is

monitored in a kinetic manner. After that, absorption at λ max 556 nm is read in a spectrophotometer, using the Hitachi 917. In this study, 103 samples, two point calibrations, and approximately 20 runs for each two quality control levels were performed. The assumed coefficient of variation was 2.1% for the low control and 2.2% for high control.

Other workups

Three sputum samples were obtained from the patients and studied using Ziehl–Neelsen staining and Lowenstein culture. Additional diagnostic procedures, such as computed tomography of the chest, bronchoscopy, echocardiography, and others, were undertaken whenever necessary, to further evaluate pleural fluid etiology.

Data collection and analysis

For each case of pleural effusion, the following variables were recorded on standard forms: demographic data, biochemical parameters in the blood, tuberculin purified protein derivative (PPD) readings, result of sputum direct smear and culture for acid-fast bacilli (AFB), characteristics of the pleural fluid and the result of fluid direct smear and culture for AFB, pleural fluid IFN- γ value, ADA activity, the histopathological and microbiological results of pleural biopsy, the etiology of the pleural effusion and duration of specific treatment, and outcome.

Quantitative variables were expressed as mean, standard deviation (SD) and range. The student *t*-test was used for continuous variables, and the Mann–Whitney *U* test was used if the quantitative variables were not normally distributed. Receiver operating characteristic (ROC) curves were plotted for various cutoff values of pleural fluid IFN- γ and ADA, to select their optimal cutoffs on the basis of the highest diagnostic accuracy with the highest sensitivity. Results were considered significant if at $P < 0.05$. Data analysis was performed with SPSS software (v 11.5; IBM Corp, Armonk, NY, USA).

Outcome of the study

The primary outcome was the estimate of the sensitivity and specificity of IFN- γ assay and ADA activity for the diagnosis of tuberculous pleuritis.

Research committee approval

The study was approved by the research committee of Hamad Medical Corporation. Informed consent was obtained from each participant before any interview or clinical examination was conducted.

Results

During the period of study, 103 patients were enrolled and tuberculous pleuritis was diagnosed in 72/103 (70%) of them. The mean age of the patients was 38.92 ± 16.52 years (range: 18–85 years). There were 87/103 (84.5%) males and 16/103 (15.5%) females. Non-Qatari residents accounted for 95/103 of the patients (92.2%).

In the tuberculous group, a pleural fluid direct smear for AFB was negative in all 72 patients, while a pleural fluid mycobacterial culture was positive in 26/72 patients (36.1%). Pleural biopsy was positive for AFB in 19/65 (29.2%), and culture grew *M. tuberculosis* in 51/65 (78.4%). Caseating granulomas were identified in 47/65 (72.3%) of specimens. Sputum smear examination for *M. tuberculosis* was positive in 6/72 (8.3%), while culture grew *M. tuberculosis* in 19/72 (26.4%).

The nontuberculous group accounted for 31/103 of effusions (30%). These included parapneumonic effusion 12/31 (38.7%), neoplastic conditions 10/31 (32.3%), and other effusions 9/31 (29%). All patients were seronegative for human immunodeficiency virus (HIV).

IFN- γ concentration

The mean IFN- γ concentration for the tuberculous group was 1.98 ± 81 pg/mL (range: 0.54–3.49 pg/mL), compared with a mean level of 0.26 ± 10 pg/mL (range: 0.02–0.47 pg/mL) in the nontuberculous group ($P < 0.0001$). The use of a cutoff level of 0.50 pg/mL as the diagnostic for pleural tuberculosis resulted in a 100% sensitivity and 100% specificity for TB in this study. The area under the ROC curve for IFN- γ was 1.0 (SE = 0.00, 95% confidence interval [CI]: 1.00–1.00) (Figure 1).

ADA activity

The mean ADA activity for the tuberculous group was 41.30 ± 20.09 U/L (range: 0.2–88.5 U/L), compared with a mean level of 14.93 ± 14.87 U/L (range: 0.10–58.5 U/L) in the nontuberculous group ($P < 0.0001$). At a cutoff of 16.65 IU/L, the sensitivity of the test was 86% and the specificity was 74%. The area under the ROC curve for ADA was 0.85 (SE = 0.043, 95% CI: 0.77–0.93) (Figure 2). ADA activity at 0.15 U/L suggests that tuberculous effusion is highly unlikely, with a sensitivity of 100% and a specificity of 32%; while ADA activity at 58.6 U/L, confirms the diagnosis of tuberculous effusion with specificity of 100% U/L and sensitivity of 18%. A comparison between the tuberculous group and nontuberculous group was carried out in Table 1, while Table 2

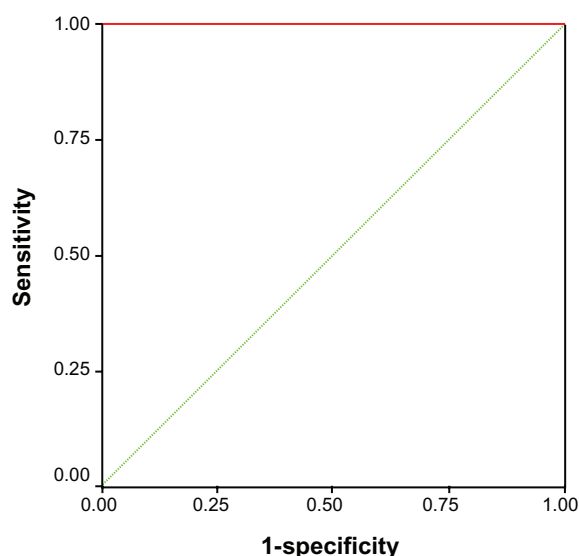


Figure 1 ROC curve plotted for pleural fluid interferon-gamma, to evaluate its utility in the diagnosis of TB pleural effusion.

Note: Area under the ROC curve: 1.0.

Abbreviations: ROC, receiver operating characteristic; TB, tuberculosis.

describes the accuracy of different diagnostic procedures in pleural TB. In the twelve patients with parapneumonic effusion, the mean ADA activity was 14.36 ± 14.80 U/L (range: 2.20–58.50 U/L).

Discussion

The characteristics of pleural tuberculosis have been well documented in Qatar,⁴ where thorascopic pleural biopsy is the main diagnostic procedure. The evaluation of IFN- γ and ADA levels in pleural fluid to determine the best cutoff

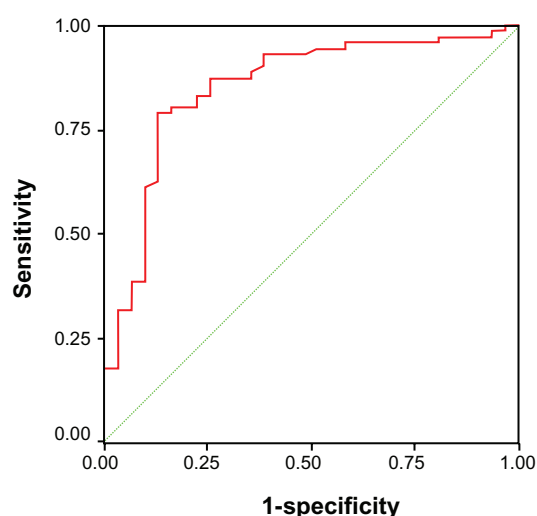


Figure 2 ROC curve of ADA levels in pleural fluid, for the diagnosis of tuberculosis.

Note: Area under the ROC curve: 0.85 (SE = 0.043, 95% CI: 0.77–0.93).

Abbreviations: ADA, adenosine deaminase; CI, confidence interval; ROC, receiver operating characteristic; SE, standard error.

Table 1 A comparison between tuberculous and nontuberculous groups, in relation to age and biochemical characteristics of pleural fluid

Parameter	Non-TB group	TB group	P value
Age (years)	55.54 \pm 16.16	31.76 \pm 10.45	<0.0001
Total protein (g/dL)	3.91 \pm 1.69	5.57 \pm 0.62	<0.0001
Albumen (g/dL)	2.22 \pm 0.98	2.63 \pm 0.47	0.006
LDH (IU/L)	429.45 \pm 508.90	925.34 \pm 724.48	0.001
WBC (/ μ L)	2162.48 \pm 2657.71	2203.54 \pm 1834.41	0.92
Neutrophils (%)	44.19 \pm 31.02	14.30 \pm 13.73	<0.0001
Lymphocytes (%)	44.51 \pm 29.74	81.61 \pm 17.61	<0.0001
IFN- γ (pg/mL)	0.26 \pm 10	1.98 \pm 81	<0.0001
ADA (U/L)	14.93 \pm 14.87	41.30 \pm 20.09	<0.0001

Note: Data are presented as mean \pm standard deviation.

Abbreviations: ADA, adenosine deaminase; IFN- γ , interferon-gamma; LDH, lactate dehydrogenase; TB, tuberculosis; WBC, white blood cells.

points to diagnose this clinical entity has not been attempted before in Qatar. This is the first study designed to investigate the clinical utility of pleural fluid IFN- γ and ADA levels in diagnosing pleural TB, in patients living in Qatar. We assessed the diagnostic utility of pleural IFN- γ and ADA level using ROC curves.

As noted, non-Qatari patients predominated the study group. This seems to be artificial as non-Qatari residents constitute the majority of the total population in this country, due to the large influx of foreign laborers.

Pleural TB occurs as a result of the entry of TB antigen into the pleural space, usually through the rupture of a sub-pleural focus, followed by a local, delayed hypersensitivity reaction mediated by CD4⁺ cells. The presence of mycobacterial antigens in the pleural space elicits an intense immune response, initially by neutrophils and macrophages, followed by IFN- γ , -producing T-helper cell type 1 lymphocytes and resulting in a lymphocyte-predominant exudative effusion.⁵ INF- γ enhances macrophage phagocytic activity against mycobacteria and increases in pleural fluid as a result of local production. It is noteworthy that, INF- γ production is not specific to mycobacterial antigens; it is also produced by T lymphocytes in response to stimulation with viral antigens.⁶ Moreover, pleural INF- γ levels may increase in hematological malignancies and in granulomatous diseases, such as rheumatoid arthritis.⁷ Nevertheless, in many studies, higher levels of IFN- γ have been found in patients with pleural tuberculous compared with nontuberculous effusions, regardless of the above mentioned conditions, but the cutoff points for pleural TB vary widely among these studies. The sensitivity of an elevated level of IFN- γ as a diagnostic marker of tuberculous pleurisy varies between 94% and 100%, while

Table 2 Accuracy of different diagnostic methods for pleural tuberculosis

Parameter	Sensitivity	Specificity	Negative predictive value	Positive predictive value
Pleural fluid TB culture	36.1%	100%	40.2%	100%
Biopsy AFB smear	29.2%	100%	40.2%	100%
Biopsy TB culture	78.4%	100%	68.8%	100%
Biopsy histology	72.3%	100%	63.2%	100%
IFN- γ (0.50 pg/mL)	100%	100%	100%	100%
ADA (16.65 U/L)	86%	74%	69.7%	88.5%

Abbreviations: ADA, adenosine deaminase; AFB, acid-fast bacilli; IFN- γ , interferon-gamma; TB, tuberculosis.

its specificity ranges from 92% to 100%.^{2,5–14} The reasons for this variation are not clearly understood. Many suggestions, such as variable cytokine response, severity of infection, malnutrition, intralaboratory method discrepancy, local epidemiological situation, and associated infections have been proposed to explain this observation.⁸ In agreement with many reports,^{12–17} our study showed that the concentration of pleural fluid IFN- γ in the tuberculous group was significantly higher than that in nontuberculous group ($P > 0.0001$). Using the optimal cutoff value of 0.50 pg/mL, the sensitivity and specificity was 100%. This result is very similar to those of other authors,^{2,12–14} although the cutoff values they adopted were different. The performance of IFN- γ tests in immunocompromised patients (eg, HIV patients) and the effect of immunosuppression on these tests remains unclear, as many studies have shown inconsistent results.^{7,8} None of our patients were positive for HIV.

On the other hand, ADA was found to be high in pleural TB in 1978;¹⁸ since then, it has been used in the diagnosis of tuberculous pleural effusions.¹⁹ ADA is an enzyme involved in purine catabolism and is responsible for the conversion of adenosine to inosine and ammonia. It is also involved in the proliferation and differentiation of lymphocytes, particularly the T subtypes. There are several isoforms of ADA, but the prominent ones are ADA-1 and ADA-2, which are located on different gene loci. ADA-1 isoenzyme is found in all cells, with highest concentration in lymphocytes and monocytes, whereas ADA-2 isoenzyme appears to be found only in monocytes/macrophages.^{20,21} Elevated ADA activity is not specific for pleural TB. It may be found in effusions due to a number of causes, including bacterial infections, rheumatic disease, and lymphoproliferative disorders.²¹ However, high levels of ADA in pleural TB are due largely to high ADA-2 activity.^{22,23} The ADA values in the HIV-positive patients did not differ significantly from those in the HIV-negative patients.²⁴ Pleural fluid ADA now forms part of the routine diagnostic workup for tuberculous effusions in many countries where TB is

endemic. It is inexpensive and easy to measure. The high diagnostic accuracy of ADA activity measurement has been reported in several studies,^{1,15–28} with variations in the cutoffs for pleural TB. The sensitivity of an elevated level varies between 56% and 100%, while the specificity ranges from 55% to 100%.^{1,15–28} The sensitivity and specificity of ADA in our study fell within the abovementioned range.

As noted in this study, and in agreement with many reports,^{8,11,13,29,30} pleural fluid IFN- γ estimation has been found to be superior to ADA as a marker for pleural TB. Moreover, many studies confirm the superiority of pleural fluid IFN- γ as a marker for tumor necrosis factor-alpha and other cytokines, such as the interleukins (IL) (IL-2, IL-6, and IL-8).^{11,14,29,30}

Some limitations can be noted in our study. First, the number of cases exceeded the number of controls, which may have led to biased results, especially regarding the cutoff values. Second, the control group was not representative for all kinds of pleural effusion as it included mainly parapneumonic effusion and malignancy.

In conclusion, our results indicate that pleural fluid ADA and IFN- γ levels have good diagnostic accuracy for pleural TB in the patient population living in Qatar, in which the prevalence of TB is high. Both are rapid noninvasive diagnostic tests for pleural tuberculosis. However, fluid IFN- γ estimation, while expensive, has been found to be superior to ADA.

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Disclosure

The authors report no conflicts of interest in this work.

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