ADENOSINE DEAMINASE IN PLEURAL FLUID — AN ENZYMATIC TEST FOR TUBERCULOUS PLEURAL EFFUSION

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SYNOPSIS

Adenosine deaminase (ADA) activity in pleural fluid was measured in 92 patients with pleural effusion of different aetiology. The mean ADA level in tuberculous effusion (88.3 U/L) is significantly higher than the levels in effusion due to malignancy (22.4 U/L), pneumonia (20.7 U/L), empyema (45.1 U/L) and SLE (36 U/L). The mean ADA level in a control group of transudative effusion was 8.0 U/L. ADA values above 50 U/L, especially in lymphocytic effusion indicate a tuberculous aetiology, while a value below 50 U/L is suggestive of malignant or other non-tuberculous effusion. The assay of ADA is useful as a diagnostic indicator of tuberculous pleural effusion and it is of particular value in differentiating tuberculous from carcinomatous effusions.

INTRODUCTION

Adenosine deaminase (ADA) is a ubiquitous enzyme involved in purine metabolism. It catalyzes the conversion of adenosine and deoxyadenosine to inosine and deoxyinosine. The importance of ADA in immune response was first recognised in 1972 when Giblett (1) detected the absence of ADA in severe combined immunodeficiency disease. On the other hand, increased ADA activity was found in diseases which stimulate T-lymphocyte response and in lymphoproliferative disorders eg infectious mononucleosis (2), typhoid (3), lymphoma (4), leukemia (5) and tuberculosis (6). It has been suggested that ADA can be of value in differentiating tuberculous from other causes of pleural effusion (6). In Singapore, tuberculosis continues to be an important cause of pleural effusion, second only to malignancy in frequency of occurrence. The differentiation of tuberculous from malignant pleural effusion is important in the management of the patient and prognosis. This paper reports our evaluation of ADA as a diagnostic test of tuberculous pleurisy in 92 patients with pleural effusion of different aetiology.

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PATIENTS AND METHODS

Patients above the age of 12 years who were admitted to Medical Units II and IV of Tan Tock Seng Hospital with radiological evidence of pleural effusion were investigated. Diagnostic evaluation included Hb, total white and differential count, estimation of serum protein levels, pleural aspiration and closed pleural biopsy using the Abrams needle. Two biopsies were done through the same puncture wound by the house or medical officer in charge of the ward, instead of by the same person, to assess the success rate under routine ward conditions. The biopsy specimens were reported by different pathologists in the Department of Pathology. Investigations of pleural fluid included gross and microscopic examination, analysis for specific gravity, sugar, protein, ADA levels and bacteriological examination of pleural fluid by smear and culture for tubercle bacilli and also culture for pyogenic organisms when indicated.

Two sputum specimens and 2 laryngeal swabs were taken and examined for Mycobacterium tuberculosis by direct smear and culture respectively. Other diagnostic procedures such as tomography, bronchoscopy, lymph node or other tissue biopsy were performed when required.

Assay of ADA

5 ml of pleural fluid was collected in a plain bottle and sent to the Clinical Biochemistry Lab, Department of Pathology, Outram Road, within the shortest time possible. The pleural fluid specimen was stored in a freezer at a temperature of −20 degrees C up to as long as 1 month and then assayed at 37C in batches using the method of Giusti (2). In this method, the ammonia liberated by the adenosine deaminase reacts with sodium hypochlorite and phenol in alkaline solution to form an intensely blue indophenol which is measured photometrically.

RESULTS

Ninety two patients aged 16—95 years (mean age 59.5 years) were admitted to the study. There were 71 males and 21 females. The causes of pleural effusion and the number of patients in each diagnostic group shown in parentheses are as follows: tuberculosis (25), malignancy (34), pneumonia (7), empyema (8), SLE (3) and transudative effusion (15). The mean ADA levels and the distribution of the ADA levels in each diagnostic group are shown in Table 1 and Fig. 1 respectively. The mean ADA activity in tuberculous effusion (88.32 U/L) is significantly higher than the levels in pleural fluid due to malignancy, ($\bar{x}$ = 22.41 U/L; $p < 0.001$); pneumonia ($\bar{x}$ = 20.71 U/L; $p < 0.001$); empyema ($\bar{x}$ = 45.13 U/L; $p < 0.001$); SLE ($\bar{x}$ = 36 U/L; $0.01 > p > 0.001$) and transudative effusion ($\bar{x}$ = 8.0 U/L; $p < 0.001$). Student's t-test was used in the statistical analysis.

There were 25 patients diagnosed to have tuberculous pleural effusion. Their ages ranged from 16 to 78 years. Diagnosis of tuberculosis was confirmed in 21 patients by bacteriology or closed pleural biopsy and in 1 patient by open pleural biopsy. In 3 patients, the diagnosis of tuberculosis was based on clinical and radiological features and response to antituberculosis chemotherapy. The success rates of diagnostic procedures used in this group of patients are as follows: pleural biopsy 73%, laryngeal swab culture 32%, sputum smear examination 20% and pleural fluid culture for acid fast bacilli 9% (Table 2).

Malignant effusion was diagnosed in 34 patients, based on cytological examination of pleural fluid or pleural biopsy (22 cases); biopsy of bronchial tissue during bronchoscopy (8 cases); lymph node biopsy (1 case) and biopsy of bone secondaries (1 case). Malignancy was diagnosed on clinical and radiological grounds in 2 patients. Primary lung cancer was diagnosed in 32 patients including the 2 patients without histological confirmation. Of the 30 confirmed cases, there were 9 cases of adenocarcinoma, 6 cases of squamous cell carcinoma, 1 case of oat cell carcinoma and 1 case of undifferentiated large cell carcinoma. In 13 cases, the histological type of malignancy could not be identified. Metastatic lung lesions were seen in 2 patients, 1 with carcinoma of breast and the other with adenocarcinoma of ovary.

Parapneumonic effusion was diagnosed in 7 patients and empyema in 8 patients. Gram negative organisms were grown from the empyema fluid of 3 patients whose ADA levels range from 43—64 U/L and from the pleural fluid of 1 patient with parapneumonic effusion whose ADA level was 28 U/L.

3 patients were diagnosed to have SLE according to the criteria of the American Rheumatism Association.

15 patients with transudative effusion were used as controls. This group consisted of 12 patients with heart failure, 2 with cirrhosis of liver and 1 with nephrotic syndrome. One patient with transudative effusion due to hypoproteinemia was excluded from the control group because of the concomitant presence of a lymphoma. This patient had an ADA level of 31 U/L.

TABLE 1: CAUSES OF PLEURAL EFFUSION IN 92 PATIENTS WITH MEAN ADA LEVELS

Diagnosis	No of Cases	ADA levels U/L ($\bar{x}$ ± SD)
Tuberculosis	25	88.3 ± 25.8
Malignancy	34	22.4 ± 11.2
Pneumonia	7	20.7 ± 12.7
Empyema	8	45.1 ± 26.1
SLE	3	36.0 ± 3.6
Transudative Effusion*	15	8.0 ± 6.5

* includes 12 cases of heart failure, 2 cases of cirrhosis and 1 case of nephrotic syndrome

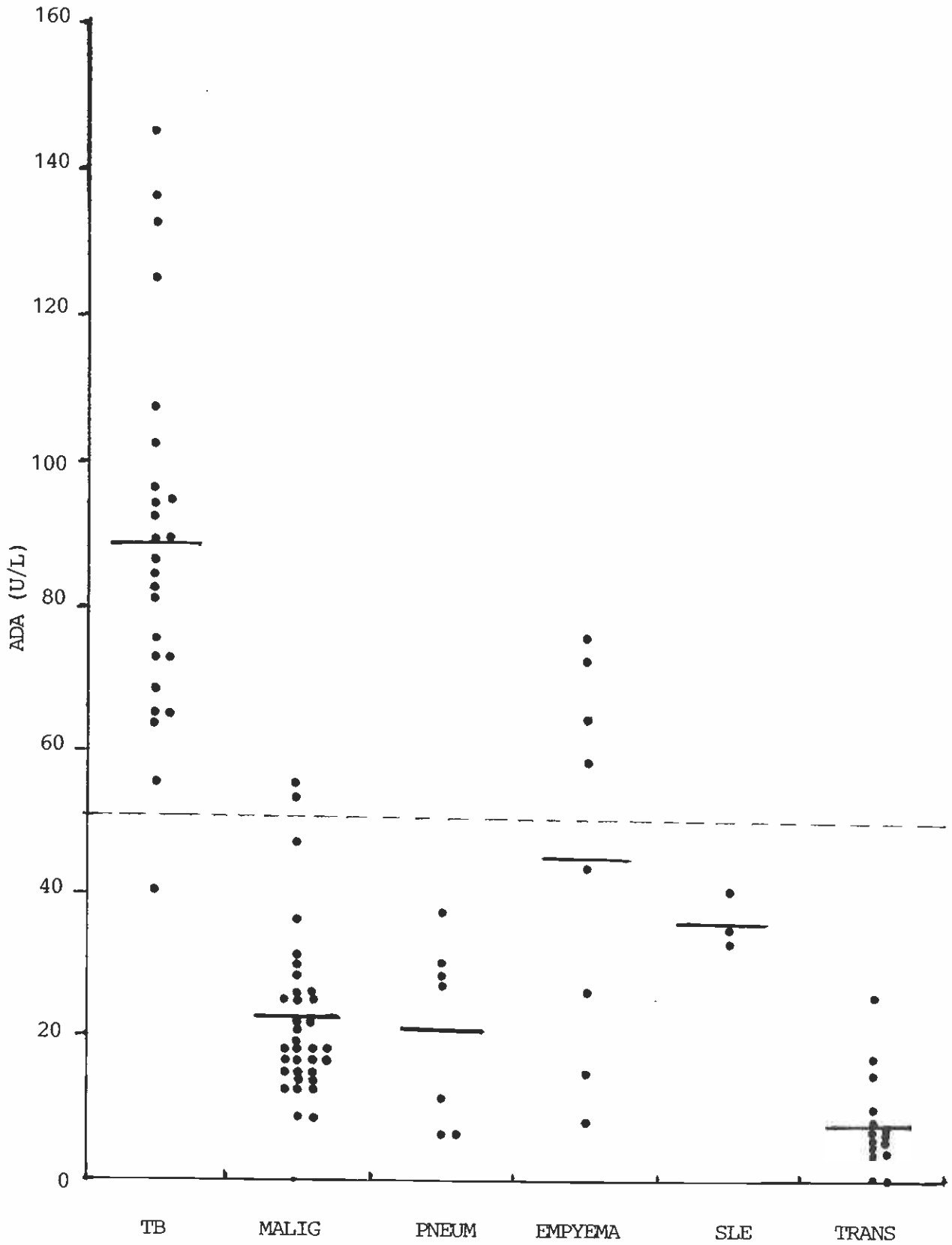

Fig. 1 **DISTRIBUTION OF ADA LEVELS IN 92 CASES OF PLEURAL EFFUSION**

Horizontal bar = mean ADA level
TB = Tuberculosis; Malig = Malignancy
Pneum = Pneumonia;
SLE = Systemic lupus erythematosus
Trans = Transudative effusion

TABLE 2: RESULTS OF DIAGNOSTIC TESTS USED IN 25 PATIENTS WITH TUBERCULOUS PLEURAL EFFUSION

Diagnostic Test	Number of Patients	Positive Test (No of Patients)	Success Rate (%)
Sputum smear*	25	5	20%
Laryngeal swab culture*	25	8	32%
Pleural fluid culture*	23	2	9%
Pleural biopsy	22	16	73%

* specimens were examined bacteriologically for Mycobacterium tuberculosis

DISCUSSION

Pleural effusion is a common clinical disorder with many diverse causes. Diagnosis of the aetiology can be difficult in spite of a careful clinical examination of the patient aided by investigative procedures and laboratory tests. In Singapore, tuberculosis and malignancy are the 2 most common causes of exudative pleural effusion which should always be considered in the differential diagnosis. Tuberculous effusion is usually diagnosed by pleural biopsy as bacteriological examination of pleural fluid, sputum and laryngeal swab specimens is of limited value because of the low diagnostic yields (9-32%) with these methods (Table 2). By comparison, pleural biopsy enabled the diagnosis to be made in 50-94% of patients with tuberculous effusion (7,8,9) and in 40-65% of patients with malignant effusion (8,9,10). One study also showed that pleural fluid cytology is more useful in diagnosis of malignant effusion compared with pleural biopsy (10). In our study, pleural biopsy was successful in establishing the diagnosis in 73% of patients with tuberculous effusion and in 42% of patients with malignant effusion (Table 2 & 3). Also, pleural fluid cytology was less successfull in detecting malignancy than pleural biopsy (Table 3). The

TABLE 3: RESULTS OF DIAGNOSTIC TESTS USED IN 34 PATIENTS WITH MALIGNANT PLEURAL EFFUSION

Diagnostic Test	Number of Patients	Positive Test (No of Patients)	Success Rate (%)
(1) Pleural fluid cytology	34	11	32%
(2) Pleural biopsy	33	14	42%
(3) Combination of (1) & (2)	34	22	65%

diagnostic accuracy of pleural biopsy is dependent on a number of factors such as the skill of the person performing the biopsy, the number of biopsy specimens taken through the same puncture site and the distribution of the pleural lesions (eg whether patchy or diffuse). These factors may account for the differences in the biopsy results reported by various groups. However, the limitations of pleural biopsy can be circumvented by using a laboratory test to detect tuberculous activity in pleural fluid. The measurement of adenosine deaminase activity in pleural fluid has been reported to be useful in the diagnosis of tuberculous effusion. This test will be of particular value in situations where it is not possible to do a pleural biopsy because of the small extent of the effusion or when standard diagnostic procedures including pleural biopsy have given negative results.

Our study showed that adenosine deaminase activity is increased in the pleural fluid of 96% of patients with tuberculous effusion, with levels ranging from 40 to 145 U/L. Similar results have been reported by other workers (6,11,12,13,14). Ocana (11) and Petersson (13) reported ADA Levels of 50 U/L or more in all their patients with tuberculous pleural effusion. If we adopt a value of 50 U/L as our cut-off point, then the sensitivity of the ADA test is 96% as there was 1 patient with a false negative result (ADA 40 U/L) in the tuberculosis group. The patient was diagnosed to have tuberculous effusion on the clinical and radiological findings and response to antituberculosis chemotherapy. However, 2 other patients with high ADA levels (86 U/L, 133 U/L) were diagnosed in the same manner. The specificity of the test is 91% as there were 6 patients with false positive results in the non-tuberculous groups of effusion. Of these, 2 had adenocarcinoma with ADA values of 53 and 55 U/L and 4 had empyema with values between 55-77 U/L. In contrast, of the 24 patients with tuberculous effusiion and elevated ADA levels, 16 (67%) had ADA values above 80 U/L with a maximum level of 145 U/L. Therefore, the magnitude of increase in the ADA levels is also much higher in tuberculous effusion. High ADA levels have been reported in patients with empyema (13) and parapneumonic effusion (14), but the reason for the increased ADA activity in these 2 conditions is not clear. The ADA levels in malignant effusion are generally low. Only 2 out of 34 patients had raised ADA levels in the pleural fluid; 1 was due to adenocarcinoma of the ovary and the other was due to primary adenocarcinoma of the lung. Our study compares well with that of Ocana (11) who reported a sensitivity of 100% and specificity of 97% for his adenosine deaminase assay.

Adenosine deaminase is widely distributed in lymphoid tissue, especially in the thymus, lymph nodes, spleen, and gastrointestinal tract. The enzyme is found in a higher concentration in T-lymphocytes than in the B-lymphocytes. In tuberculosis and other infections, the activity of adenosine deaminase is increased after activation of T-lymphocytes by antigenic stimulation. T-lymphocytes are found more abundantly in pleural fluid than in the peripheral blood of patients with tuberculous effusion (15). However, there is no correlation between ADA activity and the total number of T-cells and it is thought that ADA activity is related to the stage of maturation of the T-lymphocytes (12). It has been shown that ADA is required in the maturation of the early T-cells (17) in the thymus as well as in the differentiation and maturation of T and B-lymphocytes and monocytes (16,17,18,19) after antigenic stimulation. Adenosine deaminase activity has been found to be increased in some patients with lymphoma (11,14).

High ADA activity is also noted in patients with pleural effusion due to rheumatoid arthritis, with levels comparable to those found in tuberculous effusion (13,20). The increased ADA activity is thought to be due to a local activation of T-lymphocytes and monocytes within the pleural cavity as the activity of ADA is not increased in serum (20). In contrast, ADA activity is not elevated in pleural effusion due to SLE as shown in our study. The mean ADA activity in 3

patients with SLE was 36U/L with a range from 33-40 U/L. Our results are similar to those of Petersson (13) who reported a mean ADA level of 33.3 U/L. However, we did not encounter any cases of pleural effusion caused by rheumatoid arthritis in our study.

In conclusion, the assay of ADA is a useful test in the diagnostic evaluation of pleural effusion. The level of ADA in tuberculous pleural effusion is much higher than the levels in effusion of other etiology and it is particularly useful in differentiating tuberculous from malignant effusion because of the good separation of ADA levels in these 2 diagnostic conditions. When the ADA level in pleural fluid is $\geq$ 50 U/L, the etiology is most likely to be tuberculosis, while a value $<$ 50 U/L is indicative of malignancy or other non-tuberculous diseases. In the presence of a predominantly neutrophilic effusion (eg empyema), rheumatoid arthritis and lymphoma, high pleural fluid ADA activity is not reliable as a marker of tuberculous infection. The assay of ADA can be recommended as a useful diagnostic test of tuberculous pleurisy. Unlike pleural biopsy, ADA is not affected by sampling error eg when a non-involved area of the pleura is biopsied, and only a small volume of pleural fluid (5 ml) is required to perform the assay, which can be done in most clinical laboratories.

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