Discrimination of Tuberculous from Carcinomatous Pleural Effusion by Biochemical Markers: Adenosine Deaminase, Lysozyme, Fibronectin and Carcinoembryonic Antigen

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We measured adenosine deaminase (ADA), lysozyme, fibronectin and carcinoembryonic antigen (CEA) in the pleural fluid of tuberculous and carcinomatous pleural effusion in order to discriminate these two groups. Tuberculous pleural effusion had significantly higher levels of ADA and lysozyme than did carcinomatous effusion. When ADA activity of more than 33 IU/l is considered, diagnostic tests of tuberculous effusions showed a sensitivity of 100%, specificity of 95% and accuracy of 96%. A pleural fluid/serum ADA ratio (pl-ADA/s-ADA) above 1.1 was found in 100% of tuberculous and in 53% of carcinomatous effusions (sensitivity 100%, specificity 47%, diagnostic accuracy 70%). A lysozyme level above 12 μ g/ml, selected as the discriminating limit, was found in 100% of tuberculous and in 17% of carcinomatous effusions (sensitivity 100%, specificity 83%, diagnostic accuracy 88%). Pleural fluid/serum lysozyme ratio (PL/SL) was also valuable in the discrimination of these two groups. When the cut-off level of 1.2 was considered, diagnostic tests of tuberculous effusions showed a sensitivity of 100%, specificity of 88% and accuracy of 93%, respectively. The mean fibronectin concentration in pleural fluid with tuberculous effusion was significantly higher than that in carcinomatous effusion, but there was a marked overlap between these two groups. On the other hand, CEA was significantly higher in carcinomatous effusions than in tuberculous effusions. At a cut-off level of 5 ng/ml, 53% of patients with carcinomatous effusion showed elevated pleural fluid CEA levels, while none of the tuberculous effusion did (sensitivity 53%, specificity 100%, diagnostic accuracy 65%). The combined assay of ADA, lysozyme, pl-ADA/s-ADA, PL/SL and CEA provide valuable information in the differential diagnosis between tuberculous and carcinomatous effusion, while fibronectin concentration can not be considered to have a definite diagnostic value.

Key Words: Pleural effusion, Tuberculosis, Carcinoma, Adenosine deaminase (ADA), Lysozyme, Fibronectin, Carcinoembryonic antigen (CEA)

In recent years, the incidence of tuberculous pleural effusion has decreased, while carcinomatous pleural effusion has been more often encountered than before. However, in many areas of the world, tuberculosis remains the most common cause of pleural effusions in the absence of demonstrable pulmonary disease (1). Therefore, it is important to discriminate between these two diseases in order to

make therapeutic plans and anticipate their prognosis precisely. Conventional analysis, such as employing bacteriological and cyto-histological methods, of pleural fluid is often deceiving and yields only a low diagnostic sensitivity. Recently several immuno- and biochemical approaches to pleural fluids have been proposed to improve the discriminating accuracy of diagnosis between tuber-

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culous and carcinomatous pleural effusion.

We measured adenosine deaminase (ADA), lysozyme and fibronectin, as well as carcinoembryonic antigen (CEA) in tuberculous and carcinomatous pleural effusions and evaluated the diagnostic value of these parameters in the differential diagnosis of these two groups.

MATERIALS AND METHODS

Fourteen patients with tuberculous pleural effusion and 37 patients with carcinomatous pleural effusion were included in the study. The diagnosis of tuberculous pleural effusion was established by means of positive culture results of pleural fluid or biopsy specimens (in two cases) and/or the existence of granulomas in the biopsied specimens (in twelve cases). The diagnosis of carcinomatous pleural effusion was based on a positive pleural biopsy and/or cytological results of the examination of pleural fluid. They included 23 bronchogenic carcinomas, 12 metastatic carcinomas (stomach, pancreas, gall bladder, liver, breast, uterus), one malignant lymphoma and one acute lymphocytic leukemia. Pleural fluid samples were collected in tubes containing sodium citrate as anticoagulant and

centrifuged for 10 minutes at 3,000 rpm and were stored at -20°C until assayed. In some cases, serum (plasma) samples were obtained on the same day and processed as described above. ADA activities were measured enzymatically using a commercial kit (AD test, Maruho, Osaka, Japan). Lysozyme level was measured by turbidimetric assay (2). Fibronectin concentration was measured by enzyme-linked immunosorbent assay (MBL, Nagoya, Japan). CEA levels were measured with latex agglutination kit available commercially (RCE-70, Sysmex, Kobe, Japan). For some of the parameters, values were established in relation to sensitivity, specificity and diagnostic accuracy. The results were expressed as mean $\pm$ SD. Statistical analysis of the differences of the results was performed using Student's two-tailed unpaired t-, non-parametric and chi-square tests. Linear regression was used to assess correlation between the parameters.

RESULTS

1) ADA activity in pleural fluid and pleural fluid/serum ADA ratio

The mean ADA activity in tuberculous pleural effusion was 73.6 ± 18.6 IU/l and was significantly

Fig. 1. ADA activity in pleural fluid (left) and pl-ADA/s-ADA ratio (right) in patients with tuberculous and carcinomatous pleural effusion (closed circle, tuberculous; closed square, pulmonary carcinoma; open square, metastatic carcinoma). Horizontal line indicate mean values.

($p < 0.001$) higher than that in carcinomatous pleural effusions (16.8 ± 8.9 IU/l) (Fig. 1, left). When ADA activity of more than 33 IU/l is considered, the diagnostic test for tuberculous pleural effusion shows a sensitivity of 100%, a specificity of 95% and an accuracy of 96%. Patients with tuberculous pleural effusion had a significantly ($p < 0.001$) higher mean pleural fluid/serum ADA ratio (pl-ADA/s-ADA) than patients with carcinomatous pleural effusion (2.97 ± 1.34 vs 1.03 ± 0.44) (Fig. 1, right). The ADA activity in pleural effusion did not correlate with lactic dehydrogenase (LDH) or lymphocytes number in effusion. There was, however, a significant correlation between ADA activity in a pleural effusion and the concentration of protein in that effusion ($r = 0.51$, $p < 0.001$) (data not shown).

2) Lysozyme level in pleural fluid and pleural fluid/serum lysozyme ratio

The lysozyme level in tuberculous pleural effusion (29.8 ± 9.7 $\mu\text{g/ml}$) was significantly ($p < 0.001$) higher than that in carcinomatous pleural effusion (9.0 ± 6.6 $\mu\text{g/ml}$) (Fig. 2, left). Lysozyme levels above 12 $\mu\text{g/ml}$, arbitrarily selected as the discriminating limit, were found in 100% of tuberculous and in 17% of carcinomatous (all metastatic) pleural effu-

sions (sensitivity 100%, specificity 83%, diagnostic accuracy 88%). Pleural fluid/serum lysozyme ratio (PL/SL) in tuberculous pleural effusion (2.7 ± 0.9) was significantly ($p < 0.001$) higher than that in carcinomatous pleural effusion (0.9 ± 0.3) (Fig. 2, right). When the cut-off level of 1.2 was chosen, the diagnostic test of tuberculous pleural effusion showed a sensitivity of 100%, a specificity of 88% and an accuracy of 93%. The lysozyme level in pleural fluid did not correlate with LDH or the number of granulocytes in an effusion, although there was a significant ($r = 0.38$, $p < 0.05$) correlation between lysozyme and protein concentration in pleural fluid (data not shown).

3) Fibronectin concentration in pleural fluid and pleural fluid/plasma fibronectin ratio

The fibronectin concentration in tuberculous pleural effusion (221.4 ± 125.4 $\mu\text{g/ml}$) was significantly ($p < 0.001$) higher than that in carcinomatous pleural effusion (129.4 ± 75.7 $\mu\text{g/ml}$) (Fig. 3, left). However, there were no significant differences in the pleural fluid/plasma fibronectin ratio between the tuberculous and carcinomatous pleural effusions (Fig. 3, right).

Fig. 2. Lysozyme level in pleural fluid (left) and PL/SL ratio (right) in patients with tuberculous and carcinomatous pleural effusion.

Fig. 3. Fibronectin concentration in pleural fluid (left) and pleural/plasma fibronectin ratio (right) in patients with tuberculous and carcinomatous pleural effusion.

Fig. 4. CEA level in the pleural fluid of patients with tuberculous and carcinomatous pleural effusion.

4) CEA level in pleural fluid

The pleural CEA level was markedly raised in the carcinomatous pleural effusion compared with that in the tuberculous pleural effusion ($p < 0.005$) (Fig. 4). CEA levels above 5 ng/ml were found in 17 out of 32 carcinomatous pleural effusions, while in none of 11 tuberculous pleural effusions. There was a significant difference between these two figures ($p < 0.001$). Among carcinomatous pleural effusion, patients with adenocarcinoma of the lung had significantly higher pleural fluid CEA concentration than those with other types of lung cancer ($1,570.4 \pm 4,292.9$ vs 127.6 ± 203.4). In metastatic carcinoma, high pleural CEA level were seen in patients with breast cancer, pancreas cancer and uterine cancer, respectively.

Figure 5 shows the relationship among ADA activity, lysozyme level and fibronectin concentration in pleural fluid. There were significant correlations between ADA activity and lysozyme level ($r = 0.84$, $p < 0.001$), and ADA activity and fibronectin concentration ($r = 0.39$, $p < 0.01$). There was, however, no significant correlation between lysozyme level and fibronectin concentration in pleural fluid. Table 1 shows the mean $\pm$ SD values of pleural ADA, lysozyme, fibronectin and CEA in tuberculous and carcinomatous pleural effusion. The

Fig. 5. Relation between ADA activity and lysozyme level (left), and ADA activity and fibronectin concentration (right) in pleural fluid.

diagnostic value of the parameters differentiating tuberculous from carcinomatous pleural effusion is summarized in Table 2.

DISCUSSION

The diagnosis of tuberculous pleural effusion is established on the basis of the determination of tubercle bacilli from pleural fluid, or pleural biopsy specimens or granuloma in a biopsied specimen from

Table 1. The mean $\pm$ SD value of pleural ADA, lysozyme, fibronectin and CEA in tuberculous and carcinomatous pleural effusion.

	ADA (IU/l)	Lysozyme (μ g/ml)	Fibronectin (μ g/ml)	CEA (ng/ml)
Tuberculous	73.6 $\pm$ 18.6 (n=14)	29.8 $\pm$ 9.7 (n=14)	221.4 $\pm$ 125.4 (n=12)	1.5 $\pm$ 1.0 (n=11)
Carcinomatous	16.8 $\pm$ 8.9 (n=37)	9.0 $\pm$ 6.6 (n=35)	129.4 $\pm$ 75.7 (n=33)	712 $\pm$ 2,876.5 (n=32)

* $p < 0.001$, ** $p < 0.005$

Table 2. Diagnostic value of parameters differentiating tuberculous from carcinomatous pleural effusion.

Parameters	Tuberculous	Carcinomatous	
		Pulmonary	Metastatic
Pleural ADA activity > 33 IU/l	14/14 (100%)	0/23 (0%)	2/14 (14%)
Pleural lysozyme level > 12 μ g/ml	14/14 (100%)	0/21 (0%)	6/14 (43%)
Pleural CEA level > 5 ng/ml	0/15 (0%)	14/21 (58%)	3/11 (27%)
PL/SL > 1.2	13/13 (100%)	0/4 (0%)	2/12 (17%)
pl-ADA/s-ADA > 1.1	13/13 (100%)	7/14 (50%)	2/3 (67%)

PL/SL: pleural lysozyme level/serum lysozyme level

pl-ADA/s-ADA: pleural ADA activity/serum ADA activity

the pleura. However, the pleural fluid culture results are frequently negative (3, 4). Pleural biopsy, on the other hand, is said to be very valuable in establishing the diagnosis of tuberculous pleural effusion. However, the initial biopsy of the pleura reveals granuloma in only about 60% of the cases when examining tuberculous pleural effusion (4, 5). Conventional chemical analysis of pleural fluid, such as employing pleural pH, glucose level and pleural lymphocytosis, is of limited value in the diagnosis of tuberculous pleural effusion (6).

Carcinomatous pleural effusion is confirmed if cytological or histological results are positive, and cytological examination is widely used as an aid in the diagnosis of carcinomatous pleural effusion. But in a summary of 18 series containing 2,677 patients with malignant pleural effusions, the sensitivity was at most 52% (7). Therefore, immuno- and biochemical approaches to examination of pleural fluids are proposed for the diagnosis of tuberculous pleural effusion in place of the conventional chemical analysis of pleural fluid or in conjunction with bacteriological, cytohistological diagnostic methods, while several tumor markers are available as an adjunct in the diagnosis of carcinomatous pleural effusion.

Piras et al. (8) first stated that pleural ADA activity is elevated in tuberculous effusion, and thereafter several investigators have proposed the usefulness of pleural ADA in the discrimination of tuberculous and non-tuberculous effusion (9–11). All authors seemed to agree that ADA activity is not raised in carcinomatous effusion. We also confirmed that ADA in tuberculous pleural effusion was significantly higher than that in carcinomatous pleural effusion. However, two cases of carcinomatous pleural effusion (gastric cancer, uterine cancer) have ADA activity above 33 IU/l in our study. But this is not contradictory to previous reports because the specificity of ADA in the differential diagnosis of pleural effusion is not 100% (12, 13). The value of pl-ADA/s-ADA ratio is also examined. Maritz et al. (9) indicated that a pl-ADA/s-ADA ratio below 1.1 makes tuberculous pleural effusion highly unlikely. Pettersson et al. (11) reported that pl-ADA/s-ADA ratios over 2.5 always denoted tuberculosis, empyema or RA and there was no false positive or false negative responses. In the

current study, a pl-ADA/s-ADA ratio over 2.0 always indicated tuberculous pleural effusion and below 1.1 highly suggested carcinomatous pleural effusion, although the specificity was not 100%.

ADA is abundant in lymphocytes, especially in T-lymphocytes, so lymphocytosis may be contributable to the elevation in pleural ADA activity. We could not, however, find any correlation between lymphocyte number and ADA activity in pleural fluid. Therefore, it may be assumed that in tuberculous pleural effusion, the origin of raised ADA activity is not in the absolute lymphocyte number but in the stimulated lymphocytes, although the exact reason why ADA is elevated only in tuberculous pleurisy among lymphocytic pleurisies is unclear.

Klockars et al. (4) first described that the lysozyme level in pleural fluid was significantly higher in patients with tuberculous pleural effusion than in those with pulmonary and metastatic carcinoma. They also stated that the PL/SL ratio is significantly raised in patients with tuberculous pleurisy than in those with other causes. Asseo et al. (15) indicated that all patients with tuberculous pleurisy had a PL/SL higher than 1.0. Further, Vereia Hernando et al. (16) established the PL/SL of 1.2 as the discriminating limit and suggested the usefulness of PL/SL in the discrimination of pleural effusions; that is sensitivity 100%, specificity 94.9%, accuracy 97.3%. We also confirmed the usefulness of PL/SL as well as pleural lysozyme level, although the discriminating accuracy was somewhat lower compared with that reported by Vereia Hernando et al. (16). A PL/SL above unity suggests the local production of lysozyme in pleural cavity. But lysozyme was not detected in any lymphocyte, the predominant cell in tuberculous pleurisy, or in pulmonary carcinoma cells (14). Lysozyme was identified immunohistochemically in epithelioid granulomas, activated macrophage in tuberculosis by Klockars et al. (14), suggesting the origin of lysozyme in tuberculous effusion being in the epithelioid granuloma or activated macrophage. ADA and lysozyme behave in a similar fashion as indicated in Fig. 5, although they have different sources.

Fibronectin concentration in pleural effusion has also been studied by several workers (17, 18).

Klockars et al. (17) studied fibronectin concentration in various pleural fluids and suggested that increased fibronectin concentration reflects the presence of tuberculous or connective tissue disease rather than carcinomatous or infectious pleural effusion. In our study, the mean fibronectin concentration in tuberculous pleural effusion was significantly higher than that in carcinomatous pleural effusion. But there are considerable variations in fibronectin concentration and overlapping of tuberculous and carcinomatous pleural effusion. So the determination of fibronectin concentration of pleural effusion may not be reliably diagnostic in determining tuberculous pleural effusion.

The diagnostic value of CEA in pleural effusion has been studied extensively by many investigators (7, 13, 19–21). According to these reports, the CEA level in carcinomatous pleural effusion is significantly higher than that in non-carcinomatous pleural effusion. When the levels of significance is established as 10–26 ng/ml, the specificity becomes 100% and the resulting degree of sensitivity ranged from 34–70%. Our results agree with those findings. But statistical analysis of CEA level between tuberculous and carcinomatous pleural fluid was performed using the nonparametric test because the distribution of CEA in carcinomatous pleural effusion did not meet the requirement of parametric tests.

In conclusion, pleural fluid ADA, lysozyme, PL-ADA/s-ADA, PL/SL and CEA provide useful information in the differential diagnosis of tuberculous and carcinomatous pleural effusion because of their high specificity, sensitivity and diagnostic accuracy, while fibronectin concentration can not be considered to have a reliable diagnostic value.

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