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Increased Pleural Fluid Adenosine Deaminase Levels in Patients with Malignant Pleural Effusions: A Potential Predictor of Talc Pleurodesis Outcome

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Abstract Chemical pleurodesis using various sclerosing agents is accepted palliative therapy for patients with recurrent, symptomatic, malignant pleural effusions (MPE). However, the utility of various clinical and biochemical parameters in predicting pleurodesis outcome is still controversial. The objective of this study was to investigate the relationship between pleural fluid adenosine deaminase (Pf-ADA) levels and talc pleurodesis outcomes, and to compare Pf-ADA levels to various other biochemical variables with respect to predicting talc pleurodesis outcome in patients with MPE. In this prospective trial, 60 consecutive patients with MPE were enrolled; 35 had malignant mesothelioma (MM) and 25 had metastatic pleural carcinoma (MPC). A complete response was achieved in 49 of 60 MPE patients (81.7%). The Pf-ADA, pH, and albumin levels in patients with successful pleurodesis were significantly higher than in those with unsuccessful pleurodesis (p values < 0.001, 0.036, 0.027, respectively). ROC curve analysis revealed that optimal differentiation between successful and unsuccessful pleurodesis could be achieved with cutoff points of 17.5 U/L for Pf-ADA (area under the curve = 0.873; sensitivity = 77.6%; specificity = 90.9%); >2.5 g/dl for albumin (area under the curve = 0.715; sensitivity = 85.4%; specificity = 54.5%); and >7.26 for pleural fluid pH (area under the curve = 0.703; sensitivity = 83.7%; specificity = 54.5%). In analysis of the subgroup, Pf-ADA were found to be a good marker for discrimination between successful and unsuccessful pleurodesis in patients with MM ($p < 0.001$) but not in the MPC group ($p = 0.068$). These results indicate that Pf-ADA levels could be considered predictors of the outcome of pleurodesis, especially in patient with MM. Furthermore, the present study also demonstrated that Pf-ADA level is a superior test to predict the outcome of pleurodesis compared to pleural fluid pH and albumin level.

Keywords Malignant pleural effusions · Adenosine deaminase · pH · Pleurodesis · Talc

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Introduction

In patients with cancer, malignant pleural effusions (MPE) continue to be commonly encountered and difficult to treat. For treating patients with symptomatic MPE, several different palliative modalities are available, including repeated thoracenteses, pleurodesis, long-term drainage with a small bore catheter, pleurectomy with decortication, and pleuroperitoneal shunting [1]. One of the key management goals is to prevent recurrence. Therefore, chemical pleurodesis using various sclerosing agents has been found to be useful palliative therapy for patients with recurrent, symptomatic MPE. Since sclerosing agents can have side effects, it is important to select patients who are likely to benefit from pleurodesis. Several reports have shown an association between pleural fluid pH or pleural elastance and pleurodesis outcome [2–4]. However, the utility of various clinical and biochemical parameters for predicting pleurodesis outcome is still controversial.

The presence of high levels of pleural fluid adenosine deaminase (Pf-ADA) is the most widely used test for the

diagnosis of tuberculous pleurisy. Pf-ADA levels have been reported to be significantly higher in tuberculosis than in MPE [5, 6]. Adenosine deaminase is a cytosolic enzyme that may contribute to the proliferation, maturation, and function of lymphoid cells; it is involved in purine metabolism. Since ADA plays a critical role in lymphoid cell function, ADA has been accepted as a marker of T-lymphocyte activation [5]. There are two ADA isoenzymes (ADA1 and ADA2). ADA1 is found in most body cells, particularly in lymphocytes and macrophages; ADA2 has only been found in macrophages [7]. Elevated ADA levels also have been reported in nontuberculous pleural effusions, including malignancies such as lymphoma and malignant mesothelioma [8, 9].

The present study investigated whether the presence of elevated Pf-ADA levels could be used as a predictor of talc pleurodesis outcome. The usefulness of Pf-ADA levels was also compared to that of other biochemical parameters for predicting talc pleurodesis outcome in MPE patients.

Materials and Methods

Study Population

Between May 2003 and May 2005, 60 consecutive patients with a pathologically confirmed diagnosis of MPE were prospectively enrolled. To be included, the patients had to fulfill all of the following criteria: biopsy-proven malignancy; recurrent symptomatic MPE that improved after drainage; complete lung expansion on chest radiography after drainage; general medical condition making pleurodesis possible; and expected survival of more than 1 month. Malignant pleural effusion was diagnosed when malignant tissue was demonstrated in the pleural fluid or on pleural biopsy.

The histopathologic examination of biopsy specimens from all patients was performed by faculty members in our pathology department. These samples were treated with hematoxylin-eosin, alcian blue, and mucicarmine histochemical stains. Immunohistologic confirmation of carcinoembryonic antigen, vimentin, keratin, and Leu-M1 were obtained in all samples.

The study was approved by the institutional review board of our hospital, and all patients gave their written informed consent.

Procedures and Assessments

A chest drain was inserted in all cases, and a large bore tube (24–28 Fr) with an underwater seal was placed in the seventh or eighth intercostal space at the posterior axillary line. Pleural fluid volumes were recorded daily, and a chest roentgenogram was taken daily. Pleurodesis was attempted when the drainage fell below 150 ml/24 h and the lung had expanded completely.

After instillation of 10 ml 1% lidocaine, 5 g sterilized talc was mixed in 150 ml physiologic saline under sterile conditions and instilled through the chest tube. Talc powder was gas-sterilized using ethylene oxide. The tube was clamped for 2 h and then opened. The chest tube was removed when the drainage fell below 150 ml/24 h and the chest X-ray showed complete lung expansion.

Postpleurodesis PA radiographs were obtained immediately after tube removal and 30 days after the procedure. At day 30, the radiographic response was classified as either successful, if there was no or only minor reaccumulation of pleural fluid, or a failure, if there was fluid reaccumulation.

Parameters Assay

In all cases the pleural fluid pH and ADA levels, as well as the levels of other biochemical parameters such as glucose, lactic dehydrogenase, total protein, and albumin, were routinely measured. For each subject, at least 20 ml of pleural fluid was collected in a syringe during thoracentesis. The results of only the first thoracentesis were considered. The ADA analyses were performed using the colorimetric procedure of Guisti and Galanti [10]. A 2-ml pleural fluid sample was collected in a heparinized syringe; the pleural fluid pH was measured within 30 min after thoracentesis. The blood pH/gas analyzer Rapidlab 1265 (Bayer, USA) was used for these measurements.

Statistical Analysis

All analyses were performed using SPSS v10.0 software (SPSS, Inc., Chicago, IL, USA). Nonparametric tests were used to analyze pleural fluid variables because these variables were not normally distributed. Data are reported as median and 25–75% interquartile range (IQR) unless otherwise stated. Medians were compared using the nonparametric Mann-Whitney U test, and qualitative variables were compared using the Fisher exact test. Furthermore, the receiver operating characteristic (ROC) curves were generated using commercial software, and the optimal cutoff point was determined for the mean pleural fluid ADA and pH levels. Statistical significance was accepted at $p < 0.05$. In the combination of variables, the test was considered positive if the cutoff point for any of variables was exceeded.

Results

The study population consisted of 60 patients (31 men and 29 women), with a mean age of 62.5 ± 11.5 years (range = 35–83 years); 35 patients had malignant mesothelioma (MM) and 25 had metastatic pleural carcinomas (MPC). The study patients' demographic and laboratory data are summarized in Table 1 and Table 2. Talc slurry pleurodesis was successful in 80.0% of patients with MM and in 84.0% of patients with MPC.

Among those with successful pleurodesis, the median ADA level was 28.0 U/L (range = 1.0–82.0 U/L; IQR = 19.5–44.5 U/L); among those with unsuccessful pleurodesis, the median ADA level was 7.0 U/L (range = 0.8–32.0 U/L; IQR = 3.1–17.0 U/L) ($p < 0.001$). A significant elevation in Pf-ADA was noted in patients with MM (median = 26.5 U/L; IQR = 17.0–46.0 U/L) compared with the level in MPC patients (median = 19.9 U/L; IQR = 11.1–32.0 U/L) ($p = 0.016$). In analysis of the subgroup, median ADA levels in patients with MM were significantly higher ($p < 0.001$) in patients with successful pleurodesis (median = 35.5 U/L; IQR = 24.5–50 U/L) than in patients with unsuccessful pleurodesis (median = 11.3 U/L; IQR = 3.1–17 U/L). Considering MPC alone, there was no difference between successful (median = 20.0 U/L; IQR = 12.2–34.0) and unsuccessful (median = 11.5 U/L; IQR = 2.2–25.7) pleurodesis ($p = 0.068$) (Table 3; Fig. 1).

Median pleural fluid pH levels among patients with successful pleurodesis were significantly higher (median = 7.34; range = 6.99–7.52; IQR = 7.30–7.40) than among patients with unsuccessful pleurodesis (median = 7.26; range = 7.05–7.43; IQR = 7.20–7.34) ($p = 0.036$).

Pleural fluid albumin was significantly higher in patients with successful pleurodesis (median = 2.9 g/dl; range = 1.4–4.4; IQR = 2.7–3.4) than in patients with unsuccessful pleurodesis (median = 2.5 g/dl; range = 1.4–3.6; IQR = 1.8–2.9) ($p = 0.027$).

The area under the ROC curve for ADA was 0.873 (95% confidence interval [CI] = 0.762–0.945). The optimal cutoff value for ADA was determined to be greater than 17.5 U/L, with a sensitivity of 77.6% (95% CI = 63.4–88.2), and a specificity of 90.9% (95% CI = 58.7–98.5). The area under the ROC curve for pH was 0.703 (95% CI = 0.571–0.814); at a cutoff limit of greater than 7.26, the sensitivity was 83.7% (95% CI = 70.3–92.7), and the specificity was 54.5% (95% CI = 23.5–83.1). For albumin, the cutoff value with the best diagnostic accuracy was >2.5 g/dl, with a sensitivity of 85.4% and a specificity of 54.5%. The ROC plots for pleural fluid ADA, albumin, and pH are shown in Figure 2.

In patients with MM, at a cutoff point of ADA levels ≥ 17.5 U/L, the test had a sensitivity of 100% (95% CI = 58.9–100) and a specificity of 92.9% (95% CI = 76.5–98.9) (Fig. 3).

To determine the predictive value of the pleural fluid parameters, we also combined different variables. The operating characteristics for the three variables with their cutoff points for achieving the best individual accuracy are shown in Table 4. The best combination of variables was ADA + albumin, with a sensitivity of 98, specificity of 54.5, and accuracy of 90.

Table 1 Study population's characteristics[a]

	Pleurodesis		
	Successful ($n = 49$)	Failed ($n = 11$)	p
Age ± SD (range)	62.1 ± 11.5 (35–83)	64.1 ± 11.4 (35–77)	0.607
Gender			
Male	22	9	0.043
Female	27	2	
Primary site of tumor (n)			0.748
Mesothelioma	28	7	
Lung	12	2	
Other origin[b]	7	1	
Unknown origin	2	1	

[a] Data are presented as n or mean ± SD (range)

[b] Breast, stomach, ovary

Table 2 Comparison of pleural fluid data before pleurodesis[a]

Biochemical variables	Successful pleurodesis ($n = 49$)	Failed pleurodesis ($n = 11$)	p
ADA (U/L)	28.0 (19.5–44.5)	7.0 (3.1–17.0)	*<0.001*
PH	7.34 (7.30–7.40)	7.26 (7.20–7.34)	*0.036*
LDH (IU/L)	728 (491–1102)	793 (533–1183)	0.766
Glucose (mg/dl)	69 (36–100)	82 (52–110)	0.953
Total protein (g/dl)	4.9 (4.5–5.5)	4.1 (3.6–5.7)	0.131
Albumin (g/dl)	2.9 (2.7–3.4)	2.5 (1.8–2.9)	*0.027*

[a] Data are presented as median (25–75% IQR); p values calculated with the Mann-Whitney U test

ADA = adenosine deaminase; LDH = lactic dehydrogenase

Discussion

Pleurodesis is used to prevent reaccumulation of pleural fluid in patients with MPE. The criteria that should be used to select patients for pleurodesis have not yet been determined. In this study, the potential role of Pf-ADA levels as a predictor of pleurodesis outcome was analyzed. To the best of our knowledge no previous studies have been published that focused on the use of Pf-ADA levels to

Table 3 Comparison of pleural fluid data before pleurodesis according to tumor types

Biochemical variables	MM		*p*	MPC		*p*
	Successful (*n* = 28)	Unsuccessful (*n* = 7)		Successful (*n* = 21)	Unsuccessful (*n* = 4)	
ADA (U/L)	35.50 (23–50)	11.3 (3–17)	0.000	20 (12.2–34.0)	11.5 (2.2–25.7)	0.068
PH	7.32 (7.29–7.37)	7.26 (7.11–7.37)	0.214	7.36 (7.31–7.40)	7.28 (7.22–7.32)	0.068
LDH (IU/L)	740 (524–1158)	1004 (589–1183)	0.682	698 (391–963)	593 (326–1141)	0.803
Glucose (mg/dl)	59 (35–95)	54 (13–82)	0.399	86 (51–106)	101 (87–129)	0.262
Total protein (g/dl)	4.8 (4.3–5.5)	3.7 (3.5–5.7)	0.222	4.9 (4.6–5.3)	4.4 (4.1–5.6)	0.331
Albumin (g/dl)	2.9 (2.6–3.4)	2.5 (1.4–2.9)	0.019	2.80 (2.70–3.45)	2.75 (2.55–3.40)	0.543

Data are presented as median (25–75% IQR); *p* values calculated with the Mann-Whitney *U* test

MM = malignant mesothelioma; MPC = metastatic pleural carcinomas; ADA = adenosine deaminase; LDH = lactic dehydrogenase

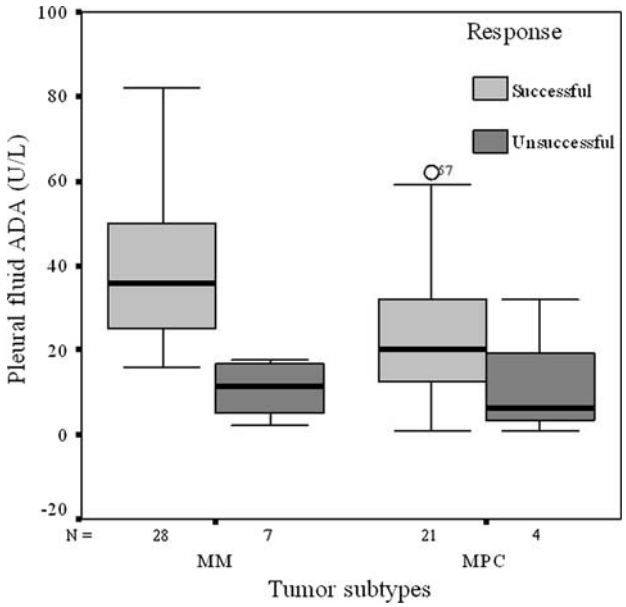

Fig. 1 Box plots showing pleural fluid ADA levels according to the outcome of pleurodesis and tumor subtypes. The difference between pleural fluid ADA levels in successful and unsuccessful pleurodesis was statistically significant in patients with MM ($p < 0.001$). In patients with MPC, there is a tendency but no significant differences in Pf-ADA level between successful and unsuccessful pleurodesis ($p = 0.068$) (MM: malignant mesothelioma; MPC: malignant pleural carcinoma)

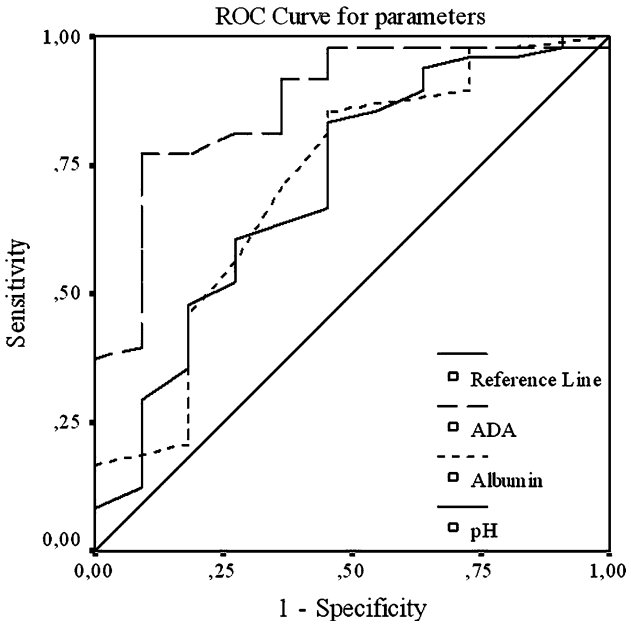

Fig. 2 ROC curve for pleural fluid ADA, pH, and albumin levels predicting successful pleurodesis. The areas under the ROC curve of pleural ADA, pH, and albumin were 0.873, 0.703, and 0.715, respectively

predict the outcome of talc pleurodesis. Using a cutoff point of >17.5 U/L, Pf-ADA levels had a sensitivity of 77.6% and a specificity of 90.9% for predicting successful pleurodesis. Pf-ADA levels were also found to be more accurate than pleural fluid pH for predicting the outcome of pleurodesis. In our study, the combination of several variables obtained better accuracy than did any one alone. We also analyzed the predictive value of these combinations. The combination of Pf-ADA and albumin offered better results for sensitivity, negative predictive value, and accuracy than individual variables or other combinations.

Though talc pleurodesis is a commonly used therapeutic option in MPE patients, serious complications such as adult respiratory distress syndrome can occur. Therefore, the availability of a marker that would be able to distinguish successful pleurodesis from unsuccessful pleurodesis would be very useful. Recently, Psathakis et al. [11] suggested that serial pleural fluid neutrophil and D-dimer values could be used to predict the outcome of talc pleurodesis. In the past, several other biochemical markers such as pleural fluid pH have been used to predict outcome. Pleural fluid pH reflects the tumor burden in the pleural cavity. Heffner et al. [3] reported that pleural fluid pH has only modest value for predicting symptomatic failure and should be used with caution when selecting patients for pleurodesis. The present study also found that pleural fluid

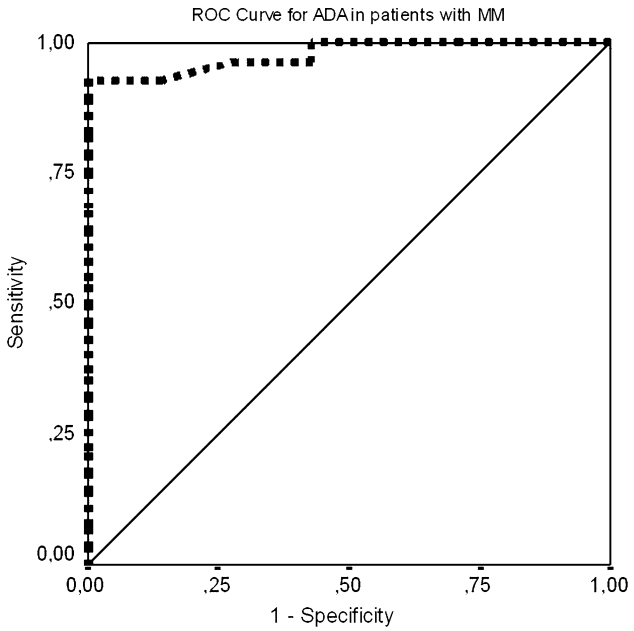

Fig. 3 ROC curve for pleural fluid ADA levels predicting successful pleurodesis in patients with MM. The area under the ROC curve of pleural fluid ADA was 0.977 (95% CI = 0.859–0.994)

pH levels were statistically significantly higher in patients with successful pleurodesis than in those whose pleurodesis failed. A pleural fluid pH value of >7.26 was the decision threshold value that had a high diagnostic accuracy for predicting the outcome of pleurodesis.

We found that patients with successful pleurodesis had significantly higher pleural fluid albumin levels than patients with unsuccessful pleurodesis. This was an unexpected finding because pleural fluid albumin levels have been found to increase depending on the degree of pleural microvascular injury [12]. It is also known that mesothelial cells are essential for good pleural symphysis because the mesothelium is the primary initiator of the biological cascade that leads to pleurodesis [13]. In the present study, pleural fluid ADA levels had the highest predictive value for differentiating between successful and unsuccessful pleurodesis compared to pleural fluid pH and albumin levels.

The major sources of Pf-ADA are lymphocytes or the monocyte-macrophage cell system. Therefore, the presence of ADA in various body fluids reflects the activity of cell-mediated immunity. In the pleural cavity, changes in the lymphocyte population may differ depending on the tumor type. Previous studies suggest that in malignant effusions secondary to non-small-cell lung cancer or breast cancer, CD4+ T lymphocytes, especially T-helper type 2, are dominant, whereas T-helper 1-type cells have been found to be the dominant CD4+ T lymphocytes in tuberculosis [14–16]. A correlation between Pf-ADA levels and CD4+ T-lymphocyte cell counts was recently documented in tuberculosis patients [17]. In contrast, De Long et al. [18] reported that the proportion of CD8+ T lymphocytes was significantly higher than that of CD4+ T lymphocytes in malignant mesothelioma patients with pleural effusions; they concluded that different tumors may trigger different responses from the host immune system.

The high incidence of malignant mesothelioma in the present study can be attributed to the fact that environmental asbestos exposure due to the use of asbestos-contaminated white soil is very widespread in our rural region. Consistent with the results of prior studies [8], in the present study the median Pf-ADA levels were significantly higher in MM patients than in MPC patients. One possible explanation for the elevated Pf-ADA level in MM patients compared to that in MPC patients may be differences in lymphocyte subgroups and consequently host immune responds as we mentioned above; however, the exact cause remains unknown. Previous studies have reported that CD8+ T lymphocytes are capable of producing several cytokines such as IL-2, IFN-γ, and TNF-α [11]. However, it should be noted that normal human mesothelial cells contain active cellular membranes that are capable of producing and secreting several cytokines, mediators, and growth factors [19]. In this study, when

Table 4 Operating characteristics of each variable and their best combination

	Cutoff point	Sensitivity (%)	Specificity (%)	PPV	NPV	Accuracy
ADA	17.5 U/L	77.6	90.9	97.4	47.6	0.8
Albumin	2.5 g/dl	83.7	54.5	83.7	42.9	0.78
pH	7.26	85.4	54.5	88.6	40	0.75
pH + ADA		98	45.5	88.9	83.3	0.88
pH + Albumin		93.9	27.3	85.2	50	0.82
ADA + Albumin		98	54.5	90.6	85.7	0.9
ADA + pH + Albumin		98	27.3	85.7	75	0.85

ADA = adenosine deaminase; PPV = positive predictive value; NPV = negative predictive value

analyses according to tumor subtypes were performed, Pf-ADA was found more effective in determining the success of pleurodesis, especially in patients with MM. In patients with MPC, there is a tendency but no significant differences in Pf-ADA level between successful and unsuccessful pleurodesis.

This study has a potential limitation in that only a small number of patients were enrolled. Studies involving more patients are needed to confirm our results. In this study, T-lymphocyte and ADA subgroups were not assessed, since our study was not designed to investigate the possible pathophysiologic mechanism of pleurodesis.

In conclusion, we have shown that Pf-ADA levels may be promising predictors to identify patients who would benefit from pleurodesis. Pf-ADA assay has an acceptable sensitivity with high specificity to predict the success of pleurodesis, especially in patients with malignant mesothelioma. However, these findings must be confirmed in larger studies.

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