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Papers

Improvement of the Diagnosis of the Cause of Pleural Effusion in Patients with Lung Cancer by Simultaneous Quantification of Carcinoembryonic Antigen (CEA) and Neuron-specific Enolase (NSE) Pleural Levels

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Carcinoembryonic antigen (CEA) and neuron-specific enolase (NSE) level determinations were carried out by radioimmunoassay in pleural fluid and plasma samples obtained from 24 patients with malignant pleural effusions and 18 patients with non-malignant pleural effusions, and compared to cytological and pathological results. Using a pathological cut-off level of 25 ng/ml for CEA and 8 ng/ml for NSE, we demonstrated that, in the diagnosis of the malignant nature of pleural effusions, the simultaneous quantification of CEA and NSE in pleural fluid possesses better discriminative values than the simultaneous quantification of both markers in plasma or the separate quantification of each marker, in pleural fluid and in plasma.
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INTRODUCTION

THE AVAILABILITY of a rapid and formal proof of malignancy by using the less invasive procedures is a constant goal in the diagnosis of malignant pleural effusions. In this respect, cytological examination of pleural fluid (PF) has low sensitivity, while histological examination of tissue samples obtained by needle, surgical or perthoracoscopic biopsies remains the most efficient diagnostic procedure. The development of a lung cancer is frequently associated with high blood levels of tumour markers such as carcinoembryonic antigen (CEA) and neuron-specific enolase (NSE). Furthermore, in lung cancer, CEA and NSE levels correlate with disease extension and return to normal values in response to efficient treatment [1–3]. Isolated CEA or NSE pleural level quantification, combined with cytological examination, has been shown to be helpful in the diagnosis of malignant pleural effusions. In order to determine whether the simultaneous measurement of both CEA and NSE pleural levels can help for the diagnosis of malignancy, CEA and NSE pleural and blood levels were analysed in a prospective study. It was demonstrated that the simultaneous pleural quantification of both markers was more efficient than the separate quantification of pleural and blood levels of each marker and than the simultaneous quantification of both markers in plasma (P).

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MATERIALS AND METHODS

Study population

CEA and NSE blood and pleural levels were quantified in 42 patients with pleural effusions: 24 malignant pleural effusions related to histologically proven neoplasms [three small cell lung cancers (SCLC), 12 non-small cell lung cancers (NSCLC), three extrathoracic carcinomas, five mesotheliomas, one lymphoma], and 18 non-malignant pleural effusions (four congestive heart failure, four serous metapneumonic effusions, one tuberculosis, six idiopathic benign hydrothorax, one lupus, two chylothoraces). Cytological examination of PF was carried out in all patients. Pleural biopsies (needle or perthoracoscopic) with histological examination were performed in 14 cases of the malignant group and 6 of the non-malignant group. All patients were followed for at least 1 year after the diagnostic procedures in order to eliminate lung or pleural cancer occurrence in the benign group.

CEA and NSE determination

CEA was quantified by radioimmunoassay (RIA; Cis-France, Gif-sur-Yvette, France); in previous series with the same RIA kit, the mean blood value in healthy controls was 20 ± 5 ng/ml (unpublished data); thus, in this study, the pathological cut-off level was defined as 25 ng/ml. NSE was determined by RIA (Pharmacia, Saint-Quentin en Yvelines, France); in previous series with the same RIA kit, the mean blood value in healthy controls was 5 ± 3 ng/ml (unpublished data); thus, in this study, the pathological cut-off level was defined as 8 ng/ml.

Statistical analysis

All values are expressed as mean ± standard error of the mean (SEM), and statistical comparisons were made using

Mann–Whitney U-test, χ^2 and Fisher tests. For all patients, the effusions correctly or incorrectly identified by the different procedures as being malignant or non-malignant are defined as "true positives" (TP), "false positives" (FP), "true negatives" (TN), and "false negatives" (FN), the term "positive" referring to histologically proven malignant pleural effusion while non-malignant effusions being referred to as "negative". Sensitivity (S) was defined as TP/(TP+FN), specificity(s) as TN/(TN+FP), positive predictive value (PPV) as TP/(TP+FP), and negative predictive value (NPV) as TN/(FN+TN). P values <0.05 were considered as significant.

RESULTS

As already known, in patients with malignant pleural effusions, CEA levels (Fig. 1a) were elevated in PF (1029 ± 570 ng/ml) and in P (639 ± 570 ng/ml) in comparison to patients with benign effusions (PF: 6.3 ± 1.7 ng/ml, $P < 0.05$; P: 8.4 ± 2 ng/ml, $P < 0.05$). While in the benign group CEA PF and CEA P levels were always <25 ng/ml, CEA PF levels were elevated in the malignant group in 10/24 patients (maximal value 13 150 ng/ml), and CEA P levels in 9/24 patients (maximal value 12 700 ng/ml). Interestingly, CEA PF and P levels were normal in the 5 cases of pleural mesotheliomas. In the malignant group, CEA PF and P levels compared as follow: PF>P, $n = 10$; PF<P, $n = 9$; PF=P, $n = 5$).

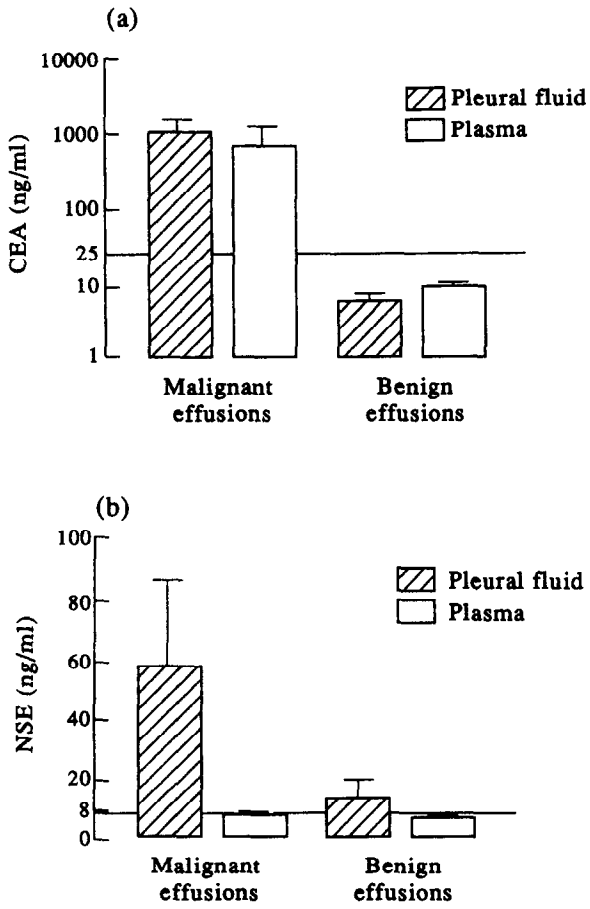

Fig. 1. Levels of tumour markers in plasma and pleural fluid of patients with malignant or non-malignant effusions. (a) Carcinoembryonic antigen (CEA): CEA was measured as described in Materials and Methods. The pathological cut-off level (25 ng/ml) is defined by a solid line. (b) Neuron-specific enolase (NSE): NSE was measured as described in Materials and Methods. The pathological cut-off level (8 ng/ml) is defined by a solid line.

Table 1. S, s, PPV and NPV of CEAP and PF levels, NSEP and PF levels, NSEP and PF levels, cytological examination of the PF (cytology), and their combination in the diagnosis of malignancy of 42 pleural effusions

	S	s	PPV	NPV
Cytology	25	100	100	50
CEA P	37.5	100	100	54.5
CEA PF	41.6	100	100	56.2
NSE P	37.5	88.9	81.8	51.6
NSE PF	54.1	88.9	86.7	59.2
CEA P and/or NSE P	58.3	88.9	87.5	61.5
CEA PF and/or NSE PF	66.7	88.8	88.8	66.7
CEA PF and/or NSE PF and cytology	71	89	89	30

The term "and/or" means that at least one of the criteria is positive, as described in Materials and Methods.

In a similar fashion, in patients with malignant effusions, NSE levels (Fig. 1b) were elevated in PF (58.5 ± 28.5 ng/ml) and in P (7.5 ± 0.8 ng/ml) in comparison to patients with benign effusions (PF: 12.5 ± 6.6 ng/ml, $P < 0.05$; P: 6.7 ± 0.9 ng/ml, $P > 0.05$, NS). In the benign group, NSE PF levels were <8 ng/ml in all patients except for one with tuberculosis (23 ng/ml), and one with lupus (123 ng/ml); NSE P levels were <8 ng/ml in all patients except for one with congestive heart failure (10.6 ng/ml), and one with idiopathic benign hydrothorax (19.6 ng/ml). In the malignant group, NSE PF levels were elevated in 13/24 patients (maximal value 640 ng/ml), and NSE P levels were only slightly elevated in 8/24 patients (maximal value 16.4 ng/ml). It is noteworthy that NSE PF levels were elevated in 12/21 malignant effusions not related to SCLC. In the malignant group, NSE PF and P levels compared as follow: PF>P, $n = 18$; PF<P, $n = 6$.

As already published, with regard to the diagnosis of malignancy, the discriminative values (S, s, PPV, NPV) of CEA PF and P levels were similar, while in contrast, the discriminative values of NSE PF levels (Table 1) were higher than those of NSE P levels (higher S and lower NPV, $P < 0.05$).

Interestingly, both S and NPV were increased when CEA and NSE PF levels were analysed together in comparison to both marker levels in P or to each marker taken alone either in PF or in P (Table 1).

The discriminative diagnostic value of cytology was markedly improved by the association of CEA and NSE quantification, and 11/18 cytology-negative pleural effusions of the malignant group had an elevation of at least one of the two markers (Table 1).

Out of the 20 patients whose diagnosis required pleural biopsy, histological examination revealed the following discriminative values: S = 85.7, s = 100, PPV = 100, NPV = 85.7and these values were improved by the combination of CEA and NSE pleural levels: S = 92.8, s = 83.3 PPV = 92.8, NPV = 83.8. In the malignant group, 12/14 of these patients had a positive histological examination for the presence of malignant cells, and out of these 12 patients, 7 exhibited a pathological elevation of at least one of the two markers.

DISCUSSION

Conventional cytological examination is highly specific for the diagnosis of malignant pleural effusions [4, 5], but has low S, due to the poor content of free malignant cells in the PF. Thus,

the most efficient diagnostic procedure remains the pathological examination of pleural biopsies. Although most of malignant pleural effusions are related to pleura invasion by tumour cells, in some cases lymphatic vessel obstruction, non-specific inflammatory effusion secondary to subpleural intraparenchymal lung tumour development or immunological mediated effusion can explain, at least in part, the apparent failure of cytological and/or histological investigations [5].

CEA represents a family of oncofetal glycoproteins (200 kDa), with blood level elevation in malignant epithelial carcinomas. In bronchogenic carcinomas, CEA plasmatic levels are elevated in 30–60% of patients (40–60% in NSCLC, and 20–40% in SCLC) [6, 7]. Recent observations [8] suggest that previously published CEA blood levels may not have been correctly quantified due to cross immunoreactivity between CEA and NCA (non-specific cross reacting antigen) and BGP (biliary glycoprotein). Furthermore, due to variations in the chosen cut-off levels and to heterogeneity in cancer types reported, some differences in CEA P and pleural diagnostic values can be noted. However, most papers confirm the efficiency of CEA pleural quantification in the diagnosis of the malignancy in pleural effusions. CEA pleural levels are elevated in 55–68% of malignant pleural effusions, whereas the simultaneous blood levels are only elevated in 30–40% of cases. CEA pleural levels are elevated more often in pleural effusions related to primary lung carcinomas than to secondary lung cancers. In most cases of malignant pleural effusions, CEA pleural levels are significantly higher than the simultaneous P levels, and about 30% of cytology-negative malignant effusions exhibit high levels of CEA in PF. In the diagnosis of the malignant nature of pleural effusions, CEA PF quantification S is 41–57%, and CEA PF quantification s is >92% [9–16].

NSE is an enolase isoenzyme found in the neurons of central and peripheral nervous system and in APUD (amine precursor uptake decarboxylation) cells; 50–70% of SCLC exhibit high NSE P and/or intracytoplasmic levels, especially in the "classic" histological subtype and in extensive diseases. But NSE blood levels are also elevated in 30–40% of NSCLC [6, 7]. Since pleural effusion is rare in SCLC, only a few data are available in respect to NSE pleural levels: in the previously published data, NSE pleural levels were elevated in 70–75% of SCLC-related pleural effusions and only 5–15% of NSCLC-related pleural effusions [19–21]. In marked contrast, 10/15 (67%) patients with malignant pleural effusions related to primary NSCLC or to extra-thoracic carcinomas exhibited high pleural NSE levels in our own series.

The fact that in 20–40% of malignant effusions CEA and NSE levels are low can be explained by phenotypic neuroendocrine differentiation (for SCLC) or oncofetal antigen reexpression (for NSCLC) not occurring in all tumoral cells, and by in some cases the production without release of tumour markers and, therefore, the absence of detectable levels in plasma or in pleural fluid. In these cases, the use of immunoperoxidase staining with monoclonal antibodies allows the detection of intracytoplasmic CEA or NSE in malignant cells, with improvement of the diagnosis of malignant pleural effusions [22–24].

Most papers confirm that CEA pleural levels show no elevation in malignant pleural mesothelioma. In our study, none of the effusions related to mesothelioma exhibited pleural or plasmatic elevation of CEA or NSE (except in 1 patient with a mild elevation of NSE P at 10.7 ng/ml). This explains the relatively lower S and s compared to other published series, which did not include mesotheliomas. In this respect, CEA quantification has been proposed as a discriminative marker to differentiate mesothelioma from metastatic pleural carcinoma (especially adenocarcinomas). Cibas [25] with immunohistochemical techniques, reported that 28/39 metastatic pleural adenocarcinomas stained for CEA whereas 0/20 malignant pleural mesotheliomas did, and Whitaker [26] reported 67% CEA pleural elevation in metastatic pleural adenocarcinomas and 0% in malignant pleural mesotheliomas, with a cut-off level of 15 ng/ml.

We conclude that the simultaneous pleural dosage of CEA and NSE demonstrates a high discriminative value for the non-invasive diagnosis of malignant pleural effusions related to SCLC, NSCLC or extra-thoracic metastatic carcinomas, but not to malignant pleural mesothelioma, and could be performed in all pleural effusions.

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Age and Prognosis of Non-small Cell Lung Cancer. Usefulness of a Relative Survival Model

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The aim of our study was the comparative evaluation of a relative survival model and a Cox model to determine the prognostic factors of survival for patients with surgically cured non-small cell lung cancer (NSCLC). We focused particularly on the exact role of age in this survival. 156 patients treated between 1975 and 1988 were studied. Both univariate and multivariate analyses were performed, using the actuarial method and the Cox model for crude survival and the Hakulinen model for relative survival. This study confirmed the poor prognosis of NSCLC, even if a curative surgical procedure has been possible, with a 5-year survival of 48% for stage I tumours but only 6% for stage III tumours. The most significant prognostic factor was the postsurgical TNM staging. The relative survival method of Hakulinen dismissed age as a significant prognostic factor. Our study underlines the usefulness of relative survival methods which should be more frequently employed to allow comparisons between series of different origin and to set up multicentre therapeutic trials.
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INTRODUCTION

DURING THE last 10 years, many survival studies of non-small cell lung carcinomas (NSCLC) undergoing surgical resection have been published. Data on prognosis factors of survival were frequently presented using univariate analysis models or, less often, multivariate analysis models. Survival rates were difficult to compare from one study to another because methods of recruitment of the cases were quite different between medical centres, especially regarding the age of the patients [1].

Indeed, for a NSCLC patient the risk of death results from the cancer-related risk of death and from the 'natural' risk of death from any other cause, which increases with age. Two adjusting methods can be used to take into account this 'natural' risk of death in survival analysis. The first one considers only death related to the disease currently studied and calculates the so-called 'net survival'. This method is rarely convenient and leads to underestimates of mortality [2]. The second useful method is 'relative survival', described by Berkson and Gage in 1950 [3] as an age-adjusted survival corrected for normal life expectancy [4]. The relative survival is the ratio of the survival rate of a group of diseased patients to the survival rate of a group of disease-free patients perfectly matched for age and sex. This method does not require any information on the true cause of death [4].

Multiple regression models of relative survival have been recently developed [2, 5] and they have already been used for the study of different types of cancer [6, 7]. Despite this, no clear data has been published so far on relative survival in NSCLC undergoing curative surgical resection. The aim of our study was the evaluation of a relative survival model compared with a Cox model, to determine the prognostic factors of survival for patients with surgically cured NSCLC. We focused particularly on the exact role of age in this survival.

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