Pleural Fluid Interferon- γ and Adenosine Deaminase Levels in Tuberculosis Pleural Effusion: A Cost-Effectiveness Analysis

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Pleural fluid levels of interferon- γ (IFN- γ) and adenosine deaminase (ADA) have been found to be high in patients with tuberculosis (TB). The present study was carried out to compare the diagnostic utility of these two markers and to carry out a cost-effectiveness analysis of performing IFN- γ estimation in comparison to ADA. A total of 52 patients with pleural effusion, 35 of which were found to have TB etiology, were prospectively included for estimation of ADA and IFN- γ levels. The difference in the cost of performing the two diagnostic tests was compared with the cost of the treatment for a patient with TB. Pleural fluid IFN- γ (median [range]: 2,100 [70–14,000]

vs. 3 [0–160]; $P < 0.001$) as well as ADA levels (mean [SD]: 93.1 [62.3] vs 15.4 [8.7]; $P < 0.001$) were significantly higher in patients with TB effusion. Even though IFN- γ estimation was more sensitive (97.1 vs. 91.4%), the extra cost of IFN- γ estimation for detecting one patient with TB was found to be equivalent to the cost of a complete course of antituberculosis treatment for six patients. In developing countries, where TB is rampant and cost is a major concern, pleural fluid IFN- γ estimation does not seem to be a cost-effective investigation method for differentiating TB from non-TB pleural effusion. *J. Clin. Lab. Anal.* 19:40–46, 2005. © 2005 Wiley-Liss, Inc.

Key words: biological markers; cytokine; diagnosis; extrapulmonary tuberculosis; paucibacillary tuberculosis

INTRODUCTION

Tuberculosis (TB) is responsible for more deaths than any other infection worldwide (1). Almost 95% of all cases of TB are present in the developing world (2). Involvement of the pleural cavity is the second most common form of extrapulmonary TB after TB lymphadenitis (3). Since TB is a paucibacillary disease, the diagnostic yield of standard modalities like pleural fluid examination or smear for *M. tuberculosis* as well as culture is low (4,5). Pleural biopsy scores higher than these tests in terms of sensitivity (6), but it is an invasive procedure and may not be feasible in all patients. Under such circumstances, the need for a noninvasive diagnostic test with a better diagnostic yield has been felt for a long time.

Attempts have been made to utilize estimation of various biological markers in pleural fluid for the diagnosis of TB. Levels of adenosine deaminase (ADA) and interferon- γ (IFN- γ) in pleural fluid of patients with TB have been extensively investigated. ADA is an enzyme involved in the breakdown of adenosine to inosine during proliferation of macrophages (7). ADA levels were reported to be elevated in

patients with TB pleural effusion in 1978 (8), after which several studies have established the diagnostic utility of ADA in TB pleural effusion (9–17). On the other hand, IFN- γ is a product of activated T lymphocytes that play a central role in mediating immune response against *M. tuberculosis*. Shimokata et al. (18) were the first to report that there is an accumulation of activated helper T cells in the pleural fluid that reacted with purified protein derivative by producing large amounts of IFN- γ . Subsequently, Ribera et al. (19) showed that levels of pleural fluid IFN- γ are significantly higher in patients

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with TB as compared to other causes of pleural effusion. These results were followed by various studies evaluating the role of IFN- γ in the diagnosis of TB pleural effusion (20–23). Many studies have also been carried out to directly compare the diagnostic utility of these two markers, and the majority of these studies have found IFN- γ to be superior to ADA (24–28) (Table 1). But the difference in the diagnostic capability of these two markers is marginal in most of the studies. It is also well established that IFN- γ estimation requires greater expertise and the cost of performing IFN- γ is much higher than the cost of ADA estimation.

These findings raise the question of the cost-effectiveness of performing a costly investigation like IFN- γ estimation in patients with a common clinical problem such as pleural effusion. The present study was designed to critically compare the diagnostic utility of ADA with that of IFN- γ in patients with TB pleural effusion and to evaluate the cost-effectiveness of performing IFN- γ estimation in comparison to ADA for patients with TB pleural effusion.

MATERIALS AND METHODS

The Institutional Ethics Committee approved the study protocol. All patients presenting to the medical outpatient department as well as those admitted to the medical wards during the period January–July 2003 were eligible for inclusion in the study. A total of 68 patients with evidence of pleural effusion on chest radiographs were screened. Patients on corticosteroids and/or immunosuppressive agents, patients having evidence of any major organ system dysfunction such as renal or hepatic disease, and all pregnant females were excluded. This left us with a study cohort of 52 patients.

Written informed consent was obtained from each study subject. Each patient was evaluated in detail by

eliciting relevant clinical history and performing pertinent physical examination. Anthropometric data were recorded in all patients. All the measurements were recorded in triplicate and the mean of the readings was taken. In addition, a complete hemogram, serum chemistry, and sputum examination for *M. tuberculosis* was done. Serology for human immunodeficiency virus (HIV) was done in all patients, as described previously (29). Each patient was subjected to thoracentesis. Pleural fluid was analyzed for total protein, albumin, and cytology. A smear for acid-fast bacilli (AFB) and a culture for *M. tuberculosis* were also done. Whenever lesser invasive means failed to yield the diagnosis, patients were subjected to pleural biopsy. In addition, pleural fluid was analyzed for IFN- γ levels using the enzyme-linked immunosorbent assay (ELISA) technique. The procedure was carried out as per instructions of the manufacturers (Predicta Human Cytokine ELISA plates; Genzyme Diagnostics, Cambridge, MA). Solid phase enzyme immunoassay employing the multiple antibody sandwich principle was employed. The detailed methodology has been described previously (30). ADA estimation was done by a spectrophotometric method described by Guisti (31). Bronchoalveolar lavage (BAL) was also performed as described earlier (30), in patients showing the presence of pulmonary parenchymal lesion but a negative smear for *M. tuberculosis*. Whenever present, enlarged peripheral lymph nodes were biopsied. Ultrasonography and computed tomography (CT) of the chest, wherever indicated, were done to establish the etiology of pleural effusion.

The diagnosis of TB pleural effusion was based on the presence of one or more of the following criteria: 1) sputum, BAL fluid, or pleural fluid examination positive for *M. tuberculosis* by Ziehl-Neelsen staining; 2) positive culture for *M. tuberculosis* (grown on Lowenstein-Jensen medium) from sputum, BAL fluid, pleural fluid,

TABLE 1. Comparison of diagnostic performance of IFN- γ and ADA estimation for the diagnosis of tuberculosis pleural effusion

	IFN- γ			ADA		
	Sensitivity	Specificity	AUC	Sensitivity	Specificity	AUC
Sharma et al. (17) (n = 75)	—	—	—	83.3%	66.6%	—
Ogawa et al. (22) (n = 50)	94%	100%	—	—	—	—
Wongtim et al. (23) (n = 66)	94.9%	96.3%	—	—	—	—
Aoki et al. (24) (n = 39)	100%	100%	—	81.80%	89.3%	—
Villena et al. (25) (n = 388)	85.7%	97.1%	—	88.1%	85.7%	—
Naito et al. (26) (n = 101)	96.2%	98.5%	0.993	88%	95.9%	0.968
Yamada et al. (27) (n = 70)	94.1%	100%	0.987	85%	91.1%	0.892
Burgess et al. (28) (n = 110)	100%	100%	—	94%	68%	—
Present study (n = 52)	97.1%	100%	0.997	91.4%	100%	0.963

IFN- γ interferon gamma; ADA, adenosine deaminase; AUC, area under the curve, as estimated by construction of receiver operator characteristics curve; n, number of patients.

or pleural biopsy specimen; and 3) histological evidence of caseating granuloma on pleural biopsy and/or enlarged lymph nodes with positive staining for *M. tuberculosis*. Patients with TB were followed up to evaluate the response to antituberculosis treatment (ATT). All patients showed clinical as well as radiological response to ATT.

All patients with effusion of non-TB etiology were diagnosed on the basis of histological/cytological evidence procured from any of the following sites using various techniques: 1) cytology for malignant cells in pleural fluid; 2) peripheral lymphadenopathy (fine needle aspiration and/or excision biopsy); 3) mediastinal adenopathy (mediastinoscopic biopsy); 4) lung mass (US- or CT-guided transthoracic fine-needle aspiration or tru-cut biopsy/video assisted thorascopic biopsy/open lung biopsy); and 5) liver biopsy.

For the cost-effectiveness analysis, the cost of performing the two tests was initially determined. It was determined that the difference in cost of the two tests would be affected by multiple factors. These included the cost of commercially available kits used for performing IFN- γ and ADA estimation, the type of equipment used for the estimation, and the level of expertise required. Estimation of ADA requires a simple spectrophotometer, which is widely available. On the other hand, estimation of IFN- γ levels requires an ELISA reader and greater technical expertise. Although all these factors would increase the cost of IFN- γ estimation in comparison to ADA, the difference in cost would vary in different settings. This is especially true for the cost of technical personnel required to perform IFN- γ estimation. Therefore, for the sake of simplicity and to permit reproducibility, only the difference in the cost of commercial kits used for performing IFN- γ and ADA estimation was taken into consideration for the present analysis. Subsequently, the number of patients left undetected by ADA estimation in comparison to IFN- γ estimation (extra false-negatives) was determined. From this, the number of IFN- γ estimations required to diagnose one patient falsely-labeled negative on the basis of ADA estimation was deduced. We could then derive the cost for detecting one ADA-negative patient by way of IFN- γ estimation. Finally, this cost was compared with the cost of a full course (six months) of ATT, estimated to cost around rupees (Rs) 2000 in India (an intensive phase consisting of rifampicin, isoniazid, pyrazinamide, and ethambutol for two months and a continuation phase consisting of rifampicin and isoniazid for four months).

Statistical Analysis

All the recorded data were entered into the computer and analyzed using STATA version 7.0 statistical

software (intercooled version, STATA Corporation, Houston, TX). All the entries were double-checked to minimize data entry error. Data were described using mean with standard deviation (SD) except for IFN- γ levels, as they were not distributed normally. The study group was divided into TB and non-TB groups on the basis of etiology of pleural effusion. Independent *t*-test was used to compare the means of continuous variables, whereas Fisher's exact test was applied for ordinal variables. The Wilcoxon rank sum test was used to compare the median values of pleural fluid IFN- γ levels. Sensitivity and 1-specificity were plotted for various cutoff levels for pleural fluid IFN- γ and ADA to construct the receiver-operator characteristics (ROC) curve. The area under the ROC curve (AUC) was estimated to compare the diagnostic utility of IFN- γ levels with ADA for the diagnosis of TB pleural effusion. Statistical significance was considered at $P < 0.05$.

RESULTS

TB etiology was confirmed in 35 out of 52 patients (67%) with pleural effusion (TB group). Diagnostic modalities used for confirmation of TB in these cases have been summarized in Table 2. The rest of the 17 patients (non-TB group) were patients with effusion secondary to carcinoma lung ($n = 15$) and non-Hodgkin's lymphoma ($n = 2$). All patients were seronegative for HIV.

Table 3 compares clinical and demographic profiles as well as pleural fluid IFN- γ and ADA levels for the two groups. Patients with TB pleural effusion were significantly younger, but there was no statistically significant difference in male to female ratio between the two groups. A highly significant difference in the IFN- γ levels was found between the two groups ($P < 0.001$).

TABLE 2. Various diagnostic modalities used to confirm the diagnosis of tuberculosis ($n = 35$)*

Diagnostic modality	Number of patients (%)
Sputum smear examination for AFB	9 (25.7%)
Smear for AFB in BAL fluid	5 (14.5%)
Culture of sputum for <i>M. tuberculosis</i>	4 (11.4%)
Culture of BAL fluid for <i>M. tuberculosis</i>	3 (8.6%)
Smear for AFB in pleural fluid	2 (5.7%)
Histological evidence of TB in pleural biopsy specimen	5 (14.3%)
Histological evidence of TB in cervical lymph nodes biopsy specimen	7 (20%)

AFB, acid-fast bacilli; BAL, broncho-alveolar lavage; TB, tuberculosis.

*Percentages in parentheses are calculated by taking denominator as the number of patients diagnosed as TB ($n = 35$).

TABLE 3. Characteristics of patients with TB and non-TB pleural effusion

Parameter	TB group mean $\pm$ SD (n = 35)	Non-TB group (n = 17) mean $\pm$ SD
Age (years)	28.5 $\pm$ 9.2	49.6 $\pm$ 13.1**
Sex (male/female)	25/10	12/5
Body mass index (kg/m ²)	18.6 $\pm$ 3.1	20.8 $\pm$ 4.1
Mid-arm circumference (cm)	22.7 $\pm$ 2.8	21.8 $\pm$ 3.6
Triceps skin-fold thickness (mm)	9.6 $\pm$ 3.1	8.9 $\pm$ 3.7
Absolute lymphocyte count (per cmm)	2430 $\pm$ 655	2560 $\pm$ 617
Serum protein (g/dl)	7 $\pm$ 1.2	5.6 $\pm$ 2.7
Serum albumin (g/dl)	3.6 $\pm$ 0.8	2.9 $\pm$ 1.4
Mantoux test (> 10 mm) ^a	18/35	1/17
Pleural fluid IFN- γ (pg/ml) ^b	2100 (70–14000)	3(0–160)**
Pleural fluid ADA (IU/L)	93.1 $\pm$ 62.3	15.4 $\pm$ 8.7**

^aMantoux test was performed with five tuberculin units (TU) of purified protein derivative (PPD).

^bData provided as median with range.

* $P < 0.05$; ** $P < 0.001$.

Fig. 1. Receiver-operator characteristic curve (ROC) plotted for pleural fluid interferon- γ (IFN- γ) and adenosine deaminase (ADA) to evaluate their utility in diagnosis of TB pleural effusion. Best cutoff was taken as the value of IFN- γ as well as ADA at the point where the curve sharply angulated (arrows).

Pleural fluid ADA levels were also significantly different for the two groups ($P < 0.001$).

ROC curves were plotted to compare the diagnostic efficacy of IFN- γ and ADA levels (Fig. 1). Both IFN- γ and ADA had high AUC values, although IFN- γ was marginally superior to ADA (IFN- γ : 0.997; 95% confidence interval [CI]: 0.988–1.006; and ADA: 0.963; 95% CI: 0.915–1.011). The best cutoff was taken at the point where the curve sharply angulated. It was found to be 167.5 pg/mL for IFN- γ whereas it was 33 IU/L for ADA. For the best cutoff of IFN- γ , the sensitivity for diagnosis of TB pleural effusion was 97.1%, whereas the negative predictive value was 94.4%. Specificity and positive predictive value were both 100%. On the other hand, the choice of the cutoff for ADA resulted in a

TABLE 4. Descriptive profile of diagnostic utility of ADA and IFN- γ estimation

	ADA ^a	IFN- γ ^b
True negative	17	17
True positive	32	34
False negative	3	1
False positive	0	0
Sensitivity	0.914	0.971
Specificity	1	1

^aADA, adenosine deaminase; Cut-off used, 33 IU/L.

^bIFN- γ , interferon gamma; Cut-off used, 167.5 pg/mL.

slightly lower sensitivity (91.4%) and negative predictive value (85%), but the specificity and positive predictive value were 100%. Difference in the sensitivity between the two markers resulted because of two extra false-negatives with ADA (Table 4). Only 1 out of 18 patients (5.5%) had IFN- γ levels below the threshold value, but that patient turned out to have TB. All of the remaining 17 patients with low IFN- γ levels did not have TB. Among the patients with low ADA, 3 patients out of 20 (15%) turned out to have TB. All patients with raised ADA as well as IFN- γ levels (above the suggested cutoff) had TB (there were no false-positives).

For 35 patients with proven TB, ADA missed diagnosis in two patients (5.7%), more than what were missed by IFN- γ estimation. That is, for every 100 patients with TB, IFN- γ estimation results in the detection of 5.7 patients that are missed by ADA estimation. Hence, 17.5 IFN- γ estimations need to be performed to diagnose one patient falsely-labeled negative on the basis of the ADA estimation.

The cost for each ADA estimation kit was found to be Rs 0.25 (less than 1 cent in U.S. currency),

whereas that for IFN- γ estimation was Rs 750 (U.S. dollars [USD] 16). This means that for detecting one case of ADA-negative TB by using IFN- γ estimation, one needs to spend nearly Rs 13,125 (USD 280.00; 16×17.5). Thus, the cost of detecting one patient with TB by IFN- γ estimation is equivalent to the cost of completing a course of ATT for more than six patients (average cost of six months of ATT is nearly Rs 2,000 [USD 42.5]).

DISCUSSION

Developing nations bear the major share of the global burden of TB (2). The HIV epidemic has resulted in a resurgence of TB and the failure of effective TB control programs in many countries. In a recent study on the interaction of HIV and TB (32), it was found that 9% of all new cases of TB were attributable to HIV. This proportion was extremely high in certain countries like the United States (26%). Since the extrapulmonary form of TB (including pleural effusion) is more common in patients with HIV, the proportion of TB patients who present with pleural effusion is also increasing. Multidrug-resistant strains are emerging and treatment failures are being seen more commonly. These developments have resulted in a marked increase in the costs associated with the management of this disease. In such a scenario, it is important that all newer diagnostic as well as therapeutic strategies that are envisaged for TB are scrutinized for cost effectiveness.

Before the findings of the present study are discussed, it is essential to highlight certain aspects about the cost-effectiveness analysis. There are three components of any cost-effectiveness analysis. The first two components are comprised of the cost itself and the outcome. The unit of measurement for these two parameters cannot be similar. An example of this concept is that the money spent (cost) could be calculated with reference to the number of lives saved (outcome). The third component of a cost-effectiveness analysis is the comparison with another intervention, or at times no intervention. This helps to establish if the extra cost of a particular intervention is worth the expenditure. It also tells us what else might be bought with the same money (33,34). A cost-effectiveness analysis should not be seen only as a means of reducing the cost of management of a particular disease. It is in fact a tool to identify and propose the most appropriate use of available funds.

In the present study, the cost of doing the two diagnostic tests (ADA and IFN- γ) and the diagnosis of TB have been taken as the first two components of the

cost-effectiveness analysis. It was shown that for detecting one patient of ADA-negative TB pleural effusion by means of IFN- γ estimation, an expenditure of nearly Rs 13,125 (USD 280) is required. It is essential to compare this extra cost with another intervention, and it was felt that the most appropriate yardstick to make such a comparison would be the cost of treatment itself.

It is essential to highlight certain shortcomings of the present study. In spite of an attempt to include consecutive patients, the control group (non-TB effusion) consisted only of patients with malignant effusion. Because of this unavoidable bias, the results cannot be generalized for all patients with pleural effusion. This is especially true since some important causes of potential false-positives with either test, such as empyema or connective tissue disorders (like rheumatoid arthritis) were not present in the control group. Nonetheless, when the issue is differentiation between tubercular and malignant etiology (a common diagnostic dilemma with most of the long-standing effusions), the cost-effectiveness of performing pleural fluid IFN- γ estimation becomes questionable. This is especially true for resource-limited settings that exist in most of the developing nations, where pleural effusion is a common problem and many of those patients are likely to have TB.

It is important to acknowledge another pertinent viewpoint for the present cost-effectiveness analysis. Although in comparison to ADA, IFN- γ estimation appeared to be a costly diagnostic investigation, ADA estimation may also result in some increase in costs associated with the management of TB. Some of the patients missed by ADA estimation (higher false-negatives) may infect others and, in the long-term, tend to increase the cost of therapy. Hence, it is essential to emphasize that in certain situations, IFN- γ estimation, by minimizing false-negatives, may, in fact, reduce the cost of therapy to some extent. Although it is almost impossible to make an assumption of this cost, it surely would not be able to compensate for the almost 3,000-fold difference in the cost of the two tests.

In spite of the deficiencies, the study highlights an important cost related issue and brings in to perspective the huge cost difference between the two diagnostic tests. Wherever a decision of carrying out IFN- γ level estimation is made, it must be kept in mind that the cost of detecting one case of ADA-negative TB effusion is equivalent to a complete course of ATT for six patients. This is especially important while formulating public health strategies for TB control programs in developing nations. It is important to stress that these strategies are not intended for application at an

individual level. On the other hand, the situation in the developed world demands a different approach. Since the incidence of TB is still low, IFN- γ estimation may be a useful modality for conclusively excluding TB etiology in cases in which the results of other tests are nonconclusive.

It is concluded that in spite of the excellent diagnostic utility of IFN- γ , the superiority over ADA estimation for differentiating TB pleural effusion from malignant effusion remains marginal. This superiority cannot justify the routine and widespread use of IFN- γ estimation, especially in developing countries where the disease is rampant and cost is a major concern. ADA estimation is a far more cost-effective diagnostic modality for differentiating TB pleural effusion from malignant pleural effusion.

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