

# The Diagnostic Value of the Adenosine Deaminase Activity in the Pleural Fluid of Renal Transplant Patients with Tuberculous Pleural Effusion

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The assessment of the adenosine deaminase activity (ADA) in the pleural effusion is used for the diagnosis of tuberculous pleural effusion (TPE). To examine whether the procedure can be applied to immunocompromised patients, we analyzed the ADA activity in the pleural fluid of renal transplant recipients. We studied 23 renal transplant patients with TPE (21 men and 2 women; the mean age, 33 years). They were treated at the Yonsei University Hospital between January 1985 and December 2001. Patients with granuloma in the pleural biopsy specimen or positive for *Mycobacterium tuberculosis* in the pleural fluid culture were recruited. The ADA activity in the pleural effusion of 23 renal transplant patients with TPE was compared with 23 immunocompetent patients with TPE. The mean ADA activity was  $69.5 \pm 4.6$  in renal transplant patients and  $65.0 \pm 4.9$  U/L in immunocompetent patients. Applying the 40 U/L cut-off point, the positivity of ADA was 91.3% in renal transplant patients, and 86.9% in immunocompetent patients. We thus concluded that the measurement of ADA in the pleural fluid is a useful means in the diagnosis of TPE in renal transplant patients.

**Key Words:** Pleural effusion, tuberculosis, adenosine deaminase, kidney transplant, immunocompromised host

## INTRODUCTION

Tuberculous pleural effusion (TPE) is the second most frequent extrapulmonary tuberculosis.<sup>1</sup> The diagnosis of TPE is difficult. The Ziehl-

Neelson staining detects Acid fast bacilli (AFB) in only about 5% of patients<sup>2</sup>; The pleural fluid cultures detects *Mycobacterium tuberculosis* only in 40% of patients.<sup>3</sup> Furthermore, the tests takes several weeks.

The assessment of adenosine deaminase (ADA) in the pleural effusion has been shown to be useful in distinguishing tuberculosis from malignant pleural effusions. However, as the ADA activity depends on the local cellular immunity, physicians were skeptical about applying the measurement of ADA to the diagnosis of TPE in immunocompromised patients. The reports describing the ADA activity of the pleural effusion in immunocompromised patients with TPE thus are limited.<sup>4,5</sup> Here, we assessed the feasibility of applying the measurement ADA in the pleural fluid to the diagnosis of TPE in renal transplant patients.

## MATERIALS AND METHODS

### Patient population

Adult kidney transplant patients with TPE treated at Severance Hospital, the Yonsei University College of Medicine, Seoul, Korea from Jan 1985 to Dec 2001 were recruited. For the diagnosis of TPE, the following criteria were used: positive for *Mycobacterium tuberculosis* in the pleural fluid smear or culture, granuloma proven in the pleural

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biopsy specimen in the absence of other pleural granulomatous disease; or positive for *Mycobacterium tuberculosis* in the sputum culture with pleural effusion. At the time of the diagnosis of TPE, all patients were taking immunosuppressive drugs; steroids and cyclosporine (14 patients), steroids with cyclosporine and azathioprine (5 patients), cyclosporine and azathioprine (2 patients), and steroids with cyclosporine and mycophenolic acid (2 patients). As the evidence of immunocompromised, cyclosporine level was between 100 to 250 ng/dL when the time of TPE.

#### Determination of the ADA activity in the pleural fluid

ADA activity was determined by Giusti's method.<sup>6</sup> The assay measures ammonia generated by ADA from adenosine in the Berthelot reaction. 1 unit of ADA is the amount of enzyme required to release 1  $\mu$ mol ammonia per minute from adenosine under the standard assay conditions. ADA activity in renal transplant patients with TPE were compared with immunocompetent patients with TPE; had no noteworthy past history and taking no immunosuppressive medication.

#### The statistical analysis

The differences of the ADA activity in pleural fluid of renal transplant patients were compared with the immunocompetent patients by Fisher's exact test, Student's t-test or chi-square test. The difference was considered statistically significant if the *p*-value were less than 0.05.

## RESULTS

Between 1985 and 2001, 1,885 patients received the kidney transplant at Severance Hospital, Yonsei University College of Medicine, in Seoul, Korea. Among them, pulmonary tuberculosis was diagnosed in fifty-two patients (2.8%). Twenty-three patients (1.2%, 21 male and 2 female) developed TPE after renal transplant. Their mean age range was 32.7 years ranged from 19 to 50 years. The mean interval between the renal transplant and the development of TPE was 40.7 months, ranged from 3 months to 131 months. Two patients had the history of tuberculosis. Prior to the transplant, however, no patients received the treatment for tuberculosis such as INH chemoprophylaxis.

The characteristic of the pleural fluid is shown in Table 1. The mean of the ADA activity in the

**Table 1.** The Characteristic of the Pleural Fluid\*

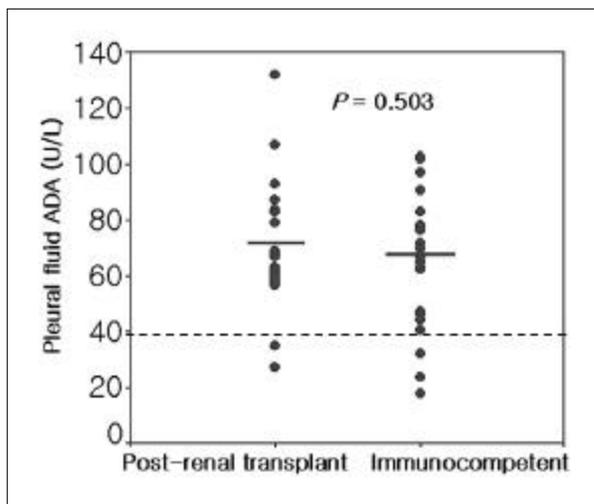
	Post-renal transplant (n=23)	Immunocompetents (n=23)	<i>p</i> value
Age, yrs	32.7 $\pm$ 1.9	37.8 $\pm$ 2.6	0.123
Sex, M/F	21/2	17/6	0.243
ADA, U/L	69.5 $\pm$ 4.6	65.0 $\pm$ 4.0	0.503
Cell count/ $\mu$ L	1567 $\pm$ 404	1868 $\pm$ 290	0.556
Lymphocytes, %	76.2 $\pm$ 6.5	87.0 $\pm$ 2.8	0.134
Neutrophil, %	24.4 $\pm$ 6.7	10.6 $\pm$ 2.6	0.066
LDH, IU/L	831 $\pm$ 141	850 $\pm$ 90	0.925
Protein, g/dL	4.7 $\pm$ 0.2	5.0 $\pm$ 0.1	0.114
Glucose, mg/dL	85 $\pm$ 13	94 $\pm$ 13	0.657
Pleural AFB smear (+)	0/17 (0%)	0/23 (0%)	1.000
Pleural AFB culture (+)	6/17 (35.3%)	3/23 (13.0%)	0.134
Granuloma in pleural biopsy	7/10 (70.0%)	15/21 (71.4%)	1.000

\*LDH, lactate dehydrogenase. Values are given as mean  $\pm$  SE.

pleural fluid was  $69.5 \pm 4.6$  U/L in renal transplant patients, which was comparable to immunocompetent patients ( $65.0 \pm 4.9$  U/L). Lymphocytes were predominant in most cases (16 out of 21, 76%). In renal transplant patients, 3 out of 11 patients with effusions (27%) had the glucose level less than 60 mg/dL. The protein level in 9 out of 19 patients (47%) was more than 5 g/dL. The followings of renal transplant patients were comparable to immunocompetent patients: the protein concentration in the pleural fluid, glucose, LDH, WBC and differential count, the positivity of AFB smear and culture, and the frequency of granuloma detected by pleural biopsy between renal transplant patients and immunocompetent patients. The distribution of the ADA activity in the pleural fluid of renal transplant patients and immunocompetent patients are shown in Fig. 1. Using the 40 U/L cut-off point, the test detected the ADA activity in 91.3% renal transplant patients and 86.9% in immunocompetent patients.

## DISCUSSION

Tuberculosis has been diagnosed traditionally by assessing the presence of bacilli in culture or AFB in clinical specimens. However, the sensi-



**Fig. 1.** The comparison of ADA activity in the pleural fluid. The ADA activity in the pleural fluid of renal transplant patients and immunocompetent patients with tuberculous pleural effusion was compared. The broken horizontal line represents the cut-off point 40 U/L. The horizontal solid line represents the mean.

tivity of AFB staining is low. The “gold standard” culture procedure takes up to 8 weeks. In recent years, the evaluation of the ADA activity in pleural fluid is proven to be sensitive, specific, and thus useful for the diagnosis of TPE.<sup>7-9</sup>

The ADA catalyses the conversion of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively. ADA is detected in lymphocytes primarily. Hence, the ADA activity in the pleural fluid reflects the cellularity of in the pleural compartment, particularly activated T lymphocytes.

The ADA activity may not be high in the TPE patients with the compromised immune system since the activity correlates to the local cellular immunity. The information about the specificity and the sensitivity of the ADA measurement in such situation is scarce. In few studies, Hsu et al.<sup>4</sup> investigated the ADA activity in the pleural fluid of 10 immunocompromised patients with TPE. The sensitivity of the procedure ADA was only 40%, using the cut-off level at 80 U/L. This suggests that the diagnostic value of the ADA assay in immunocompromised hosts is not useful as in immunocompetent hosts. However, this study was performed with a limited number of cases (n=10) with various underlying diseases: 4 diabetes mellitus, 2 uremia, 2 liver cirrhosis, 1 liver cirrhosis with diabetes mellitus, 1 chronic lymphocytic leukemia.<sup>4</sup> Hence, this study was not accepted generally. Another study show that HIV infection does not alter the ADA activities.<sup>10,11</sup> In addition, it has been reported that the level of interferon- $\gamma$  in the pleural fluid, another good diagnostic marker of TPE, was useful in immunocompromised patients.<sup>12</sup>

In this study, we investigated the ADA activity in renal transplant patients with TPE, whose immunity was compromised by immunosuppressive drugs. The chemistry reports of the pleural fluid in renal transplant patients was compatible to other reports. Lymphocytes were predominant in the effusion in 76% patients. The glucose level was less than 60 mg/dL in 27%.<sup>2</sup> Regarding the ADA activity, renal transplant patients were compatible to immunocompetent patients. Furthermore, the ADA levels in 2 patients were below the cut-off point. This sensitivity was 91%, which is comparable to other studies.<sup>7-9</sup>

The ADA activity in pleural fluid was measured by various methods. First, the relationship of the ADA activity in TPE patients was not correlated to the number of CD3, CD4 or CD8 positive cells, the total lymphocyte count, the ration of CD4:CD8 positive cells, or the proportion of CD3, CD4 or CD8 positive cells in the total lymphocyte. However, the ADA activity has been shown to correlate to the number of CD3 and DR positive T cells in the fluid.<sup>13</sup> Further studies are required, however, to determine the source of ADA in the pleural fluid and it's significance.

ADA is produced by T lymphocytes primarily. The ADA activity did not correlate to the number of T-lymphocytes in the pleural effusions.<sup>14</sup> The ADA activity was considered to be related primarily to the activity, proliferation, and differentiation of the T-lymphocytes in the pleural effusions. If ADA activity represented the rate of T lymphocytes proliferation, this may explain why the ADA level in the pleural fluid of malignant effusion containing predominantly lymphocytic cells is low. The immune system in the pleural space has been reported to be separated from other systems functionally. For example, significant defect was not detected in developing pleural granuloma formation in patients with very low CD4 counts.<sup>15</sup>

In conclusion, the ADA test is a useful method for the diagnosis of tuberculous pleural effusions, in immunocompromised patients such as renal transplant patients.

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