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ORIGINAL ARTICLE

# Differential diagnosis of tuberculous and malignant pleurisy using pleural fluid adenosine deaminase and interferon gamma in Taiwan

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Accurately differentiating tuberculous pleurisy from lung cancer is important for disease management but difficult using conventional laboratory methods. This study assessed the value of adenosine deaminase (ADA) and interferon gamma (IFN-$\gamma$) for differentiating the two conditions in a region of Taiwan with a high prevalence of tuberculosis. The study population comprised patients with lymphocytic exudative pleural effusions: tuberculous ($n = 24$) and malignant ($n = 42$). Mean levels of ADA and IFN-$\gamma$ in pleural fluid, measured with commercial standardized kits, were significantly higher for tuberculous than for malignant pleurisy ($p < 0.001$ for both). For differentiating the two effusions, results for ADA versus IFN-$\gamma$ were: sensitivity, 70.8% versus 91.7%; specificity, 95.2% versus 97.6%; positive predictive value, 89.5% versus 96.7%; and negative predictive value, 85.1% versus 95.3%. IFN-$\gamma$ allows precise diagnosis of pleural tuberculosis, but ADA is easier to use, has a low cost, and results are quickly available. Our study confirms previous studies and extends the usefulness of these diagnostic methods to a wider group of clinical laboratories by showing the

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reliability of standardized relatively inexpensive commercial kits. We recommend that initial ADA screening be used in conjunction with IFN-γ measurements for differential diagnosis of tuberculous pleurisy.

## Introduction

There are many causes of pleural effusion, and an effusion caused by tuberculous pleurisy may be difficult to distinguish from one caused by some form of cancer. A rapid precise diagnosis is important as the prognosis and therapy for these two diseases are markedly different. Unlike some other studies, we restricted our analysis to these two groups to mimic the common clinical situation. A definitive diagnosis of tuberculous pleural effusion can be difficult because of nonspecific clinical presentation and the relatively poor efficiency of traditional diagnostic methods. In clinical practice, the predictive value is an important measure of diagnostic validity. The positive predictive value (PPV) is the probability of the presence of a disease, given a positive test result. Likewise, the negative predictive value (NPV) is the probability of absence of a disease, given a negative test result.

Detection of *Mycobacterium tuberculosis* using primary culture or the polymerase chain reaction usually allows definitive diagnosis, although the positive rate of pleural fluid culture for *M tuberculosis* is low in tuberculous pleurisy.[1,2] Because of the long culture period, clinical and therapeutic decisions often have to be made before culture results become available. The sensitivities of pleural fluid culture, pleural biopsy culture, and histological examination of a pleural biopsy sample, are reported to be on the order of 23%, 55%, and 63%, respectively.[3] Another method, acid-fast staining of pleural effusion fluid, has the advantage of being rapid and inexpensive but lacks sensitivity and often produces negative results even in patients with a confirmed diagnosis of tuberculous pleurisy.

The inefficiency of conventional laboratory methods has resulted in the development and evaluation of alternative diagnostic strategies. In recent years, both adenosine deaminase (ADA) activity and interferon gamma (IFN-γ) concentrations have been reported as useful diagnostic markers of tuberculous pleurisy.[4–12] However, one limitation of predictive values is their dependence on disease prevalence.[13] If the sensitivity and specificity of a diagnostic test were constant, then the PPV would increase with disease prevalence, whereas the NPV would decrease with disease prevalence. Thus, the prevalence of tuberculosis (TB) has a strong impact on the predictive values of clinical diagnostic tests. The value of ADA in the diagnosis of tuberculous pleurisy in a country with a high burden of TB, such as Taiwan, has not been reported previously.

A previous prospective study showed that using ADA in combination with the lymphocyte/neutrophil (L:N) ratio increased the specificity of tuberculous pleurisy diagnosis.[4]

In this report, the study population was restricted to one of the most diagnostically challenging categories of pleural effusions, namely, patients with lymphocyte-predominant exudative pleural effusions. The goal of the present study was to evaluate the diagnostic value of using rapid commercial ADA and IFN-γ quantification kits for differentiating between tuberculous pleurisy and malignant pleurisy in patients with lymphocytic exudative pleural effusions. Use of such kits would make these tests available to a wider range of clinical laboratories. We evaluated the sensitivity, specificity, and PPVs and NPVs of the ADA and IFN-γ tests in this setting and provided recommendations for routine clinical practice.

## Materials and methods

### Patients

This study was approved by the Institutional Review Board of Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan. Informed consent was obtained from patients who participated in the study. Pleural effusions were collected from consecutive patients who were admitted to Kaohsiung Veterans General Hospital between July 2006 and January 2007. Samples from consecutive patients ($n = 177$) with lymphocytic exudative pleural effusions were screened for inclusion in this study. Patients with exudative effusion and lymphocyte counts less than 50% were excluded from further analyses ($n = 54$). Among the remaining 123 patients, patients with TB and malignancies were eligible for further analysis. Clinical signs and symptoms, demographic data, and radiologic results were recorded. Results of ADA and IFN-γ tests were blinded to the physicians to avoid bias in determination of patient clinical diagnosis.

### Diagnostic criteria

Patients were included in the study if they had exudative pleural effusions with lymphocyte counts of 50% or more. Pleural effusions were classified as exudates if the pleural fluid-to-serum ratio of total protein was 0.5 or more, the pleural fluid level of absolute lactic dehydrogenase (LDH) was 200 IU/L or more, or the pleural LDH fluid-to-serum ratio was 0.6 or more.[1] A diagnosis of pleural TB was made if patients had one of the following clinical manifestations suggestive of TB: *M tuberculosis* isolated from pleural fluid or pleural tissue, granulomas in the pleural tissue that stained positive for acid-fast bacilli (AFB), or granulomas in the pleural tissue that did not stain positive for AFB but did show a response following antituberculous treatment. The diagnosis of malignant pleurisy was made

if malignant cells were found in pleural fluid by cytological study, or if histopathology examination found malignant cells in a biopsy sample of pleural tissue. Causes of pleural effusion were determined using established clinical criteria.[1,14,15]

## Specimen collection and processing

At least 40 mL of pleural fluid was collected from each patient during thoracentesis using a sterile syringe. Aliquots of each sample were submitted for AFB staining; bacteriological examination; cytological examination; and measurements of protein, albumin, total bilirubin, LDH, and glucose concentrations. A separate aliquot was centrifuged at 2,000 revolutions per minute for 10 minutes and the supernatant was frozen at $-80°C$ for further analyses within 2 months.

## ADA activity

ADA activity was determined at $37°C$ using a commercial kit (Adenosine Deaminase Assay Kit; Diazyme Laboratories, San Diego, CA, USA) based on the Berthelot reaction according to the method described by Giusti and Galanti.[16] Briefly, a colored indophenol complex formed from ammonia liberated from adenosine was quantified spectrophotometrically. One unit of ADA was defined as the amount of enzyme required to release 1 μmol of ammonia/min from adenosine under standard assay conditions. Results are expressed in international units of enzyme activity in a standard volume of pleural fluid.

## IFN-γ levels in pleural fluid

IFN-γ levels were measured using a commercial Cytometric Bead Array (CBA) kit (Becton Dickinson, San Diego, CA, USA) according to the manufacturer's instructions. Briefly, dyed microparticles with a maximal emission wavelength of approximately 650 nm fluorescence-3 (FL-3) were coated with antibodies specific to human IFN-γ (Ab-bead reagent). IFN-γ was detected directly, using the antibody fused with phycoerythrin (PE), which emits fluorescence at 585 nm (FL-2). The intensity of FL-2 was proportional to the cytokine concentration in the sample, and the results were derived from a calibration curve. For each pleural fluid sample and cytokine standard mixture, 50 μL of sample or standard was added to a mixture of 50 μL each of capture Ab-bead reagent and detector Ab-PE antibody reagent. The mixture (150 μL) was incubated for 2 hours at room temperature to form sandwich complexes and washed to remove unbound detector Ab-PE antibody before data acquisition using flow cytometry.

Two-color cytometric analysis used a FACSCalibur flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA). Data were analyzed using CBA software. Dot-plot forward versus side scatter was used to distinguish between particles and to detect cytokine particles. Human IFN-γ concentrations were determined from standard curves (cytokine calibrator concentration vs. FL-2 median fluorescence intensity) using a four-parameter logistic curve-fitting model.

## Culture and identification

Mycobacterium culture was performed by inoculation of individual specimen samples into Lowenstein-Jensen medium and into one mycobacteria growth indicator tube and tested using a mycobacteria growth indicator tube 960 instrument (Becton Dickinson Diagnostic Systems, Sparks, MD, USA). Positive growth was identified using the BDProbeTEC ET CTB assay (Becton Dickinson Microbiology Systems, Maryland, MD, USA).

## Statistical analyses

Results are given as the mean ± standard deviation for normally distributed, continuous variables, or as the median (interquartile range) for non-normally distributed variables. Results for categorical variables are given as percentages (%). Normally distributed continuous variables were compared using an independent, two-sample $t$ test. A Mann-Whitney $U$ test was used for non-normally distributed variables. Categorical variables were compared by a $\chi^2$ test. The optimal cutoff values for ADA and IFN-γ in the diagnosis of tuberculous pleurisy were determined using receiver operator characteristic curves. The McNemar test was used to test for significant differences between two diagnoses of tuberculous pleurisy. All statistical assessments were two sided, and analyses used SPSS 15.0 statistical software (SPSS Inc., Chicago, IL, USA). Significance was accepted for $p$ values less than 0.05.

## Results

From an initial population of 123 patients with lymphocytic exudative pleural effusions, samples from 24 patients (36.4%) with tuberculous pleurisy and 42 patients (63.6%) with malignant pleural effusions were subjected to further analyses. Baseline demographic characteristics and pleural effusion biochemistry findings are shown in Table 1. Patients with malignant pleural effusions were significantly older than those with tuberculous pleural effusions ($70.4 \pm 12.50$ years vs. $61.0 \pm 18.96$ years, $p = 0.037$). There were no statistically significant differences between these two groups for effusion concentrations of protein, glucose, or LDH.

Importantly, as shown in Table 2, ADA levels and IFN-γ concentrations were significantly higher in patients with tuberculous pleurisy than in patients with malignant pleurisy ($p < 0.001$ for both). Among patients with tuberculous pleurisy, IFN-γ concentrations were significantly higher in patients with positive cultures than in patients with negative cultures (929 pg/mL vs. 280 pg/mL, $p = 0.012$). There was no significant difference for ADA levels from patients who had positive or negative cultures. In addition, there was a significant difference in IFN-γ between culture-negative TB and malignant effusions (279 pg/mL vs. 3.49 pg/mL, $p < 0.001$). No significant difference for ADA levels between culture-negative TB and malignant effusion was found.

Figure 1 shows the receiver operator characteristic curves for the diagnostic parameters of ADA and IFN-γ. The area under the curve for ADA was 0.76 ($p < 0.001$, 95% confidence interval: 0.612–0.909), whereas the area under

**Table 1** Demographics and biochemistry findings from pleural fluid for patients with tuberculous and malignant pleurisy (n = 66)[a]

| Variable | Tuberculous pleurisy (n = 24) | | | Malignant pleurisy (n = 42) | | |
|---|---|---|---|---|---|---|
| | Total (n = 24) | Culture positive (n = 13) | Culture negative (n = 11) | Total (n = 42) | Lung cancer (n = 32) | Other malignancy (n = 10) |
| Age (yr) | 61.00 ± 18.96[b] | 60.31 ± 20.81 | 61.80 ± 17.49 | 70.40 ± 12.5[b] | 72.06 ± 11.50 | 65.10 ± 14.67 |
| Sex, male | 18 (75.0) | 8 (72.7) | 10 (76.9) | 28 (66.7) | 23 (71.9) | 5 (50.0) |
| Protein (g/dL) | 4.50 ± 1.00 | 4.65 ± 1.09 | 4.35 ± 0.92 | 4.28 ± 0.98 | 4.35 ± 0.87 | 4.07 ± 1.26 |
| LDH (IU/L) | 368 (232–653) | 322 (192–653) | 393 (281.5–783) | 282.5 (186–551) | 282.5 (186–441) | 382 (213–649) |
| Glucose (mg/dL) | 95.5 (69–130) | 118 (73–155) | 83 (60–115) | 113 (96–128) | 106 (93–141) | 118.5 (100–127) |

[a] Data are presented as mean ± standard deviation, n(%) or median (interquartile range).
[b] Significant difference between tuberculous and malignant groups, $p < 0.05$.
LDH = lactic dehydrogenase.

**Table 2** Mean IFN-γ and ADA levels for patients with tuberculous and malignant pleurisy (n = 66)[a]

| Parameter | Tuberculous pleurisy (n = 24) | | | Malignant pleurisy (n = 42) | | |
|---|---|---|---|---|---|---|
| | Total (n = 24) | Culture positive (n = 13) | Culture negative (n = 11) | Total (n = 42) | Lung cancer (n = 32) | Other malignancy (n = 10) |
| ADA (IU/L) | 42.76 (17.19–64.34)[b] | 44.43 (39.82–63.71) | 19.70 (10.06–64.97) | 16.35 (12.99–21.38)[b] | 16.35 (12.16–21.38) | 17.82 (14.26–23.05) |
| IFN-γ (pg/mL) | 580.61 (174.64–1,611.39)[b] | 929.02 (722.40–2,781.03)[c] | 279.55 (118.37–427.98)[c] | 3.49 (0.00–8.39)[b] | 3.41 (0.00–8.14) | 5.23 (0.00–16.07) |

[a] Data are presented as median (interquartile range).
[b] Significant difference between tuberculous and malignancy groups, $p < 0.05$.
[c] Indicates significant difference between culture positive and negative in tuberculous groups, $p < 0.05$.
The p values are based on Mann-Whitney U tests.
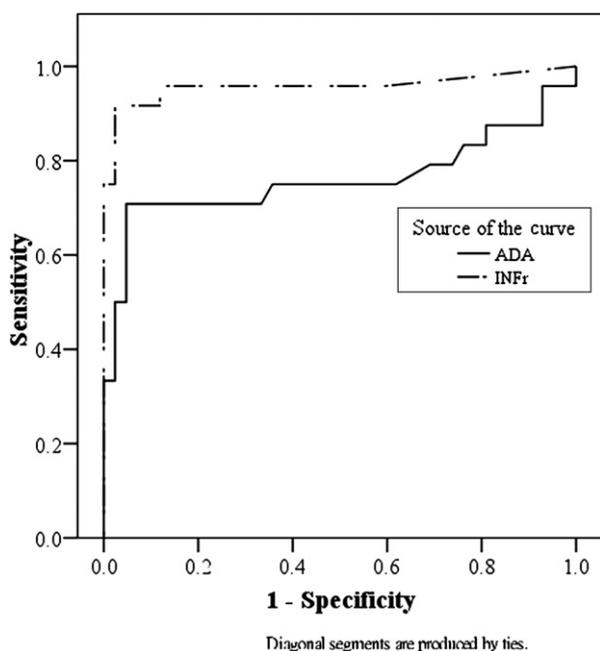ADA = adenosine deaminase; IFN-γ = interferon gamma.

**Figure 1.** Receiver operator characteristic curves for determining optimal ADA and IFN-γ cutoff values for diagnosing tuberculous pleurisy. ADA = adenosine deaminase; IFN-γ = interferon gamma.

the curve for IFN-γ was 0.96 ($p < 0.001$, 95% confidence interval: 0.892—1.024). The cutoff values determined to give the best diagnostic accuracy were 30 IU/L for ADA and 70 pg/mL for IFN-γ. The results for comparing ADA versus IFN-γ were: sensitivity, 70.8% versus 91.7%; specificity, 95.2% versus 97.6%; PPVs, 89.5% versus 97.5%; and NPVs, 85.1% versus 95.3% (Table 3). Based on the results of a McNemar test, there was no significant difference between ADA and IFN-γ as diagnostic parameters ($p = 0.289$).

## Discussion

A prompt accurate differential diagnosis of pleural exudates is vital for initiating treatment, particularly for cases with TB. A definitive diagnosis of tuberculous pleurisy is traditionally based on one or more of the following: identification of *M tuberculosis* in the pleural fluid or in a biopsy sample or from histopathological observation of granulomas in the pleural tissue. These conventional

methods generally have poor sensitivity and are time consuming because the cultures require time to grow. Although a biopsy sample of the pleura is more sensitive, it is also more invasive, which increases the possibility of associated morbidity and is subject to sampling error.[17]

Other methods, such as thoracoscopy[18] or polymerase chain reaction,[11,19] may enhance diagnostic sensitivity and specificity and may shorten the time to differential diagnosis. Yet, these procedures may be invasive or may be costly in terms of required equipment and trained personnel. Thus, these methods may not be readily available, particularly for resource-poor clinics or hospitals. An alternative is the examination of pleural exudates for biomarkers that can be reliable for differential diagnoses.

ADA activity is principally because of ADA produced by monocytes[20] and is indicative of a local, active, inflammatory response. IFN-γ, secreted by antigen-triggered CD4+ lymphocytes, is a key lymphokine that activates macrophages, increasing their bactericidal activity against *M tuberculosis*.[21] Methods, such as measurements of ADA activity and IFN-γ levels, have proven to be sensitive and specific for pleural TB in populations with a high prevalence of TB.[22—28]

The criteria for exudates were first proposed by Light et al.[5] and have become the accepted standard for accurate identification of exudative effusions, although the marker LDH has subsequently been demonstrated to have no role in differentiating between transudates and exudates.[29] In addition, a predominance of small lymphocytes in a pleural effusion is thought to indicate that the patient most likely has cancer or tuberculous pleuritis. The combined use of ADA activity and differential cell counts provides a more efficient means for diagnosing tuberculous pleurisy[27] than the use of ADA levels alone.[16,30]

Therefore, only exudates containing more than 50% lymphocytes were included in the present study. Previous studies have shown that more than 90% of TB effusions are lymphocytic, that is, with a lymphocyte count of more than 50%.[14] In our study, none of the 54 patients who had exudative pleural effusions and lymphocyte counts less than 50% were diagnosed as tuberculous pleurisy.

The present study demonstrates that ADA and IFN-γ concentrations in pleural fluid are useful tools for the differential diagnosis of tuberculous pleurisy. IFN-γ was a better diagnostic marker than ADA for tuberculous pleurisy, with a higher sensitivity and specificity and a PPV. A recent meta-analysis of the diagnostic value of ADA in

**Table 3**  Accuracy of ADA and IFN-γ cutoff values for the diagnosis of clinical tuberculous pleurisy (*n* = 66)

| Cutoff values | TB (*n* = 24) | Malignancy (*n* = 42) | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | AUC (95% CI) |
|---|---|---|---|---|---|---|---|
| ADA (IU/L) | | | | | | | |
| ≥30 | 17 | 2 | 70.8 | 95.2 | 89.5 | 85.1 | 0.76 (0.61—0.91) |
| <30 | 7 | 40 | | | | | |
| IFN-γ (pg/mL) | | | | | | | |
| ≥70 | 22 | 1 | 91.7 | 97.6 | 95.7 | 95.3 | 0.96 (0.89—1.02) |
| <70 | 2 | 41 | | | | | |

ADA = adenosine deaminase; AUC = area under the curve; CI = confidence interval; IFN-γ = interferon gamma; NPV = negative predictive value; PPV = positive predictive value; TB = tuberculosis.

tuberculous pleural effusion reported sensitivities ranging from 47.1% to 100%.[6] Interestingly, better ADA diagnostic performance was observed in studies conducted in Europe, whereas performance was poorer in studies from East Asia than from other regions.[6] A study from Hong Kong also showed lower ADA sensitivity for diagnosis of tuberculous pleurisy.[7] This suggests the possibility that population ethnicity has an influence on the diagnostic value of ADA.[31] Lower ADA levels among Asians may compromise its usefulness in TB detection in this population.[6]

Reported ADA and IFN-γ cutoff values for the diagnosis of tuberculous pleurisy vary greatly. This discrepancy can be attributed, in part, to the use of different methods. With the most frequently reported colorimetric ADA assay, described by Giusti and Galanti,[16] the reported cutoff value for ADA varies from 40 IU/L to 60 IU/L.[5,27,29,30,32-34] Our cutoff value of 30 IU/L was lower than this range, although this may be because of the ethnicity of our study population. The cutoff values for IFN-γ vary from 60 pg/mL to 240 pg/mL, based on an enzyme-linked immunosorbent assay.[35-37] The only report using the CBA method listed a cutoff of 100 pg/mL.[38]

In the present study, two commercial kits were used to determine ADA and IFN-γ. The main advantages of these kits are that they are standardized and can be incorporated easily into the routine workflow. In addition, specialized equipment and personnel training are not required. Our study using these kits not only confirms results reported previously by others but also shows further that similar diagnostic results can be obtained using commercial kits. Thus, many more clinical laboratories can expect reliable results even without sophisticated equipment or highly trained personnel. More general use of standardized test kits also can be reasonably expected to lead to more uniform cutoff values for improved comparability between laboratories and to help determine the effect of ethnicity.

ADA has the advantage of cost effectiveness, efficiency, noninvasiveness, and ease of use. This established marker has proven utility in distinguishing between tuberculous and nontuberculous respiratory disease.[39-41] IFN-γ concentrations in pleural effusions were determined with a CBA kit. When compared with conventional enzyme-linked immunosorbent assay methods, this microparticle-based flow cytometric immunoassay has been proven to have comparable analytical sensitivity.[38] In addition, CBA is a suite of products that has been optimized for ease of use and time efficiency.

In conclusion, the present study compared ADA and IFN-γ concentrations in pleural effusion fluids from patients with lymphocyte-predominant exudative pleural effusions. Although both markers were useful for distinguishing between tuberculous and malignant pleurisy, results indicate that the IFN-γ level was a better diagnostic marker of tuberculous pleurisy than ADA activity. When measurement of IFN-γ is not readily available because of cost or other reasons, ADA remains useful for initial differential diagnosis. Should diagnostic uncertainty remain, IFN-γ measurement should be considered. However, neither ADA nor IFN-γ can replace bacterial culture, which remains necessary for susceptibility testing to guide antituberculous therapy.

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