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Lung surfactant protein-A and carcinoembryonic antigen in pleural effusions due to lung adenocarcinoma and malignant mesothelioma

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Lung surfactant protein-A and carcinoembryonic antigen in pleural effusions due to lung adenocarcinoma and malignant mesothelioma. N. Shijubo, Y. Honda, T. Fujishima, H. Takahashi, T. Kodama, Y. Kuroki, T. Akino, S. Abe. ©ERS Journals Ltd 1995.

ABSTRACT: Lung surfactant protein-A (SP-A) is a major phospholipid-associated glycoprotein in surfactant, and is a useful immunohistochemical marker for lung adenocarcinoma. Carcinoembryonic antigen (CEA) has not been immunohistochemically detected in mesothelioma. In pleural effusions due to malignant mesothelioma, very low concentrations of SP-A and CEA can be expected. We studied the value of combined determinations of CEA and SP-A in pleural fluid to distinguish between lung adenocarcinoma and mesothelioma.

SP-A and CEA concentrations were measured in pleural effusions from 78 patients with lung adenocarcinoma and 10 with malignant mesothelioma.

SP-A concentrations in pleural effusions due to lung adenocarcinoma and mesothelioma were 516±140 and 16.9±3.6 ng·ml^{-1} (mean±SEM), respectively. CEA concentrations in pleural effusions due to lung adenocarcinoma and mesothelioma were 239±92.4 and 1.7±0.3 ng·ml^{-1}, respectively. SP-A values did not exceed 100 ng·ml^{-1} in any of 10 mesotheliomas, whilst in 37 of 78 lung adenocarcinomas they did. CEA values did not exceed 10 ng·ml^{-1} in any of 10 mesotheliomas, whilst in 53 of 78 lung adenocarcinomas they did. Increased values of SP-A and/or CEA were found in pleural effusions from 67 of 78 lung adenocarcinomas.

It is concluded that a combination of CEA and SP-A assays in pleural effusions will be helpful for discriminating lung adenocarcinoma from mesothelioma.

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Lung surfactant is a lipoprotein complex which is synthesized and secreted into the alveoli of the lung by type II pneumocytes [1]. Its major protein component, surfactant protein-A (SP-A), is a glycoprotein with a reduced denatured molecular mass of 35 kD [2]. We have developed an enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies against human SP-A [3]. The ELISA has been clinically applied to measure SP-A as a marker of surfactant in amniotic fluid [3, 4], sputum [5], pleural effusion [6], serum [7], and bronchoalveolar lavage fluid [8, 9]. In malignant pleural effusions, we have found high concentrations of SP-A only in patients with lung adenocarcinoma [6].

Carcinoembryonic antigen (CEA) is one of the best characterized tumour-associated markers [10]. Plasma levels of CEA are increased in patients with various cancers, and CEA has been expressed in cancer tissues. By contrast, CEA has not been found immunohistochemically in mesothelioma [11–13], and CEA levels are not increased in malignant effusions due to malignant mesothelioma [14, 15].

The aim of the present study is to clarify whether the determination of SP-A and CEA in pleural effusions is helpful for distinguishing lung adenocarcinoma from malignant mesothelioma.

Materials and methods

Patients

Seventy eight patients had a lung tumour on chest radiograph and computed tomography. A histological diagnosis of lung adenocarcinoma was obtained by transbronchial biopsy (n=38), percutaneous biopsy (n=7), surgical resection (n=21) or autopsy (n=12). The pleural effusions from these patients were found to contain malignant cells, and the cytological diagnosis was confirmed by two pathologists.

Ten patients with a history of asbestos exposure had malignant mesothelioma. The diagnosis, which was based on histological and immunohistochemical examination of pleuropneumonectomy (n=8) and autopsy (n=2), was confirmed by two pathologists. In the specimens, there was no histological evidence of a primary lung tumour.

The pleural effusion samples of these patients were collected before therapy. All tumours were immunohistochemically negative for CEA, when using the monoclonal antibody A5B7 against CEA (Dako A/S, Glostrup, Denmark). Histologically, seven tumours were epithelial, and three showed a biphasic pattern. Neither immunohistochemistry nor determination of SP-A were performed at the time of the diagnosis.

Pleural effusion samples were centrifuged at 400×g for 10 min at room temperature. After centrifugation, the supernatants of pleural effusions were collected and then centrifuged again at 550×g for 30 min. Supernatants were collected and then cryopreserved at -30°C until use.

ELISA for determination of human SP-A

ELISA kits were provided by the Teijin Institute of Bio-Medicine (Hino, Japan). The assay, which uses two distinct monoclonal antibodies PE10 and PC6 against human SP-A, was performed as described by SHIMIZU *et al.* [4]. Briefly, samples (200 µl) were serially diluted at 1/4 to 1/128 using buffer solution I (0.01 mol·l^{-1} phosphate-buffered saline (PBS), pH 7.2, containing 0.6% sodium dodecyl sulphate and 2% Triton X-100). Each sample was mixed thoroughly with 200 µl of peroxidase-labelled monoclonal antibody PE10 dissolved with buffer solution II (PBS containing 0.25% skimmilk). A plastic bead, coated with monoclonal antibody PC6, was added to each tube containing the above mixture. The tubes were then incubated at 37°C for 120 min. After incubation, the beads were washed sufficiently with distilled water. We added 400 µl of substrate solution (5 mmol·l^{-1} of hydrogen peroxide and phosphate citrate buffer, pH 4.0) and developer (0.6% tetramethylbenzidine HCl) to each tube. The tubes were incubated at 37°C for 15 min. The reaction was stopped by adding 1 ml of reaction stopper (1 N sulphuric acid). The absorbance of each tube was measured at 450 nm with a spectrophotometer. Several concentrations of purified SP-A with linear absorbance were used as standards. Human SP-A was purified from whole lung lavage fluids in patients with pulmonary alveolar proteinosis as described previously [7]. All determinations were made in duplicate, and data were expressed as mean values.

Determination of CEA in pleural effusions

CEA concentrations in pleural effusions were measured with radioimmunoassay using a commercial CEA kit (Dainabot Co., Tokyo, Japan). This assay system, which contains two distinct monoclonal antibodies, reacts with CEA, but shows almost no reaction against nonspecific cross-reacting antigen [16]. All determinations were made in duplicate, and results were given as mean values.

Statistical analysis

The data were expressed as means±standard error of the mean. The Mann-Whitney U-test was used to determine the statistical differences. The relationship between concentrations of SP-A and CEA in pleural effusions was assessed with the Pearson product-moment correlation analysis.

Results

Pleural fluid SP-A and CEA concentrations and their correlation

The average SP-A concentrations in pleural effusions due to lung adenocarcinoma and mesothelioma were 516±140 ng·ml^{-1} (range 3.0–9,530 ng·ml^{-1}; median 87.6 ng·ml^{-1}) and 16.9±3.5 ng·ml^{-1} (range, 2.0–38.2 ng·ml^{-1}, median 17.0 ng·ml^{-1}), respectively (fig. 1). SP-A values in pleural effusions due to lung adenocarcinoma were significantly higher than those due to mesothelioma ($p<0.001$).

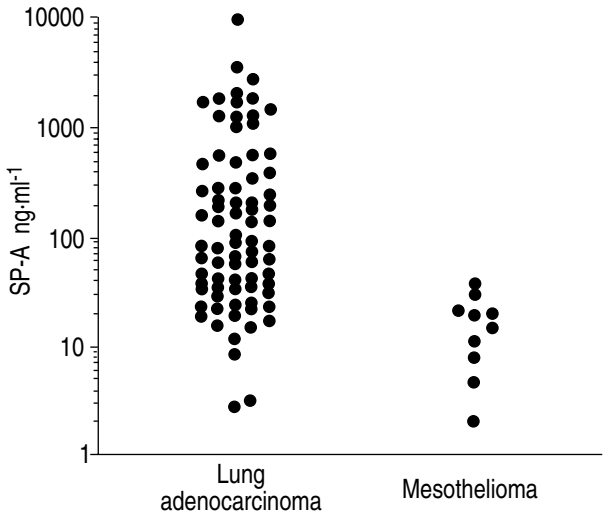

Fig. 1. – Lung surfactant protein-A (SP-A) concentrations in pleural effusions due to lung adenocarcinoma and malignant mesothelioma.

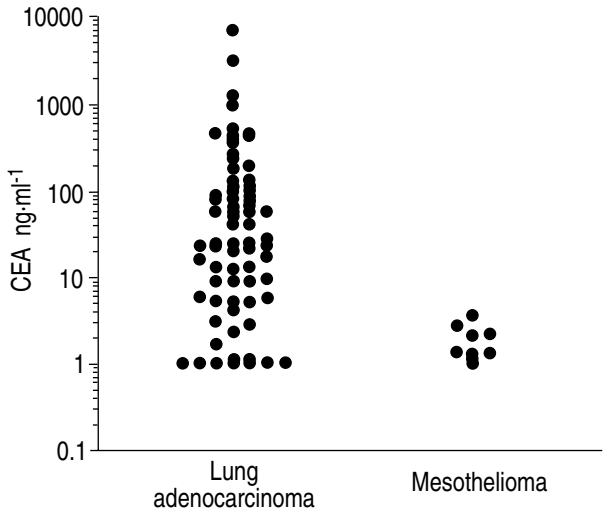

Fig. 2. – Carcinoembryonic antigen (CEA) concentrations in pleural effusions due to lung adenocarcinoma and malignant mesothelioma.

Table 1. – Combination of SP-A and CEA assay in malignant pleural effusions

	Lung adenocarcinoma	Mesothelioma
CEA > 10 ng·ml^{-1}	53/78 (64)	0/10
SP-A > 100 ng·ml^{-1}	37/78 (47)	0/10
CEA > 10 ng·ml^{-1} and or SP-A > 100 ng·ml^{-1}	67/78 (86)	0/10

Values are n/total population. The values in parentheses are percentages. Cut-off values of CEA and SP-A assay were set at 10 and 100 ng·ml^{-1}, respectively. CEA: carcinoembryonic antigen; SP-A: surfactant protein-A.

The average CEA concentrations in pleural effusions due to lung adenocarcinoma and mesothelioma were 239±92.4 ng·ml^{-1} (range 1.0–6,530 ng·ml^{-1}; median 26.3 ng·ml^{-1}) and 1.7±0.3 ng·ml^{-1} (range 0.9–3.4 ng·ml^{-1}; median 1.7 ng·ml^{-1}) (fig. 2). CEA values in pleural effusions due to lung adenocarcinoma were significantly higher than those due to mesothelioma ($p<0.001$).

The correlation between concentrations of SP-A and CEA were also analysed in all patients. There was no significant correlation ($r=0.129$).

Discrimination between lung adenocarcinoma and mesothelioma

The usefulness of combining SP-A and CEA assays for discriminating lung adenocarcinoma from mesothelioma was analysed (table 1). The CEA assay system in this study showed a relatively low cut-off value (2.5 ng·ml^{-1}) in plasma for cancers [15]. The cut-off value (100 ng·ml^{-1}) of SP-A assay in pleural effusions corresponds to the prior cut-off value (500 ng·ml^{-1}) when using human SP-A purified full-term amniotic effusions as standards [6]. CEA assay alone could discriminate 64% of malignant effusions due to lung adenocarcinoma from those due to mesothelioma. When using CEA and SP-A assays, the discrimination rate rose to 86%.

Discussion

Plasma levels of CEA are elevated in patients with various epithelial tumours, and have been used clinically in the diagnosis of certain types of cancer and for monitoring of disease progression [10]. Several studies [14, 15, 17, 18] have also demonstrated that measurements of CEA are of diagnostic value in malignant pleural effusions. By contrast, reactivity against CEA is negative or only weakly positive in mesothelioma [11–13, 19, 20]. Weak immunoreactivity to anti-CEA antibodies in mesothelioma may influence cross-reactivity of commercial anti-CEA antibodies with nonspecific cross-reactivity antigen. The current study demonstrated very low values of CEA in pleural effusions due to malignant mesothelioma. This finding is in agreement with previous studies [14, 15]. However, as shown here, CEA values in a number of malignant pleural effusions due to lung adenocarcinoma do not exceed the cut-off value.

SP-A is a major lung surfactant-associated hydrophilic glycoprotein [1, 2], and complementary deoxyribonucleic acid (DNA) for human SP-A has been characterized [21]. SP-A appears to be synthesized and secreted from type II pneumocytes and nonciliated bronchiolar epithelial cells in the lung under normal condition [22]. SP-A has been immunohistochemically found in approximately half of primary lung adenocarcinomas, but not in other histological types of lung carcinomas or in metastatic lung tumours [23, 24]. SP-A messenger ribonucleic acid (mRNA) was detected in 5 of 6 lung adenocarcinomas by *in situ* hybridization, but was not detected in a large-cell lung carcinoma or six lung squamous cell carcinomas [22]. Recently, however, immunoreactivity to antibody against human SP-A was found in a minor population of primary lung squamous cell carcinoma and large-cell lung carcinoma tissues [25]. GAZDAR *et al.* [26] reported that SP-A mRNA was found in 7 of 15 lung adenocarcinoma lines, whilst it was found in only one (a lung squamous cell carcinoma line) of 11 other histological types of lung carcinoma lines. Although it remains unclear whether SP-A synthesis from malignant cells is specific for lung adenocarcinoma, very high values of pleural fluid SP-A have been observed only in primary lung adenocarcinoma, and not in other histological lung cancer types [6].

NOGUCHI *et al.* [27] reported that SP-A expression can be used to distinguish lung adenocarcinoma from malignant mesothelioma, since SP-A expression has never been detected in mesothelioma immunohistochemically. Immunohistochemical studies with the monoclonal antibody PE10 against human SP-A demonstrated negative expression of SP-A in our series of 10 mesotheliomas (data not shown). The present study showed very low levels of pleural fluid SP-A in malignant mesothelioma. This result is in accordance with the immunohistochemical examinations, suggesting that mesothelioma cells do not produce SP-A.

We also demonstrated that SP-A values are independent of CEA values in malignant pleural effusions due to lung adenocarcinoma. KODAMA *et al.* [28] reported that in 2 of 28 goblet cell type mucus-producing lung adenocarcinomas (7%), SP-A was immunohistochemically expressed, whereas 20 of 28 (71%) had CEA. SP-A was detected in 18 of 24 (75%) bronchial gland cell type mucus-producing lung adenocarcinomas, and 21 of 24 (88%) had CEA, immunohistochemically [29]. Hence, SP-A expression in lung adenocarcinomas may be related to the subtype of lung adenocarcinomas in contrast to CEA.

In conclusion, we examined the value of combining SP-A and CEA determinations in pleural fluid. We found that in a malignant pleural effusion without an apparent lung tumour, the combined assay of pleural fluid SP-A and CEA will be helpful for discriminating lung adenocarcinoma from mesothelioma.

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