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The Role of Concurrent Determinations of Pleural Fluid and Tissue Carcinoembryonic Antigen in the Distinction of Malignant Mesothelioma from Metastatic Pleural Malignancies

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Abstract—*A combined determination of pleural fluid and tissue carcinoembryonic antigen (PF-CEA and T-CEA) by radioimmunoassay and immunoperoxidase staining technique respectively was performed in patients with malignant mesotheliomas* (12), *metastatic pleural carcinomas* (17) *and benign pleural diseases* (*seven*). *All PF-CEA-positive* (>39 ng/ml) *cases were T-CEA-positive metastatic carcinomas. In contrast,* 4/30 *PF-CEA-negative* (<39 ng/ml) *cases were T-CEA-positive metastatic carcinomas* (*three cases*) *and idiopathic pleuritis* (*one case*). *These results suggest that CEA, though present in the tumour, is not always released in measurable amounts in effusions. Hence T-CEA content should be determined in the PF-CEA-negative cases when an early and definite diagnosis of tumour type is required to enable correct management of these patients. These ancillary tests aim at enhancing the level of confidence of the routine morphological diagnosis of serous surface malignancies in living patients using minimal intervention instead of resorting to open chest surgery.*

INTRODUCTION

MORPHOLOGICALLY, the diagnosis of malignant mesothelioma is often difficult since it has a broad spectrum of histological appearances [1, 2]. Both benign reactive mesothelial proliferations and metastatic carcinoma, particularly pulmonary adenocarcinoma metastatic to the pleura [3], must be excluded with certainty in histological material. Light microscopic study can be supplemented by cytochemistry, immunocytochemistry, fluid cytology and chemistry, and ultrastructure according to a protocol of diagnostic approach to the tissue diagnosis of pleural disease [4]. These ancillary tests may prove to be useful, in order to increase the accuracy of the pathological diagnosis, particularly when tissue sampling is limited, such as in needle biopsy specimens [5]. The results of the tissue CEA test [6] are claimed to have value in distinguishing between malignant mesothelioma, which is invariably negative, and pleural metastatic adenocarcinoma, which is frequently CEA-positive [7–10]. We previously reported the value of CEA determination in a rapid screen approach to diagnosis of pleural effusions, showing that, in our experience, mesothelioma is not consistent with a PF-CEA greater than 15 ng/ml and specificity of the PF-CEA assay reaches 100% with the cut-off level at 39 ng/ml [11].

The purpose of this report is to correlate pleural fluid CEA (PF-CEA) concentration with tissue CEA (T-CEA) content as determined by the immunoperoxidase technique and to ascertain the role of the determination of PF-CEA and T-CEA in a practical approach to the diagnosis of serosal malignancies.

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MATERIALS AND METHODS

Seventy-two biopsy specimens of pleural tissues were obtained from 46 patients (39 men and seven women, aged 25–82 yr) admitted to the hospital with suspected pleural malignancies. Based on history and clinical studies, other primary sites of neoplasma were excluded. Pleural effusions were present in 41/46 patients, while five patients showed only radiological evidence of pleural thickening. Biopsy specimens were obtained by Abrams needle (ten cases), thoracoscopy (33 cases) and thoracotomy (three cases).

Light microscopic review of biopsy specimens was performed by three of the authors (A.D., P.G.B., R.B.) who had no clinical information, or results of the PF-CEA assay or immunohistochemical staining. Mesotheliomas were classified according to the scheme proposed by Clemmesen [12] and adopted by the Commission of the European Communities (CEC) Mesothelioma Panel.

PF-CEA was determined by radioimmunoassay, as previously described [11].

Immunoperoxidase staining for CEA was performed using the peroxidase anti-peroxidase (PAP) method on formalin-fixed, paraffin-embedded tissue specimens. Dako rabbit anti-CEA serum diluted 1:200 was used. Four-micron-thick deparaffinized sections were pretreated with 0.6% hydrogen peroxidase in methanol for 30 min to block endogenous peroxidase activity and, after rehydration, were subsequently treated with 0.1% trypsin in Tris buffer for 1 hr to unmask the antigens. The remainder of the staining procedure was carried out in the usual way using 30-min incubations at 37°C with antisera. For all test cases, both positive and negative control slides for CEA were made simultaneously. Staining intensity was estimated according to the following guide based on the proportion of stained cells and the intensity of staining: positive: >75% of the cells stained moderately or >50% of the cells stained intensely; equivocal: <50% of the cells were weakly stained; and negative: focal weak staining or no staining.

RESULTS

A definite diagnosis of pleural disease was made in 41 patients (Table 1), since tissue sampling was inadequate in five cases. Five more cases were excluded because no effusion was present. Only 36 cases were assessed for simultaneous determinations of PF-CEA and T-CEA. Twenty-nine out of 36 patients had primary or metastatic pleural malignancies, while seven had benign pleural diseases. There were ten cases of mesothelioma belonging to Clemmesen's A or B classes, i.e. definite or probable. Two cases were diagnosed as mesothelioma C, i.e. possible. Mesothelioma E (definitely not mesothelioma) included 16 metastatic carcinomas and a case of pleural involvement in disseminated non-Hodgkin's lymphoma. Ten of them were metastases of primary lung cancer, including seven adenocarcinomas and three squamous cell carcinomas, and six were from an unknown primary site.

Cytologic examination was positive for malignant cells in 66.6% of all pleural tumours. Needle biopsy, thoracoscopy and thoracotomy yielded a definite diagnosis in 80, 93.2 and 100% of cases, respectively.

In non-malignant pleural effusions the mean CEA concentration was 5.9 ng/ml (median 1.5 ng/ml; range 0–19.6 ng/ml). In mesothelioma-associated pleural effusions the mean CEA concentration was 3.2 ng/ml (median 3 ng/ml; range 0–7 ng/ml). In metastatic pleural effusions the mean concentration was 118.6 ng/ml (median 3.1 ng/ml; range 0–500 ng/ml).

Results of immunohistochemical staining for tissue carcinoembryonic antigen in the 36 patients with pleural effusion are shown in Table

Table 1. *Etiological diagnoses of pleural diseases*

Diagnoses	No. of patients
Malignant disease	34
Mesothelioma A or B	11 (1)*
Mesothelioma C	3 (1)*
Mesothelioma E:	20
metastasis from lung	11 (1)*
metastasis from unknown primary site	8 (2)*
disseminated non-Hodgkin's lymphoma	1
Benign disease	7
Tubercolosis	3
Trauma	1
Idiopathic	3

*The No. of cases with no effusion is shown in parentheses.

2. All of the ten mesotheliomas (A or B) exhibited negative staining for CEA. Nine of the 16 metastatic carcinomas were positive for CEA, five were negative and two were equivocal. All of the metastatic pulmonary adenocarcinomas, i.e. seven cases, were definitely positive. Of the two possible mesotheliomas (C), one demonstrated negative staining whereas one was equivocal. Tissue samples from all the benign cases of pleural disease were CEA-negative, except one case of idiopathic benign pleuritis.

Mean pleural fluid CEA in tissue-CEA-negative or equivocal cases was 3.8 ng/ml (median 2 ng/ml; range 0–19.6 ng/ml). In tissue-CEA-positive cases mean pleural fluid CEA was 199.7 ng/ml (median 139 ng/ml; range 0–500 ng/ml). Of the 30 cases in which PF-CEA was lower than 39 ng/ml staining for CEA was positive in four, negative in 23 and equivocal in three. In all of the six cases with PF-CEA greater than 39 ng/ml staining for CEA was positive (Fig. 1).

DISCUSSION

This study is consistent with our previous report [11] which suggested that malignant mesothelioma is not consistent with PF-CEA greater than 15 ng/ml. Mean PF-CEA was 3.2 ng/ml (median 3 ng/ml; range 0–7 ng/ml) in all mesotheliomas included in this second series. In the former study [11] 50% of patients with metastatic pleural effusions had PF-CEA concentrations which were lower than the cut-off level of 39 ng/ml. This percentage is higher in the present series, i.e. 64.7%, since only cases without clinical evidence of primary site of malignancy other than pleura were included.

Immunoperoxidase study of CEA in pleural tissue specimens seems to be of diagnostic value in the subset of PF-CEA-negative (<39 ng/ml) patients, based on the finding of four falsely PF-CEA-negative cases which showed postive T-CEA staining. In contrast, all PF-CEA-positive (>39 ng/ml) cases were T-CEA-positive. These findings therefore suggest that CEA, although present in the tumour, is not always released in measurable amounts into pleural fluid. Certain factors which may affect the concentration of PF-CEA include: tumour mass, tumour lysis, cellular turnover rate and degree of differentiation of the tumour. On the other hand, a possible alternative explanation for falsely negative PF-CEA levels might be the formation of CEA-anti-CEA complexes such that at least part of the CEA is no longer radioimmunoassayable. Hence T-CEA evaluation is useful in PF-CEA-negative patients with histologically proved pleural malignancy, when immunohistochemical study is performed in order to define the specific tumour type. The particular significance of the T-CEA assay in studying the origin of tumours is that whereas the test is frequently positive in endodermal- or ectodermal-derived neoplasms, it is invariably negative in neoplasms of mesodermal origin, including mesotheliomas. Wang *et al.* [6] first found that nine cases of malignant mesothelioma were all negative for a CEA-like substance but 12 cases of adenocarcinoma and bronchiolo-alveolar carcinoma were positive. Since then, other reports in the literature [7–10] have confirmed that staining for CEA may be a useful method for distinguishing mesotheliomas from carcinomas.

We believe that combined PF-CEA and T-CEA determinations can play a useful role in the diagnostic approach to neoplasms involving serosal membranes, and therefore in the development of a definitive diagnosis of malignant mesothelioma. This is especially valid when the amount of available pleural tissue is limited, and the clinical presentation, lacking a history of asbestos exposure, recurrent pleural effusion, or chest pain or pleural thickening, is not typical of mesothelioma. The peroxidase-anti-peroxidase technique has the distinct advantage of easy application on routinely processed histological sections and its importance is enhanced in the face of recent data [13] which challenge the value of histochemical stains for mucopolysaccharides in the diagnosis of mesothelioma [14]. Moreover, diagnostic criteria based on limited biopsy specimens and pleural fluid meet the increasing

Table 2. Results of immunohistochemical staining for tissue-CEA in patients with pleural effusion

	Negative	Equivocal	Positive
Mesothelioma A or B (10)	10	0	0
Mesothelioma C (2)	1	1	0
Mesothelioma E (17):			
metastatic pulmonary carcinoma (10)	3	0	7
metastasis from unknown primary site (6)	2	2	2
disseminated non-Hodgkin's lymphoma (1)	1	0	0
Benign pleural disease (7)	6	0	1

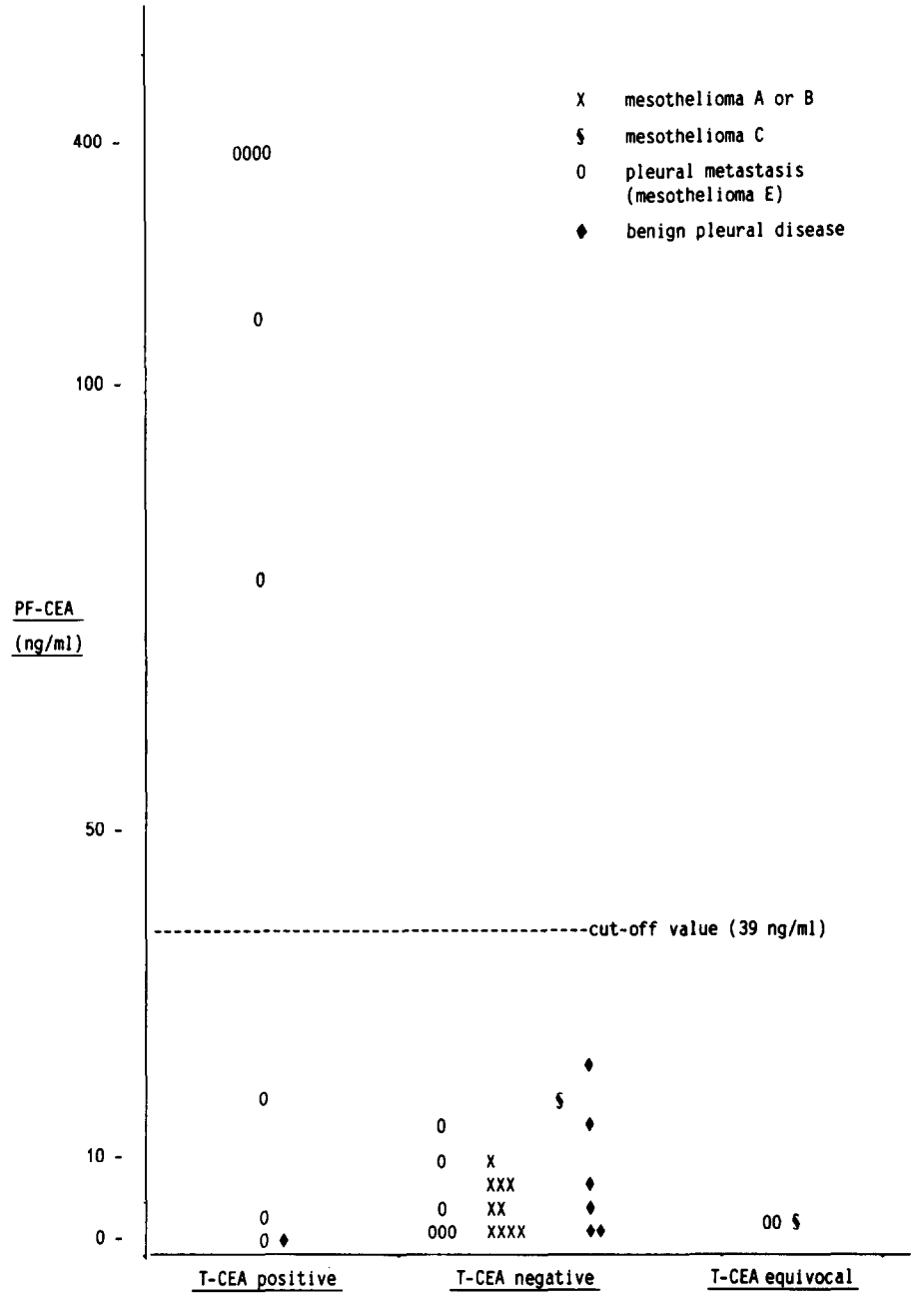

Fig. 1. Pleural fluid carcinoembryonic antigen (PF-CEA) values correlated with tissue carcinoembryonic antigen (T-CEA) staining results.

need to establish an early tissue diagnosis using minimal intervention instead of open chest surgery. This latter procedure may prove hazardous in mesothelioma patients because of possible neoplastic spread into the surgical wound and from there into the chest wall and nearby structures [15].

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