DIAGNOSTIC ROLE OF PLEURAL FLUID ADENOSINE DEAMINASE IN TUBERCULOUS PLEURAL EFFUSION

Wipa Reechaipichitkul[1], Theerasuk Kawamatawong[1], Yaovalak Teerajetgul[2], Boonsong Patjanasoontorn[1]

[1]Department of Medicine, Srinagarind Hospital, Faculty of Medicine; [2]Department of Clinical Chemistry, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand

Abstract. Between June 1998 and June 2000, 132 consecutive patients with symptomatic exudative lymphocytic pleural effusion were studied to evaluate the diagnostic role of pleural fluid adenosine deaminase (ADAPF) levels. The mean age was 52.2 (SD 16.3) years. The male to female ratio was 1.4:1. The analysis of ADAPF levels was measured base on Giusti's method. Tuberculous pleural effusion was diagnosed in 50 patients (37.9%). Another 59 patients (44.7%) had malignancies, 23 patients (17.4%) had miscellaneous other etiologies (including; 19 with chronic inflammations, 3 with melioidosis, and 1 with systemic lupus erythrematosus). The percentages of pleural fluid lymphocytes and pleural fluid protein in the tuberculous pleural effusion were similar to those with malignancies, but higher than those in the miscellaneous group. The mean value of ADAPF in the tuberculosis group was 93.2 (SD 56.5) U/l, which was significantly higher than for the malignancy and miscellaneous groups ($p<0.05$, one-way ANOVA). The mean values of ADAPF in the malignancy group were 36.7 (SD 39.2) U/l, and 31.3 (SD 23.4) U/l in miscellaneous group. Three patients were diagnosed with melioidosis and had ADAPF levels of 15, 46.9, and 49.8 U/l, respectively. One patient with systemic lupus erythrematosus had ADAPF levels of 24.1 U/l. A receiver operating characteristic (ROC) curve identified ADAPF level of 48 U/l as the best cut-off value, which in turn yielded a sensitivity of 80% (95% CI, 73 to 87%) and specificity of 80.5% (95% CI, 73.6 to 87.4%). The positive and negative predictive values at this cut-off value were 71.4% and 86.8%, respectively. The likelihood ratios for the diagnosis of tuberculous pleural effusion in patients with ADAPF levels less than 45 U/l were 1:4, between 45 and 100 U/l were 5:2, and greater than 100 U/l were 7:1. We concluded that ADAPF levels are a useful diagnostic test for tuberculous pleural effusion. In addition, The analyis of ADA levels can be done simply, quickly, and cheaply.

INTRODUCTION

The diagnosis of tuberculous pleural effusion is important because tuberculosis is normally a treatable cause of exudative lymphocytic pleural effusion (David *et al*, 1987; Ferrer, 1997). Other differential diagnosis of exudative lymphocytic pleural effusions are malignancy, fungal infection, melioidosis, sarcoidosis, and connective tissue diseases (Yam, 1967; Reechaipichitkul *et al*, 1999). The primary difficulty in getting a diagnostic confirmation of tuberculous pleural effusion is the identification of mycobacteria in the pleural fluid (<50%) (Berger and Mejia, 1973; Epstein *et al*, 1987; Bueno *et al*, 1990). Pleural biopsy is usually the main diagnostic support in 51 to 88% of cases (Epstein *et al*, 1987; Bueno *et al*, 1990; Seibert *et al*, 1991), but its invasive nature and the difficult technique that limits its practice, especially in children (Merino *et al*, 1999). Many biologic parameters have been introduced. One such marker is adenosine deaminase (ADA), which has been proposed as a useful diagnostic tool (Antony, 1996; Valdes *et al*, 1998; Light, 1998) because ADA levels can be ascertained quickly and cheaply (Gakis, 1996; Light, 1998; Perez-Rodriguez *et al*, 2000).

In Srinagarind Hospital, pleural tuberculosis accounts for 37% of exudative lymphocytic pleural effusion (Reechaipichitkul *et al*,

1999). Other than malignancy, melioidosis is one of the differential diagnosis. Previous reports of ADA levels for this infection have not been published. The purpose of our study is to define the diagnostic role of pleural fluid ADA (ADAPF) in areas where tuberculosis and melioidosis are prevalent, and what the optimum cut-off levels should be.

MATERIAL AND METHODS

Between June 1998 and June 2000, 132 consecutive patients with symptomatic exudative lymphocytic pleural effusion according to Light's criteria (Heffner *et al*, 1997) were studied. Informed consent was obtained before beginning the study. Patients with bleeding tendency were excluded. A detailed history, a thorough physical examination, and a chest radiograph were taken of each patient. A standard diagnostic thoracocentesis was performed. In cases of free-flow pleural tapping, three pieces of pleural biopsy by Abram's needle was taken for histopathologic examination.

Pleural fluid was sent for conventional diagnosis, including gram staining, AFB staining, aerobic culture, culture for *Mycobacterium tuberculosis* on Lowenstein-Jensen media, and cytology. Ten milliliters of additional pleural fluid was sent for ADA assay, using Giusti's method (1974). The laboratory technician was blinded to the tentative diagnosis of each patient; furthermore, the clinicians were unaware of the ADAPF levels when the diagnoses were assigned.

Diagnostic classification

A diagnosis of tuberculous pleural effusion was defined by positivity of any one of the following : 1) *Mycobacterium tuberculosis* identified in a culture of the pleural fluid and/or sputum; 2) Caseating granulomas in the absence of any clinical evidence of sarcoidosis, tularemia or fungal infection in the pleural tissue; 3) A response to antituberculous drugs revealed by an improvement of clinical symptoms and/or a clearing of chest radiograph.

A malignancy was diagnosed when neoplastic pleural tissue and/or fluid cytology were identified. Melioidosis was diagnosed when pleural fluid and/or hemoculture isolated *Burkholderia pseudomallei*. Other diagnoses depended on the final outcome of investigation.

Ethics

This research was approved by the Ethics Committiee of the Faculty of Medicine, Khon Kaen University.

Statistical analysis

Means and standard deviations were given for continuous data, and number and percentages were given for catecorical data. Group comparisons were made using a one-way ANOVA for continuous variables, and χ^2 test for catecorical variables. P-values <0.05 were considered significant.

Following standard definitions, sensitivity and specificity of ADAPF were calculated by 2x2 table at each level. The best cut-off value was selected using a receiver operating characteristic (ROC) curve. The sensitivity (95% CI), specificity (95% CI), positive and negative predictive value at this cut-off value were calculated. The likelihood ratio of this diagnostic test was also calculated.

RESULTS

During the study period, 132 patients with exudative lymphocytic pleural effusion were investigated. Tuberculous pleural effusion was diagnosed in 50 patients (37.9%). Other causes of exudative lymphocytic pleural effusion were malignancy (44.7%), chronic nonspecific inflammation (14.4%), melioidosis (2.3%), and systemic lupus erythrematosus (0.7%) (Table 1). Of the 50 patients diagnosed with tuberculosis, the pleural biopsy exhibited granulomatous inflammation in 48% of them, pleural fluid staining was positive in 6%, pleural fluid culture for *M. tubercu-*

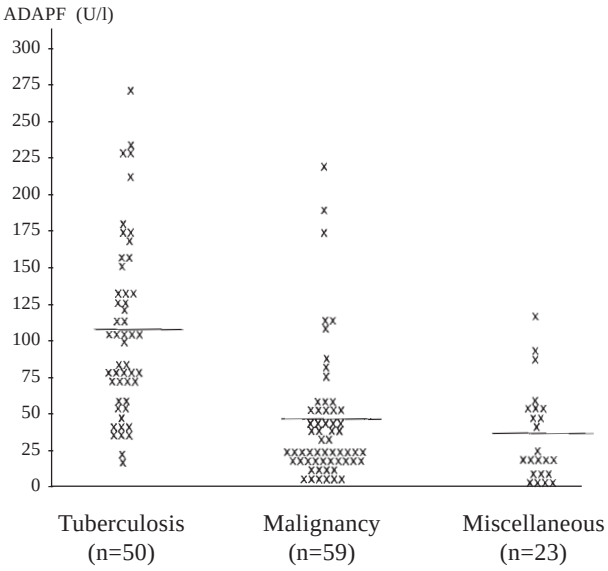

Fig 1–ADAPF levels in 132 patients with various final diagnosis.

Table 1
Etiology of exudative lymphocytic pleural effusion.

Etiology	Number of patients	%
Tuberculosis	50	37.9
Malignancy	59	44.7
Miscellaneous		
-Chronic nonspecific inflammation	19	14.4
-Melioidosis	3	2.3
-Systemic lupus erythrematosus	1	0.7
Total	132	100

losis was positive in 16%, and all of the patients clinical symptoms subsided after taking antituberculous drugs. The demographic data and pleural fluid profiles of the patients were summarized in Table 2. The mean age, male to female ratio, pleural fluid leukocytes and LDH levels did not significantly differ among patients with tuberculous pleural effusion, malignancy, and miscellaneous etiologies. The percentage of lymphocytes and pleural fluid protein in tuberculous pleural effusion were similar to malignancy, but higher than miscellaneous group.

Pleural fluid ADA levels were significantly higher ($p<0.05$, one-way ANOVA) in patients with tuberculous pleural effusion than in any other groups (Fig 1). The mean values of ADAPF were 93.2 (SD 56.5) U/l in patients with tuberculous pleural effusion, 36.7 (SD 39.2) U/l in those with a malignancy, and 31.3 (SD 23.4) U/l in the miscellaneous group (Table 3). Only 3 patients were diagnosed as having melioidosis. ADAPF levels in these three patients were 15, 46.9, 49.8 U/l respectively. One case with systemic lupus erythrematosus had ADAPF levels 24.1 U/l.

The ROC curve identified ADAPF levels of 48 U/l as the best cut-off value (Fig 2). The area under ROC curve was equal to 86.2%. At this cut-off for patients with tuberculous pleural effusion, the sensitivity of ADAPF was 80% (95% CI, 73 to 87%) and specificity was 80.5% (95% CI, 73.6 to 87.4%). Whereas, the positive and negative predictive

Table 2
Clinical and pleural fluid profiles of patients.

Patient characteristics	Tuberculosis	Malignancy	Miscellaneous	p-value
No. of patients	50	59	23	-
Age, year	49.6 (16.9)	53.9 (15.0)	53.3 (17.9)	0.35
Male : female ratio	31 : 19	34 : 25	12 : 11	0.72
Leukocytes, cells/μl	1,992.4	1,673.1	1,152	0.38
Lymphocytes, %	78.8 (28)	75.1 (16.1)	63.6 (22.9)	0.01*
LDH, IU/l	558.0	696.3	413.2	0.35
Protein, g/dl	5.3 (1.07)	4.7 (1.3)	4.2 (1.9)	0.007*

*p-value < 0.05 (One - way ANOVA for continuous variables, χ^2 test for catecorical variables)

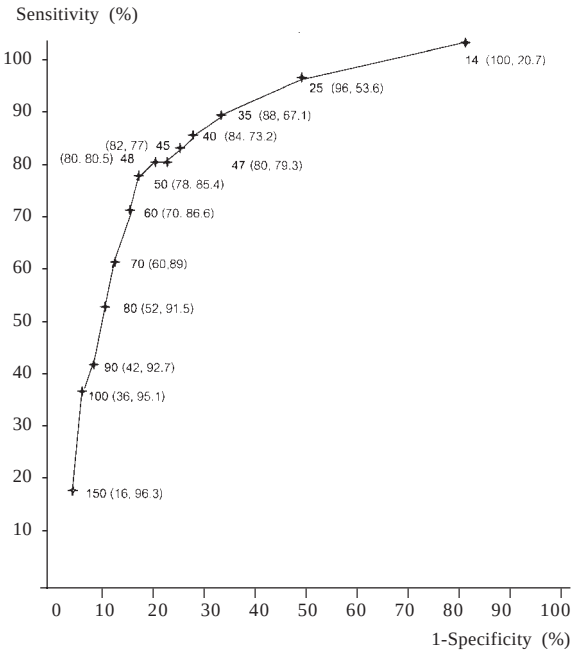

Fig 2–An ROC curve showing sensitivity and 1-specificity at various cut-off values for ADAPF (in paracentesis was sensitivity and specificity).

values at this cut-off value were 71.4% and 86.8%, respectively.

The likelihood ratio of this diagnostic test was also calculated (Table 4). The likelihood ratio for the diagnosis of tuberculous pleural effusion in patients with ADAPF levels less than 45 U/l were 1:4. Similarly, the likelihood ratio for ADAPF levels between 45 and 100 U/l were 5:2, and the likelihood ratio for ADAPF levels greater than 100 U/l were 7:1.

DISCUSSION

Exudative lymphocytic pleural effusions are commonly encountered in clinical practice but they often constitue difficult diagnostic problems. The two most common causes are malignancy and tuberculous effusions (Valdes *et al*, 1996). In northeast of Thailand, melioidosis is one of subacute infections which

Table 3
Pleural fluid ADA levels of each group.

Etiology	Pleural fluid ADA levels (U/l)	95% CI
Tuberculosis	93.2 (56.5)	77.1-109.2
Malignancy	36.7 (39.2)	26.5-47.0
Miscellaneous	31.3 (23.4)	21.2-41.5

$p<0.05$ (One-way ANOVA)

Table 4
The likelihood ratio of this diagnostic test.

ADAPF levels (U/l)	Likelihood ratio
<45	1:4
45-100	5:2
>100	7:1

mimic tuberculosis (Chaowagul *et al*, 1994). The attempt to diagnose these conditions is to be proper management of the treatable diseases, such as tuberculosis and melioidosis. For tuberculosis, the limitation of diagnostic tests are few positive staining and culture from pleural fluid, and time consuming for identification (Roth, 1999).

Adenosine deaminase is considered an indicator of cell-mediated immunity and is found mainly in T lymphocytes and macrophages (Piras *et al*, 1978). Since the initial proposal of Piras *et al*, many studies have confirmed the utility of ADA for diagnosis of tuberculous pleural effusion (Ocana *et al*, 1983; Banales *et al*, 1991; Valdes *et al*, 1993; 1995; Ungerer *et al*, 1994) though some have questioned its diagnostic value (van-Keimpema *et al*, 1987; Maartens *et al*, 1991; Rodriguez *et al*, 1993; Sahoo, 1994). The discrepancy of the ADAPF cut-off values depended on characteristic of the populations studied.

In our study, we investigated 132 cases of exudative lymphocytic pleural effusions. Our findings seem to confirm that ADA activity is a useful parameter for the diagnosis of tuberculous efftusion. The mean levels of pleural

Table 5
The utility of ADAPF levels in diagnosis of tuberculous pleural effusion.

Authors	Year	Cut-off ADAPF (U/l)	Sensitivity (%)	Specificity (%)
De Oliveira *et al*	1994	40	91	96
Perez-Rodrigues *et al*	1995	40	88	97
Ocana *et al*	1983	45	100	97
Maartens *et al*	1991	45	77	83
Valdes *et al*	1993	47	100	95
Our study	2001	48	80	80.5
Burgess *et al*	1996	50	91	81
Riantawan *et al*	1999	60	95	96
Banales *et al*	1991	70	98	96

ADA in tuberculous effusion were higher than in any other group. Some cases in the malignancy group also had high levels of ADAPF (the maximum ADAPF for a malignancy was 206.7 U/l in a non-Hodgkin's lymphoma which responded to chemotherapy). Only three cases of exudative lymphocytic pleural effusion were according to melioidosis. The ADAPF ranged from 15 to 49.8 U/l, may be small number of the patients. Since, it had some overlapping of ADAPF levels among these conditions. Rodriguez *et al* (1993) reported very high levels of ADAPF due to bronchoalveolar carcinoma, while van-Keimpema *et al* (1987) reported high levels for mesothelioma, Valdes *et al* (1995) for those with lymphoma, and Orriols *et al* (1992) for those with psittacosis. No previous studies reported about ADAPF levels due to melioidosis. Nevertheless, tuberculosis is the leading cause of exudative lymphocytic pleural effusion in the high prevalence of tuberculosis and melioidosis infection. It may be careful to use ADAPF levels for differential diagnosis between tuberculosis and melioidosis. Only one patient in this study was diagnosed systemic lupus erythrematosus and had low levels of ADAPF, which was the same as previously reported (Valdes *et al*, 1995).

Many studies have reported the utility of ADA in the diagnosis of tuberculous pleurisy with a wide range of cut-off values (*ie*, from 40 to 70 U/l) (Table 5). In our study, based on the ROC curve, an ADAPF levels of 48 U/l was the most suitable cut-off value yielding a sensitivity of 80% (95% CI, 73 to 87%) and a specificity of 80.5% (95% CI, 73.6 to 87.4%) for diagnosis of patients with tuberculous pleural effusion. The explanation for the variability could be differences in : 1) the population, 2) the diagnostic criteria for tuberculous pleural effusion, and/or 3) the method of ADAPF analysis. In our study, we inculded symptomatic exudative lymphocytic pleural effusion patients and used Giusti's colorimetric method for the measurement ADA levels. We also calculated the likelihood ratio of this diagnostic test. We found that if ADAPF levels were high, the likelihood ratio for the diagnosis of tuberculous pleural effusion was higher.

In conclusion, the diagnosis of tuberculous pleural effusion should be excluded in every patient with exudative lymphocytic pleural effusion. Patients with tuberculous pleural effusion tend to have high levels of ADA in their pleural fluid. The measurement of ADA levels provides simple alternative to needle biopsy of the pleural for establishing the diagnosis of tuberculous pleural effusion. In addition, the analysis of ADA levels requires only a few hours and a lower cost compared with other diagnostic test. The delay in establishing diagnosis of this condition increases patient morbidity. Interpretation of ADAPF results must take into account the overall clinical setting.

ACKNOWLEDGEMENTS

This work was supported by a research grant from the Faculty of Medicine, Khon Kaen University. The authors thank Mr Bryan Roderick Hamman for his assistance with the English-langue presentation of the paper and Mr Bandit Thinkhamrop for his assistance with the statistical analysis.

REFERENCES

Antony VB. Adenosine deaminase isoenzymes and pleural tuberculosis, *J Lab Clin Med* 1996; 127: 326-7.

Banales JL, Pineda PR, Fitzgerald JM, Rubio H, Selman M, Salazar-Lezama M. Adenosine deaminase in the diagnosis of tuberculous pleural effusions: a report of 218 patients and review of the literature. *Chest* 1991; 99: 355-7.

Berger HW, Mejia E. Tuberculous pleurisy. *Chest* 1973; 63: 88-92.

Bueno CE, Clemente G, Castro BC, *et al*. Cytologic and bacteriologic analysis of fluid and pleural biopsy specimens with Cope's needle. *Arch Intern Med* 1990; 150: 1190-4.

Burgess LJ, Maritz FJ, Le Roux I, Taljaard F. Combined use of pleural adenosine deaminase with lymphocyte/neutrophil ratio: increased specificity for the diagnosis of tuberculous pleuritis. *Chest* 1996; 109: 414-9.

Chaowagul V, Suputtamongkol Y, White NJ. Clinical presentations of melioidosis. In: Puthucheary SD, Malik YA, eds. Melioidosis: prevailing problems and future directions. Kuala Lumpur: SP Muda Printing, 1994: 130-4.

David ME, Lewis RK, Steven MA, Wallance TM. Tuberculous pleural effusion. *Chest* 1987; 91: 106-9.

De Oliveira HG, Rossato ER, Prolla JC. Pleural fluid adenosine deaminase and lymphocyte proportion: clinical usefulness in the diagnosis of tuberculosis. *Cytopathology* 1994; 5: 27-32.

Esptein DM, Kline LR, Albelda SM, Miller WT. Tuberculous pleural effusions. *Chest* 1987; 91: 106-9.

Ferrer J. Pleural tuberculosis. *Eur Respir J* 1997; 10: 942-7.

Gakis C. The low cost of the adenosine deaminase assay. *Chest* 1996; 111 : 970-80.

Giusti G. Adenosine deaminase. In: Bergmeyer HU, ed. Methods of enzymatic analysis. New York: Academic Press, 1974: 1092-9.

Heffner JE, Brown LK, Barbieri CA. Diagnostic value of tests that discriminate between exudative and transudative pleural effusions. *Chest* 1997; 111: 970-80.

Light RW. Establishing the diagnosis of tuberculous pleuritis. *Arch Intern Med* 1998; 158: 1967-8.

Maartens G, Bateman ED. Tuberculous pleural effusions: increased culture yield with bedside inoculation of pleural fluid and poor diagnostic value of adenosine deaminase. *Thorax* 1991; 46: 96-9.

Merino JM, Carpintero I, Alvarez T, Rodrigo J, Sanchez J, Coello JM. Tuberculous pleural effusion in children. *Chest* 1999; 115: 26-30.

Ocana J, Martinez-vazquez JM, Segura RM, Fernandez-De-Sevilla T, Capdevila JA. Adenosine deaminase in pleural fluids: test for diagnosis of tuberculous pleural effusion. *Chest* 1983; 84: 51-3.

Orriols R, Munoz X, Drobnic Z, Ferrer J, Morell F. High adenosine deaminase activity in pleural effusion due to Psittacosis. *Chest* 1992; 101: 881-2.

Perez-Rodriguez E, Oriz de Saracho J, Sanchez JJ, *et al*. Pleural adenosine deaminase (ADAp) and its iso-enzymes in tuberculous effusion. *Eur Resp J* 1995; 8 (19S): 554S.

Perez-Rodriguez E, Castro DJ. The use of adenosine deaminase and adenosine deaminase isoenzymes in the diagnosis of tuberculous pleuritis. *Curr Opin Pulm Med* 2000; 6: 259-66.

Piras Ma, Gakis C, Budroni M, Andreoni G. Adenosine deaminase activity in pleural effusions: an aid to differential diagnosis. *Br Med J* 1978; 2: 1751-2.

Reechaipichitkul W, Lulitanond V, Patjanasoontorn B, Kawamatawong T. Diagnostic role of pleural biopsy as compared to high volume pleural fluid study in tuberculous pleural effusion. *Thai J Tuberc Chest Dis* 1999; 20: 145-50.

Riantawan P, Chaowalit P, Wongsangiem M, Rojanaraweewong P. Diagnostic value of pleural fluid adenosine deaminase in tuberculous

pleuristis with reference to HIV coinfection and a Bayesian analysis. *Chest* 1999; 116: 97-103.

Rodriguez E, Martinez JA, Buges J, Torres M. High adenosine deaminase level in pleural effusion due to bronchoalveolar carcinoma. *Chest* 1993; 103: 978-9.

Roth BJ. Searching for tuberculosis in the pleural space. *Chest* 1999; 116: 3-4.

Sahoo RC. Raised pleural adenosine deaminase: Does it mean tuberculosis? *Chest* 1994; 107: 1911-2.

Seibert AF, Haynes JJr, Middleton R, Bass JB. Tuberculous pleural effusion: twenty-year experience. *Chest* 1991; 99: 883-6.

Ungerer JPJ, Oosthuizen HM, Retief JH, Bissbort SH. Significance of adenosine deaminase activity and its isoenzymes in tuberculous effusions. *Chest* 1994; 106: 33-7.

Valdes L, Alvarez D, Valle JM, Pose A, San Jose E. The etiology of pleural effusions in an area with high incidence of tuberculosis. *Chest* 1996; 109: 158-62.

Valdes L, Alvarez D, San Jose E, Penela P, Valle JM, Garcia-Pazos JM. Tuberculous pleurisy: a study of 254 patients. *Arch Intern Med* 1998; 158: 2017-21.

Valdes L, San Jose E, Alvarez D, *et al*. Diagnosis of tuberculous pleurisy using the biologic parameters adenosine deaminase, lysozyme, and interferon gamma. *Chest* 1993; 103: 458-65.

Valdes L, Alvarez D, San Jose E, *et al*. Value of adenosine deaminase in the diagnosis of tuberculous pleural effusions in young patients in a region of high prevalence of tuberculosis. *Thorax* 1995; 50: 600-3.

van-Keimpema AR, Slaats EH, Wagenaar JP. Adenosine deaminase activity, not diagnostic for tuberculous pleurisy. *Eur J Respir Dis* 1987; 71: 15-8.

Yam LT. Diagnostic significance of lymphocytes in pleural effusions. *Ann Intern Med* 1967; 66: 972-82.