DIAGNOSTIC YIELD OF ADENOSINE DEAMINASE IN BRONCHOALVEOLAR LAVAGE

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Abstract. Adenosine deaminase (ADA) activity rises in various body fluids in patients with tuberculosis. A prospective study was conducted to determine the diagnostic value of ADA activity in bronchoalveolar lavage. Between March 2001 and February 2003, 148 patients were enrolled in our study, mean age 55.6 years (SD 14.6), and a male to female ratio of 2.4:1. The mean duration of symptoms was 66.2 days. All patients were either sputum-smear negative for AFB or failed to produce sputum. The final diagnosis resulted in three patient groups: 43 with pulmonary tuberculosis, 70 malignancy, and 35 miscellaneous causes. The mean ADA activity in the bronchoalveolar lavage for the pulmonary tuberculosis, malignancy, and miscellaneous causes groups was 8.98 (95% CI, 3.79-14.17), 7.63 (95% CI, 4.12-11.14), and 11.61 U/l (95% CI, 3.59-19.62), respectively. No difference was detected in the ADA level in the pulmonary tuberculosis *vs* other groups (p=0.56, one-way ANOVA). A high level of ADA activity was found in non-tuberculous conditions such as bronchogenic carcinoma, pulmonary hemosiderosis, chronic pneumonia with empyema thoracis and chronic myeloid leukemia. We concluded that ADA activity in the bronchoalveolar lavage was not clearly diagnostic of smear-negative pulmonary tuberculosis. Early diagnosis required histopathology of biopsied transbronchial specimens obtained by fiberoptic bronchoscopy.

INTRODUCTION

Pulmonary tuberculosis remains an important world health problem, especially where HIV is endemic. Microscopy of sputum smears is the most widely used method for diagnosing pulmonary tuberculosis. However, many patients fail to produce sputum and others have smears that are repeatedly negative for acid-fast bacilli because of the low sensitivity of the test. In these patients, diagnostic confirmation depends on identifying *Mycobacterium tuberculosis* in culture, but this laboratory work requires 6 to 8 weeks and negative results occur in about one third of cases (Van der Kuyp, 1998). Fiberoptic bronchoscopy with transbronchial biopsy and bronchoalveolar lavage have proved a valuable tools (So *et al*, 1982; Charoenratanakul *et al*, 1995).

With these techniques, a definitive diagnosis can be made in most cases of active tuberculosis, but because direct microscopy is insensitive, diagnosis may be delayed until culture results are available. An alternative rapid test for these specimens is the assay of adenosine deaminase (ADA) activity (Kurata, 1995). ADA is an enzyme involved in the purine catabolism, produced by mononuclear cells and lymphocytes. The evaluation of ADA activity has proven a useful diagnostic tool for tuberculous pleural effusion (Valdes *et al*, 1993; Burgess *et al*, 1995; Reechaipichitkul *et al*, 2001). The possible value of these enzymes in other biological fluids has not been fully investigated. Our aim, therefore, was to determine whether ADA activity in bronchoalveolar lavage could be employed for differential diagnosis of pulmonary tuberculosis, especially in patients with sputum smears negative for AFB or failing to produce sputum.

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MATERIAL AND METHODS

Between March 2001 and February 2003, we enrolled patients aged ≥15 years with an abnor-

mal chest radiograph and negative for acid-fast sputum staining or no sputum production. Patients with a bleeding tendency or arterial hypoxemia were excluded. Written consent was obtained from all patients before undergoing bronchoscopy. Bronchial biopsy specimens were taken from the abnormal area (s) for pathological examination.

Bronchoalveolar lavage was performed in the most affected lobe in case of localized disease and in the right middle lobe when the disease was diffuse. Three aliquots of 50 ml of normal saline solution at room temperature were instilled and immediately aspirated using a handheld syringe. Bronchoalveolar lavage (BAL) was sent for acid-fast staining and culture for *M. tuberculosis*, Gram's staining and aerobic culture, Wright's staining, fungal culture and cytological examination. Additional BAL was analyzed for ADA using Giusti's colorimetric method (Giusti, 1974). The laboratory technician was not apprised of the tentative diagnosis of each patient; furthermore, the clinicians were unaware of the ADA level when the diagnoses were decided.

Measurement of adenosine deaminase (ADA)

ADA was determined in all BAL specimens according to the method described by Giusti (1974). This is the colorimetric method based on the measurement of the formation of ammonia by Berthelot reaction: ammonia which is produced when ADA acts on excess adenosine forms an intensely blue indophenol with sodium hypochlorite and phenol in alkaline solution. Sodium nitroprusside is used as the catalyst of the reaction. The ammonia concentration is directly proportional to the absorbance value of the indophenol at wavelength 628 nm. One unit of ADA is defined as the amount of enzyme required to release 1 μmol ammonia per minute from adenosine under standard assay conditions.

Diagnostic classification

A diagnosis of pulmonary tuberculosis requires one of the following: 1) *M. tuberculosis* positive culture from the bronchoalveolar lavage or biopsied bronchial specimens; 2) A stained acid-fast bacilli from the bronchoalveolar lavage or bronchial biopsy specimens; 3) The presence of granulomas in the absence of any clinical evidence of sarcoidosis, tularemia, or fungal infection in the lung tissue; 4) A response to antituberculous drugs revealed by an improvement of clinical symptoms and/or clearing of chest radiograph.

A malignancy was diagnosed when a neoplasm was identified by histopathology of the lung tissue and/or cytology of bronchoalveolar lavage. Melioidosis was diagnosed when bronchoalveolar lavage and/or hemoculture grew *Burkholderia pseudomallei*. Other diagnoses (*ie* fungal infection, *Pneumocystis carinii* infection or interstitial lung diseases) depended on the outcome of the investigations.

Ethics

The Ethics Committee of the Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand, approved the research.

Statistical analysis

Means and standard deviations were calculated for the continuous data, and number and percentages for the categorical data. Group comparisons were made using a one-way ANOVA for continuous variables. P-values <0.05 were considered significant.

RESULTS

During the study period, 148 patients were enrolled (104 males): 43 with pulmonary tuberculosis; 70 bronchogenic carcinoma; and, 35 miscellaneous causes (Table 1). Of the 43 cases of pulmonary tuberculosis, the bronchoalveolar la-

Table 1
Etiology of the 148 patients.

Etiology	Number of patients	%
Tuberculosis	43	29.1
Malignancy	70	47.3
Miscellaneous[a]	35	23.6
Total	148	100

[a]Including: interstitial lung diseases, lupus pneumonitis, systemic sclerosis, radiation pneumonitis, sarcoidosis, chronic myeloid leukemia, melioidosis, *Pneumocystis carinii* pneumonia, aspirated pneumonia, chronic pneumonia with empyema thoracis, cryptococcal pneumonia, aspergylloma, pulmonary hemosiderosis.

Table 2
Clinical and patients characteristics.

Patient characteristics	Tuberculosis	Malignancy	Miscellaneous
No. of patients	43	70	35
Age (year)	56.8 (14.9)	58 (13.4)	49.2 (14.8)
Male : female ratio	28:15	55:15	21:14
Mean duration of symptom (day)	62.5	74.7	50.9
Abnormal chest radiographs (%)			
Alveolar infiltrate	51.2	27.1	34.3
Interstitial infiltrate	18.6	4.3	45.7
Mass	30.2	68.6	20.0

Table 3
ADA levels in the bronchoalveolar lavage of each group.

Etiology	ADA levels (U/l)	95% CI
Tuberculosis	8.98 (16.85)	3.79-14.17
Malignancy	7.63 (14.73)	4.12-11.14
Miscellaneous	11.61 (23.32)	3.59-19.62

p = 0.56 (one-way ANOVA)

vage of 22 grew *M. tuberculosis*. The 35 miscellaneous causes comprised: interstitial lung diseases, lupus pneumonitis, systemic sclerosis, radiation pneumonitis, sarcoidosis, chronic myeloid leukemia, melioidosis, *Pneumocystis carinii* pneumonia, aspirated pneumonia, chronic pneumonia with empyema thoracis, cryptococcal pneumonia, aspergylloma, and pulmonary hemosiderosis.

Patients averaged 55.6 years (SD 14.6). Symptoms lasted an average 66.2 days. Characteristic chest radiographs revealed alveolar infiltration (35.8%), interstitial infiltration (18.3%) and lung mass (45.9%). Patient characteristics and chest radiographic findings for each group are presented in Table 2.

The mean values (and SD) of ADA levels in the bronchoalveolar lavage for the tuberculosis, malignancy, and miscellaneous causes groups were 8.98 (16.85), 7.63 (14.73), and 11.61 (23.32), respectively (Table 3). The ADA activity in the bronchoalveolar lavage of patients with pulmonary tuberculosis was not significantly different from the other patient groups (p = 0.56, one-way ANOVA). The individual and mean concentrations of ADA in the bronchoalveolar lavage were shown in Fig 1. High levels of ADA activity were found in the malignancy and miscellaneous groups. The highest level in the malignancy group

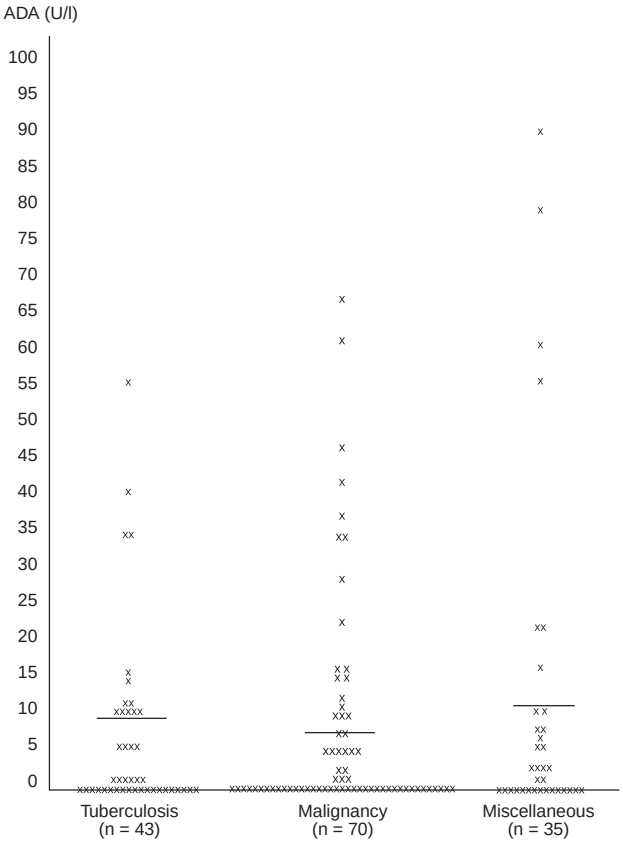

Fig 1–ADA levels in the bronchoalveolar lavage of the 148 patients with various final diagnoses.

was 65.75 U/l, while it was 91.75 U/l in the miscellaneous group, in a patient with pulmonary hemosiderosis. Melioidosis was confirmed in three patients in the miscellaneous causes group: all of whom had zero ADA activity in their bronchoalveolar lavage.

DISCUSSION

Patients with no sputum or sputum-smear-negative for AFB represent a clinical challenge for the pulmonologist. In order to provide rapid diagnosis of pulmonary tuberculosis; several techniques have been proposed such as: radiometric culture method (Russell *et al*, 1986), assay for tuberculostearic acid (Pang *et al*, 1989), and polymerase chain reaction (PCR) for TB (Chen *et al*, 2002). Measurement of ADA is another diagnostic test; simple, quick, and inexpensive (Gakis, 1996). Several reports indicate the value of ADA in the pleural fluid for diagnosis of tuberculous pleural effusion (Valdes *et al*, 1993; Burgess *et al*, 1995; Reechaipichitkul *et al*, 2001). However, few articles describe its diagnostic role in the bronchoalveolar lavage (Pushpakom *et al*, 1988; Orphanidou *et al*, 1998; Kayacan *et al*, 2002), all had a small sample size and reported the ADA in the bronchoalveolar lavage of pulmonary tuberculosis was seemed to be greater than in other conditions.

In our study, 148 patients were enrolled. The mean ADA activity in the bronchoalveolar lavage of patients with pulmonary tuberculosis was greater than in the malignancy group but lower than in the miscellaneous causes group. The difference, however, is not statistically significant. Nearly half (20 of 43) of the pulmonary tuberculosis patients had zero ADA activity; by contrast, some patients in the malignancy and miscellaneous causes groups had significantly higher levels of ADA activity. Two patients in the malignancy group had high ADA activity, 65.75 and 62.5 U/l, respectively, and transbronchial biopsy identified adenocarcinoma and squamous cell type bronchogenic carcinoma. High levels of ADA activity were also found in some cases of the miscellaneous causes group: pulmonary hemosiderosis (91.7 U/l), chronic pneumonia with empyema thoracis (81.9 U/l), chronic myeloid leukemia (60.3 U/l) and systemic sclerosis (54.3 U/l). A wide variation of ADA activity was found for tuberculosis, malignancy, and miscellaneous causes when applied clinically. This may depend on the severity of illness and the procedures used to obtain the bronchoalveolar lavage. Since lymphocytes and mononuclear phagocytes contain high amounts of ADA (Albera *et al*, 1993), diseases which respond to this cell type or bronchoalveolar lavage with large amounts of this cell type will demonstrate high levels of ADA.

In conclusion, the use of ADA levels in the bronchoalveolar lavage for rapidly diagnosing smear-negative pulmonary tuberculosis has limited value. However, fiberoptic bronchoscopy was consistently useful and should be performed. Histopathology of transbronchial biopsied specimens had the major role for early diagnosis. Mycobacterial culture of the bronchoalveolar lavage remains the gold standard for diagnosis although it requires several weeks to complete.

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