Clinical Utility of Interferon-γ in Tuberculous Pleural Effusion

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ABSTRACT

Introduction: Tuberculosis (TB), the single most frequent infectious cause of **death worldwide, also is a major cause of pleural effusion, which in TB usually has lymphocytic and exudative characteristics. Differential diagnosis between TB and nontuberculous pleural effusion can be sometimes difficult, representing a critically important clinical problem.**

Aim of the work: to evaluate the clinical utility of pleural IFN- γ level in pleural fluid for diagnosing tuberculous pleuritis.

Subjects & methods: the study was conducted in kaser El-Aini hospital, Cairo University in the period from January 2011 to January 2012. It was carried on 40 patients. The patients included in the study were classified into group I (included 20 cases with tuberculous pleural effusion) and group π (included 20 cases with non tuberculous pleural effusion). All patients were subjected for complete history taking and clinical examination, chest X-rays PA and lateral views, pleural fluid aspiration and analysis.

Result: our results demonstrate that the pleural fluid concentrations of ADA, INF-γ in patients with tuberculous pleural effusions are significantly higher than in other effusions. Most importantly, ROC analysis clearly demonstrated ADA to sensitive and specific rather than INF- γ for diagnosis of tuberculous pleuritis.

Key words: adenosine deaminase, interferon-γ, pleural fluid, tuberculous pleuritis.

Abbreviations: ADA **:**adenosine deaminase, INF **:**interferon, LDH **:** lactate dehydrogenase, ROC **:** receiver operating characteristic and TB: tuberculosis.

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Introduction

Worldwide, tuberculosis (TB) is the single most frequent cause of death by an infectious agent.[1] TB also is a major cause of pleural effusion; tuberculous effusions usually are lymphocytic and exudative. The diagnosis of tuberculous pleuritis should be considered in any patient with an exudative pleural effusion. Management of patients with tuberculous pleuritis who have acquired pleural effusion requires an effective treatment plan based on timely and accurate diagnostic information.

The diagnosis of tuberculous pleuritis commonly is made from observation of granulomas in pleural biopsy specimens or a culture finding positive for Mycobacterium tuberculosis from pleural tissue or pleural fluid. However, sensitivity of these methods is sufficiently low that even when histopathology and culture are combined, the diagnosis can be uncertain or missed in "negative" cases. [2] [3] While repeating invasive diagnostic procedures ultimately may yield positive results, such an approach places patients at increased risk of complications and also increases costs. A reliable clinical marker providing physicians with rapid and accurate diagnosis of tuberculous pleuritis is greatly needed.

A variety of biological markers have been proposed to aid in the diagnosis of tuberculous pleuritis, including increased pleural fluid concentrations of adenosine deaminase (ADA),[4] [5] [6] interferon (INF)-γ,[7] [8] [9] [10] [11] [12] [13] However, which of these markers is most useful for diagnosis of tuberculous pleuritis has not been determined. Here we studied 40 patients with pleural effusion to determine whether ADA, INF-γ concentrations in pleural fluid show associations with the cause of pleural effusion.

Materials and Methods

Patients:

Forty inpatients presenting with pleural effusions who were admitted to Kasr El Aini Hospital between January 2011 and January 2012 were studied. Informed consent was obtained from the patients. Clinical signs and symptoms, demographic data, and radiologic results were recorded. Of these 18 men and 22 women ranging age from 21 to 70 years old, 19 patients had tuberculous pleurisy, 11 patients had malignant pleuritis, 5 patients had parapneumonic pleural effusions and 5 patients had pleural effusion with various nontuberculous, nonmalignant etiologies (liver cell failure in 1 patient, metastatic adenocarcinoma in 1 patient and undetermined etiology in 3 patients).

Specimen Collection and Processing

For each subject, at least 40 mL of pleural fluid was collected in a syringe during thoracentesis. A portion of the sample was submitted for acid-fast staining, bacteriologic examination, cytologic examination, and measurement of protein, lactate dehydrogenase (LDH), and glucose. Another part of the sample was centrifuged at 2,000 revolutions per minute for 10 min. The supernatant was frozen at − 20° until assays for markers.

Diagnosis of Tuberculous, Malignant, and Miscellaneous Pleural Effusions

Patients were classified into one of following diagnostic groups:

A. Tuberculous pleural effusion. These patients were subcategorized into two groups to the diagnostic tests for tuberculosis: (i) patients with granulomas in the pleural biopsy specimen in the absence of other pleural granulomatous diseases; (ii) patients aged 40 years or younger, with a constitutional manifestation with either a positive purified protein derivative test or at least 95% lymphocytes in the pleural fluid. Patients were classified according

to the highest group for criteria met.

B. Neoplastic pleural effusions. These patients had a cytologic or histologic diagnosis of neoplasm of the pleural space or a histologic diagnosis of a tumor in another organ, and no other cause of pleural effusion.

C. Parapneumonic effusions. These patients presented with cough, fever, and a radiographic pulmonary infiltrate that resolved with antibiotic treatment. Patients with empyema, defined as pus in the pleural cavity, were included in this group.

D. Pleural effusions of unknown (nontuberculous) etiology. Patients with no known cause of pleural effusion who had nonspecific pleuritis by pleural biopsy, thoracoscopy.

Determination of Pleural Fluid Levels of ADA, INF-γ:

ADA activity was measured by autoanalyzer using commercially available kits. INF-γ was measured using commercially available enzyme-linked immunosorbent assay kits.

Statistical Analysis

Quantitative data were presented as mean and standard deviation (SD) values. For parametric data, Student's

t-test was used for comparisons between mean values of two groups. One way ANOVA (Analysis of Variance) was used to compare between mean values of more than two groups. Tukey's post-hoc test was used for pair-wise comparisons between mean values when ANOVA test is significant.

For non-parametric data, Mann-Whitney U test was used to compare between two groups. This test is the non-parametric alternative to Student's t-test. Kruskal-Wallis test was used to compare between more than two groups. This test is the non-parametric alternative to one-way ANOVA. Mann-Whitney U test was used for pair-wise comparisons between the groups when Kruskal-Wallis test is significant.

Qualitative data were presented as frequencies and percentages. Chi-square (x^2) test was used for studying the comparisons between different qualitative variables.

Spearman's correlation coefficient was used to determine significant correlations between the different variables.

ROC (Receiver Operating Characteristic) curve was constructed to establish the optimal cut-off points and the likelihood ratios (LRs) of ADA and Interferon.

Results:

Table (1): Mean, standard deviation (SD), frequency, percentage values and results of One-way ANOVA, Tukey's test and Chi-square test for comparison between ages and gender distributions in the studied groups

	TB (n = 19)	Malignancy (n = 11)	Pneumonia (n = 5)	Idiopathic (n = 3)	Others (n = 2)	*P*-value
Age (Mean ± SD)	29.2 ± 12.2 [b]	55.2 ± 11.9 [a]	54.6 ± 11.5 [a]	51.7 ± 16.1 [a]	58.5 ± 2.1 [a]	<0.001*

Gender (Frequency, %)						
Male	9 (47.4)	5 (45.5)	3 (60)	1 (33.3)	0 (0)	0.681
Female	10 (52.6)	6 (54.5)	2 (40)	2 (66.7)	2 (100)	

*: Significant at P ≤ 0.05, Different letters are statistically significantly different according to Tukey's test

Diagnostic accuracy of ADA

At cut-off point of 30 IU/L, the sensitivity of ADA was (84.2%), specificity was (71.4%) and the diagnostic accuracy was 77.5%.

Table (2): Sensitivity, specificity, predictive values and diagnostic accuracy of ADA in detecting TB

ADA	TB		Total
	+ ve	- ve	
+ ve	16 (True +ve)	6 (False +ve)	22
- ve	3 (False –ve)	15 (True –ve)	18
Total	19	21	40

Sensitivity (%) = $\dfrac{16}{16+3}$ x 100 = **84.2%**

Specificity (%) = $\dfrac{15}{6+15}$ x 100 = **71.4%**

Positive predictive value (PV^{+}) (%) = $\dfrac{16}{16+6}$ x 100 = **72.7%**

Negative predictive value (PV^{-}) (%) = $\dfrac{15}{3+15}$ x 100 = **83.3%**

Diagnostic accuracy (%) = $\dfrac{16+15}{40}$ x 100 = **77.5%**

ROC curve analysis

Interferon

ROC curve analysis of Interferon values for the diagnosis of TB in the present study showed that the optimal cut-off point was determined at 0.25. The likelihood ratios (LRs) were 1.88 and 0.20 for values above or below this cut-off point.

Table(3) Results of ROC curve analysis for Interferon in diagnosis of TB

Area under the ROC curve (AUC)	0.713
Standard error	0.085
95% Confidence interval	0.548 to 0.845
P-value	0.013*

: Significant at P ≤ 0.05
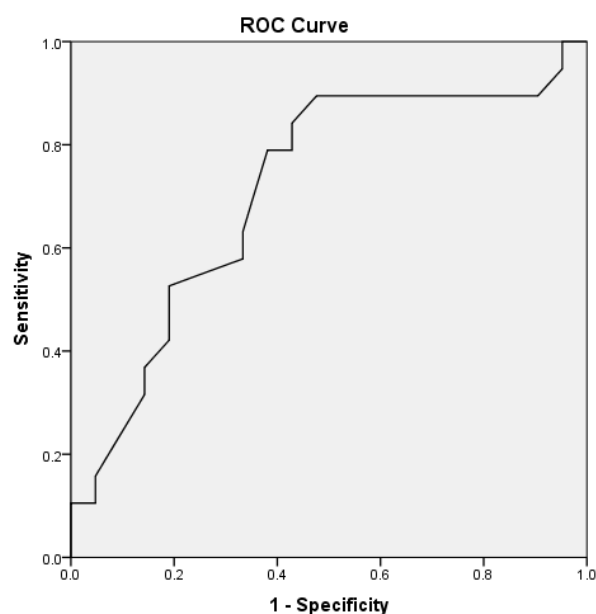
Diagnostic accuracy of Interferon

The sensitivity of Interferon was (84.2%), specificity was (57.1%) and the diagnostic accuracy was 70%.

Table (4): Sensitivity, specificity, predictive values and diagnostic accuracy of Interferon in detecting TB

Interferon	TB		Total
	+ ve	- ve	
+ ve	16 (True +ve)	9 (False +ve)	25
- ve	3 (False –ve)	12 (True –ve)	15

Total	19	21	40

Sensitivity (%) = $\dfrac{16}{16 + 3}$ x 100 = **84.2%**

Specificity (%) = $\dfrac{12}{9 + 12}$ x 100 = **57.1%**

Positive predictive value (PV^{+}) (%) = $\dfrac{16}{16 + 9}$ x 100 = **64%**

Negative predictive value (PV^{-}) (%) = $\dfrac{12}{3 + 12}$ x 100 = **80%**

Diagnostic accuracy (%) = $\dfrac{16 + 12}{40}$ x 100 = **70%**

Table (5): Sensitivity, specificity, predictive values and diagnostic accuracy of different tests used in detecting TB

	Sensitivity	Specificity	PV $^{+}$	PV $^{-}$	Diagnostic accuracy
ADA	84.2%	71.4%	72.7%	83.3%	77.5%
Interferon	84.2%	57.1%	64%	80%	70%

Discussion :

Differential diagnosis between tuberculous and nontuberculous pleural effusions represents a critical clinical problem. Conventional methods for diagnosis of pleural tuberculosis have proven insufficient. Direct examination of pleural fluid by Ziehl-Neelsen staining requires bacillar densities of 10,000/mL and, therefore, has low sensitivity (0 to 1%).[2] Although culture is more sensitive (11 to 50%), 2 to 6 weeks are required to grow M tuberculosis, and a minimum of 10 to 100 viable bacilli are needed. The sensitivity of pleural biopsy reportedly is higher than thoracentesis whether in terms of culture (39% vs 79%)[2] or histologic evaluation (71 to 80%). [2] [3] However, biopsy requires greater expertise, is more invasive, and is subject to sampling error.

Pleural levels of a number of biomarkers have been proposed as aids in the diagnosis of TPE, including those of ADA, interferon-gamma, interleukin-12, interleukin-18, immunosuppressive acidic protein, and soluble interleukin-2 receptor, the levels of which are all significantly higher in TPE than in non-TPE[4]

An ideal test for tuberculous pleurisy should be economic, minimally invasive, of high accuracy, and quick to perform. The ADA test has been hailed lately as the ideal test for the diagnosis of TPE. However, this test has some limitations that should be taken into consideration when facing the challenge of diagnosing a pleural effusion of unknown origin[5].

ADA activity is found in pathologic conditions other than TB. Nevertheless, high levels of the enzyme are more strongly associated with pleural TB, (ADA), released by activated lymphocytes, macrophages and neutrophils, is a nonspecific marker of inflammation. The ADA2 isoenzyme released from monocytes and macrophages is the predominant contributor to total ADA [6].

IFN-gamma, a cytokine that exhibits both antiviral and cytotoxic activities, It is produced by T lymphocytes in response to stimulation with specific antigens or nonspecific antigens, and is capable of modifying the response of other cells to the immune system. INF-γ is known to activate macrophages, increasing their bactericidal capacity against M tuberculosis. Therefore, INF-γ detected in pleural fluid may be the result from stimulation of T lymphocytes by tuberculous antigens [7].

The interferon- γ release assays (IGRAs) are technically more complicated and expensive than established biomarkers and their diagnostic performance for active TB is highly variable between studies.. In developing countries where the burden of TB is high and cost is a major issue, pleural fluid IFN- γ does not seem to be an attractive means of differentiating TB from non-TB aetiology. In fact, the World Health Organization Strategic and Technical Advisory Group for Tuberculosis (WHO STAG–TB) has not yet endorsed the use of IGRAs in resource-limited countries[7].

According to the final diagnosis the patients of our work were classified into two groups:

group I (included 20 cases with newly diagnosed TB. Pleural effusion who did not receive any treatment.

group п (included 20 cases with non tuberculous pleural effusion), which sub classified into:

group па: included 11 patients with malignant pleural effusion who did not receive any radiotherapy or chemotherapy.

group пb: included 5 patients with parapneumonic effusion.

group пc: included 4 patients with Pleural effusions of unknown (nontuberculous) etiology. Patients with no known cause of pleural effusion who had nonspecific pleuritis by pleural biopsy, thoracoscopy.

All of the above, subgroups па, пb, пc were considered as control cases.

In this study the mean age of tuberculous patients was 29.2 ± 12.2 years while those of malignant patients were 55.2 ± 11.9 years, 54.6 ± 11.5 years in parapneumonic subgroup and 51.7 ± 16.1 years, so Tuberculous patients showed the statistically significantly lowest mean age these result agreed with **Valdes et al**[8].

Valdes et al, found that the mean age of tuberculous group was 33.9 ± 13.2 years that of malignant group was 45.5 ± 16.8 years .

In our study the level of ADA activity was the most sensitive method (84.2%) for the diagnosis of pleural TB. The specificity of ADA activity was 71.4% for pleural TB, ADA activity had the highest NPV (83.3%).

These result agreed with **Greco et al** who found that measurement of ADA

activity was the single most sensitive method (88.1%) for the diagnosis of pleural TB. The specificity of ADA activity was 85.7% for confirmed and probable pleural TB combined, a value that is inferior to but approximates that of culturing, pleural biopsy, and PCR. Nevertheless, ADA activity had the highest NPV (88.2%), which remained high over a range of prevalence from 0.01 to 0.5[9].

In a study done by **Trajman** using histopathology as the reference standard, pleural fluid ADA activity, PCR and immunoglobulin (Ig)A-ELISA tests were evaluated in a high TB incidence country among 77 patients with pleural effusion, 60 of whom had TB pleuritis[10].

ADA activity was the only test that had a significantly higher sensitivity than histopathological examination[11].

In our work the level of IFN- γ was measured in the pleural fluid , we found that ROC curve analysis of Interferon values for the diagnosis of TPE showed that the optimal cut-off point was determined at 0.25. The likelihood ratios (LRs) were 1.88 and 0.20 for values above or below this cut-off point, in order The sensitivity of Interferon was (84.2%), specificity was (57.1%) and the diagnostic accuracy was 70%.

This result was agreed with **Ogawa** using 2.5 IU/ml as cutoff point, had a sensitivity of 44% and specificity of 60%[12].

In astudy done by **Kaled et al**, using cutoff point 6.5 IU/ml , the sensitivity was 89%, specificity 0f 83 %, accuracy 86%[13].

We reviewed the literatures and found a difference in the diagnostic capacity of the pleural IFN gamma between different study, this might be due to the different cutoff point level were used and also may be due to inter-laboratory variability.

There have been many reports on the usefulness of ADA activity and IFN- γ level in the diagnosis of pleural TB. These reports have shown that the range of sensitivity and specificity of ADA activity are 69–100% and 72–100%, respectively, , whereas the sensitivity and specificity of the IFN- γ level are 80–100% and 90–100%, respectively[14].

Sharma study comparing the cost-effectiveness of performing interferon (IFN)- γ estimation in comparison to adenosine deaminase (ADA) for pleural effusion found that even though it was more sensitive, the cost of using IFN- γ for detecting one additional patient was equivalent to the cost of complete TB treatment for six patients[14]

In our study we further compared the sensitivity, specificity and the area under the ROC curve among ADA and IFN-γ in tuberculous pleural effusion, and this comparison showed that ADA activity was 84 % in sensitivity, 71.4 % specificity, 77.5 % in accuracy and the IFN gamma activity was 84.2 % in sensitivity, 57.1 % specificity, 70 % in accuracy , we found that that the pleural fluid ADA and IFN-γ are useful indicators for diagnosis of tuberculous pleuritis.

Although studies[16]have shown IFN-γ levels to be more sensitive and specific than ADA, it is less preferred in resource-limited settings as it is more expensive and less readily available compared to .

However, further studies including larger numbers of patients are needed to verify this result. In addition, results of testing for these biological markers should be compared with polymerase

chain reaction findings in pleural fluids.

In conclusion, IGRAs, although potentially useful tools for diagnosing extrapulmonary TB, are still not suitable for high- TB burden, low-resource countries. A possible practical solution would be to use ADA measurement as the test of choice at the community level and IFN- γ in tertiary referral institutions.

. Further studies including larger numbers of patients should be undertaken confirm this result.

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