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Clinical Usefulness of Serum Assays of Neuron-specific Enolase, Carcinoembryonic Antigen and CA-50 Antigen in the Diagnosis of Lung Cancer

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Serum concentrations of neuron-specific enolase (NSE), carcinoembryonic antigen (CEA) and CA-50 antigen were determined in 168 consecutive patients with lung cancer. All three markers were significantly elevated compared with levels in 102 patients with non-malignant chest diseases. NSE and CEA varied significantly across histological lung cancer types, with most highly elevated serum levels in small cell lung cancer and adenocarcinomas, respectively. The overall diagnostic accuracy was 0.66 for NSE, 0.74 for CEA, and 0.62 for CA-50, implying that CEA best discriminated between lung cancer and benign chest diseases, while CA-50 was less efficient as a diagnostic marker. In multivariate analysis of the three markers combined, a positive predictive value of 95% for lung cancer could be achieved with a diagnostic sensitivity of 57%, with a cut-off level defined as $0.037 \cdot NSE + 0.052 \cdot CEA + 0.011 \cdot CA\text{-}50 > 1$. In 22% of the cancer patients, the time from admission to histological or cytological lung cancer diagnosis exceeded 1 month. In 52% of these patients, the initial weighted tumour marker index was > 1, strongly implying the cancer diagnosis. The study lends support to the potential use of combined analysis of NSE, CEA and CA-50 as a complementary tool in the diagnosis of lung cancer.
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INTRODUCTION

DURING THE last decade a considerable interest has been taken in defining the role of tumour markers in the clinical management of lung cancer, e.g. diagnosis, staging, prognosis and monitoring of treatment [1, 2]. Patterns of correlations between various tumour markers and different histological types of lung cancer have been investigated, but no single substance has yet been identified as a diagnostic marker of major clinical importance in relation to established diagnostic procedures. Multiple-marker assays have been performed to enhance the diagnostic efficacy [3–8]. However, there is still a need for a clinically useful multivariate model for diagnostic purposes in the evaluation of lung cancer.

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For lung cancer patients in general, carcinoembryonic antigen (CEA) was one of the first markers described [9]. CEA is a glycosylated protein that is primarily associated with carcinomas within the gastrointestinal tract. In larger lung cancer series (> 100 patients), elevated serum CEA levels have been detected in 50–55% of the patients [10, 11]. CEA has been related to tumour burden in groups of patients, with possible prognostic implications in the preoperative assessment of lung cancer patients [12].

Neuron-specific enolase (NSE) has been extensively tested in small cell lung cancer (SCLC) [13]. NSE is a glycolytic enzyme found in neurons, peripheral neuroendocrine tissue and in tumours of the amine precursor uptake and decarboxylation system (APUD) cell series, the most important of which are SCLC and neuroblastoma. Elevated levels of NSE have been found in approximately 70% of SCLC patients. It has been related to the stage of disease both in groups of patients and in individuals, and has been shown to be an important prognostic factor in the treatment of SCLC [14]. In non-small cell lung cancer (NSCLC), elevated serum NSE levels have been reported in 10–20% of the patients. Recent studies imply that the

expression of NSE in NSCLC predicts a favourable response to chemotherapy [15–17].

The CA-50 antigen is present in carcinomas of various sites and histological types [18]. In larger, although not clinically representative, series of lung cancer patients, elevated serum concentrations were found in 44–51% [6, 13]. However, high rates (> 33%) of elevated serum CA-50 levels have been demonstrated also in non-malignant lung diseases [19], and its diagnostic properties, alone or in combination with other serum markers, are not satisfactorily established in the evaluation of lung cancer.

The aims of the present study were to evaluate the diagnostic properties of CA-50 in a clinically representative population of lung cancer patients; to compare the diagnostic properties of CA-50 with those of CEA and NSE; to test a simple multivariate model for optimal utilisation of the three tumour markers combined in the diagnosis of lung cancer in a standard clinical setting and to estimate to what extent tumour marker analysis provides additive diagnostic information in comparison with standard diagnostic procedures.

MATERIAL AND METHODS

The study comprises 311 consecutive patients who were referred to the Department of Pulmonary Medicine at Renströmska Hospital or to the Department of Thoracic Surgery at Sahlgrenska Hospital, Göteborg, between November 1987, and December 1988, for investigation of suspected lung cancer. 61% were men, and the age varied between 21 and 88 years (mean 64 and median 67 years). Diagnostic procedures varied depending on individual treatment considerations. A cancer diagnosis was established only if histologically and/or cytologically proven during the primary investigation or at a later point of time. The tumour stage was classified according to the TNM system [20]. The criteria of a benign diagnosis were no cytological or histological evidence of a malignant diagnosis, *and* either (a) a cytological, histological or microbiological non-malignant diagnosis with adequate therapeutic response, or (b) a complete disappearance of symptoms and signs of the disease. The follow-up period was ≥ 36 months (December, 1991).

Blood samples were collected at the referral or during the primary investigation before any cancer treatment was initiated. Quantitative measurements of NSE, CEA and CA-50 antigen in serum were performed, using the DELFIA system provided by Pharmacia. The DELFIA system is a solid-phase two-site fluoroimmunometric assay based on the direct sandwich technique, in which monoclonal antibodies are directed against antigenic sites on the antigen molecule. Then europium-labelled antibodies directed against a different antigenic site on the antigen molecule are reacted with the bound molecule. Finally, europium ions are dissociated from the labelled antibody by an enhancement solution, forming highly fluorescent chelates. The fluorescence from each sample is proportional to the concentration of the antigenic molecule in the sample.

The diagnostic accuracy of serum analyses of NSE, CEA and CA-50 antigen for lung cancer vs. benign diagnoses was assessed in three ways. First, a receiver operating characteristic (ROC) curve [21] was constructed for each of the tumour markers by plotting the sensitivity against the 1-specificity at different cut-off values of serum concentrations, defined by the percentile distributions in lung cancer and benign diagnoses, respectively. The area below the curve was used as a measure of the diagnostic accuracy of the test. The algorithm for calculating this area is:

$$1 + \frac{n_s + 1}{2n_f} - \frac{R_s}{n_f n_s},$$

where n_s is the number of cases (i.e. lung cancer patients), n_f the number of controls (i.e. patients with benign diagnoses), and R_s the rank sum of the cases.

Second, the sensitivity of each tumour marker was estimated at the minimum serum cut-off level yielding a positive predictive value (PPV) for lung cancer of at least 95%, which was considered as a prerequisite for clinical application.

Third, the sensitivity of the three markers combined was analysed, using a multivariate technique which is graphically represented by a correlation scattergram of all three markers on orthogonal axes (x, y and z) crossing through an area defined as $ax + by + cz = 1$. A solution for this discrimination function was sought with the same prerequisites as in the univariate analysis, i.e. a cut-off level yielding a PPV ≥ 95%, combined with an optimal sensitivity.

We also assessed the diagnostic yield from serum marker analysis in lung cancer patients with regard to the time span from admission to histologically or cytologically proven cancer.

The Mann–Whitney U-test was used for two-group comparisons, while the Kruskal–Wallis non-parametric analysis of variance was applied for comparison of three or more groups. The χ^2-test was used for comparison of proportions. Significance testing of correlations was performed with the Spearman rank correlation analysis, while the correlation coefficients given in the text are Pearson's product moment correlation coefficients. The significance level was set to 95% throughout.

RESULTS

In 168 of the 311 patients, a primary pulmonary malignancy was diagnosed (squamous cell cancer 42%, small cell cancer 21%, adenocarcinoma 23%, large cell cancer 6%, undifferentiated lung cancer 7%, carcinoid 1%, mucoepidermoid cancer 1%), while in another 9 patients a lung cancer was suspected but not histologically or cytologically verified (patients died without a postmortem examination or were otherwise lost to follow-up). 32 patients had extrapulmonary malignancies with thoracic engagement. In 102 patients a benign diagnosis was established.

The mean age was significantly higher in the lung cancer patients than in patients with benign diagnoses [67 ± 0.7 vs. 59 ± 1.5 years (mean ± S.E.M.), $P < 0.001$]. No significant age differences were seen between the various histological lung cancer types. The lung cancer rate was higher, but not significantly so, in men compared with women (68 vs. 58%, $P > 0.1$).

The percentile distributions of serum levels of NSE, CEA and CA-50 by diagnosis are shown in Figs 1–3. All three markers were elevated in lung cancer in comparison with benign diagnoses ($P < 0.001$ for NSE and CEA, respectively; $P < 0.005$ for CA-50), but only NSE and CEA varied significantly across different lung cancer types. NSE was elevated primarily among SCLC patients ($P < 0.001$), while CEA levels were most elevated in adenocarcinomas ($P < 0.05$).

There was no significant difference between the sexes with regard to the distribution of NSE and CEA, but CA-50 was more highly elevated in women than in men ($P < 0.05$), both in lung cancer and in benign chest diseases. CEA concentrations correlated moderately with age, but this correlation was mainly confined to patients with benign chest diseases ($r = 0.28$, $P < 0.05$).

In Table 1, the numbers of patients with elevated serum levels of NSE, CEA and CA-50 are displayed, using different cut-off

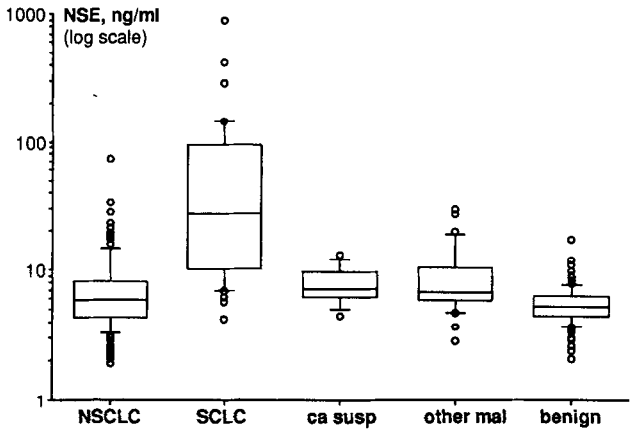

Fig. 1. Percentile distribution of serum concentrations of NSE by diagnosis. Each box contains the variable distribution between the 25th and 75th percentiles with the median value indicated with a line in the box. The bars extending above and below the box indicate the 90th and 10th percentiles, respectively. ca susp, Suspected lung cancer; other mal, other malignancies.

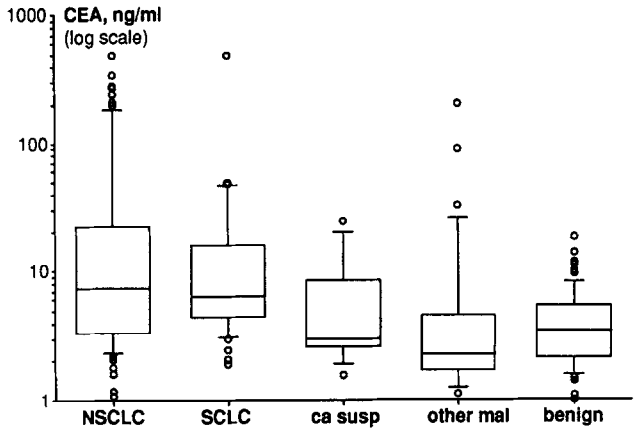

Fig. 2. Percentile distribution of serum concentrations of CEA by diagnosis. For explanation see Fig. 1.

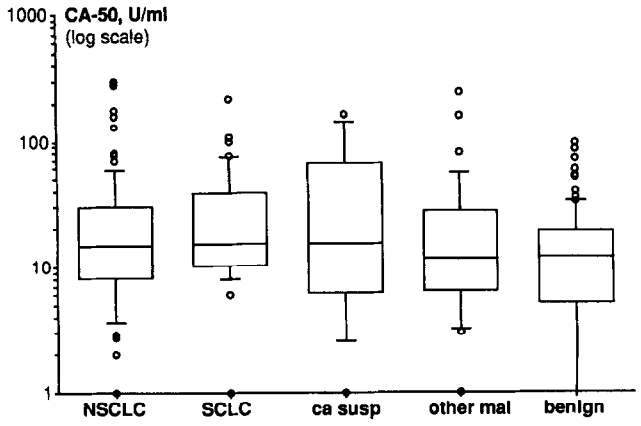

Fig. 3. Percentile distribution of serum concentrations of CA-50 by diagnosis. For explanation see Fig. 1.

Table 1. No. of patients (%) with elevated serum levels of NSE, CEA and CA-50

		Diagnosis		
Marker	Cut-off	NSCLC (n = 132)	SCLC (n = 36)	Benign disease (n = 102)
NSE	12.5 ng/ml	16 (12)	25 (69)	1 (1)
	25 ng/ml	4 (3)	20 (56)	0
CEA	5 ng/ml	82 (62)	25 (69)	28 (27)
	10 ng/ml	54 (41)	12 (33)	6 (6)
CA-50	15 U/ml	65 (49)	18 (50)	38 (37)
	25 U/ml	41 (31)	14 (39)	18 (18)

NSCLC, Non-small cell lung cancer; SCLC, small cell lung cancer.

levels. NSE levels > 12.5 were rarely seen in benign diagnoses but occurred in 12% of the NSCLC patients, while NSE levels exceeding 25 were seen almost exclusively among SCLC patients. CEA levels > 10 were seen in ≈40% of the lung cancer patients, while only 5% of patients with benign diagnoses had serum CEA elevated beyond this level. More than a third of the patients with benign diagnoses had elevated levels (> 15) of CA-50 and 18% had highly elevated CA-50 levels (> 25).

In Table 2 the distribution of serum concentrations of NSE, CEA and CA-50 by lung cancer stage is shown. The variance by tumour stage was statistically significant for all three markers ($P = 0.0005$, $P = 0.012$ and $P = 0.029$ for NSE, CEA and CA-50, respectively), with more highly elevated serum levels in stage 4 compared with stages 1–2 (all markers) or stage 3 (NSE and CA-50 only).

ROC curves for NSE, CEA and CA-50 as diagnostic markers for lung cancer vs. benign diagnoses are shown in Figs 4, 5 and 6, respectively. The diagnostic accuracy was 0.66 for NSE, 0.74 for CEA, and 0.62 for CA-50. Thus, CEA best discriminated between lung cancer and benign chest diseases, while CA-50 was less efficient as a diagnostic marker. Choosing a cut-off point at the 90% specificity level resulted in a sensitivity of 40% for NSE, 44% for CEA and 22% for CA-50, while the sensitivity values at the 95% specificity level were reduced to 31% for NSE, 38% for CEA and 12% for CA-50.

The cut-off levels yielding a PPV ≥ 95% were 12, 12 and 100 for NSE, CEA and CA-50, respectively, with sensitivity values of 28% (NSE), 33% (CEA) and 7% (CA-50). In other words, to predict a lung cancer diagnosis from an elevated serum value of

Table 2. Distribution of serum concentrations (mean values ± S.E.M.) of NSE, CEA and CA-50 by lung cancer stage (n = 168)

	Stage		
Marker	1–2	3	4
NSE (ng/ml)	10.9 ± 3.4	20.0 ± 6.3	78.8 ± 29.6
CEA (ng/ml)	31.7 ± 11.1	49.9 ± 18.9	78.9 ± 23.1
CA-50 (U/ml)	25.7 ± 4.8	22.2 ± 4.9	60.5 ± 13.3

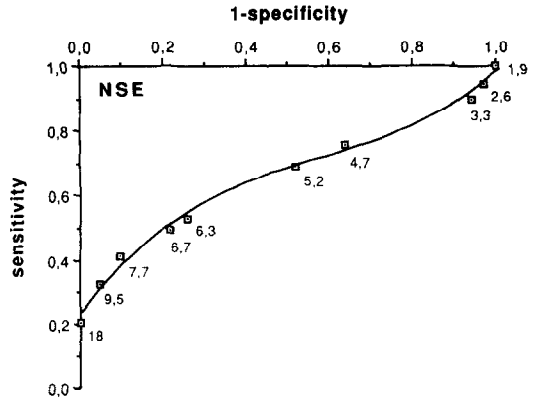

Fig. 4. ROC (receiver operating characteristic) curve for NSE. The curve plot is indicated with the series of cut-off points that yielded either specificity or sensitivity levels of 95, 90, 75 and 50%. The diagnostic accuracy, depending both on specificity and sensitivity and represented by the area beneath the curve, was 0.66.

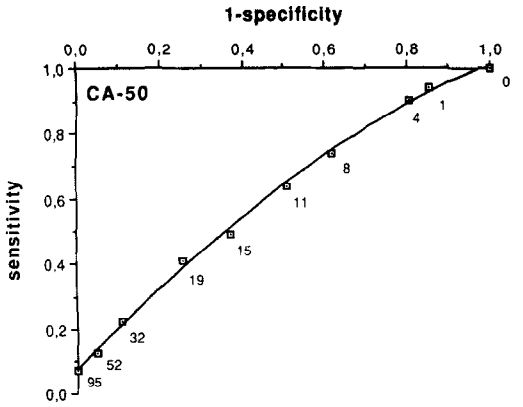

Fig. 6. ROC curve for CA-50; diagnostic accuracy was 0.62. For explanation see Fig. 4.

a single marker with a probability of at least 95%, only 7–33% of all lung cancer patients would be discriminated from patients with benign pulmonary diseases.

In multivariate analysis of the three tumour markers combined, the optimal solution for a weighted cut-off index with a prerequisite of a PPV for lung cancer ≥ 95% was: 0.037 · NSE + 0.052 · CEA+0.011 · CA-50 > 1, yielding an overall sensitivity of 57%. Thus, the diagnostic efficacy was significantly enhanced using a multiple marker assay, although 43% of the lung cancer patients remained undetected at this PPV level. When the multivariate model was applied in different tumour stages, the sensitivity was 39% in stages 1–2, 59% in stage 3 and 64% in stage 4. The tumour stage significantly influenced the variance of the weighted index ($P < 0.001$).

The time span from hospital admission to a histologically or cytologically proven diagnosis of lung cancer varied between 0 and 316 days. 50% of the lung cancer diagnoses were established within 7 days, 28% between 8 and 30 days, and 22% more than 1 month from admission. The diagnostic yield from multiple marker analysis in the corresponding patient groups was 63, 68 and 52%, respectively. Thus, in more than half of the lung cancer patients with a late diagnosis, the initial serum analysis strongly supported a cancer diagnosis.

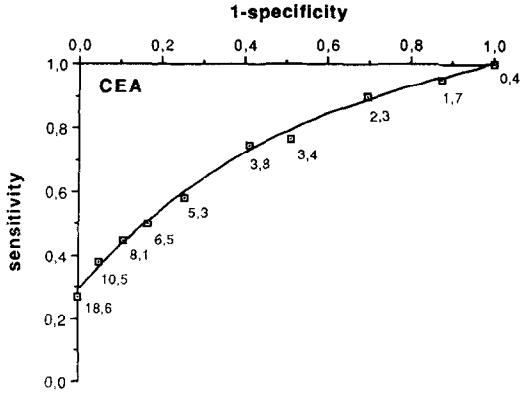

Fig. 5. ROC curve for CEA; diagnostic accuracy was 0.74. For explanation see Fig. 4.

DISCUSSION

The standard diagnostic procedures in the evaluation of suspected lung cancer include sputum cytology, bronchoscopy, and transthoracic needle aspiration (TTNA). The diagnostic sensitivity of sputum cytology varies between 40 and 70% of samples that are representative for the lower respiratory tract. In many patients, such representative samples are difficult to obtain, resulting in a considerably lower overall sensitivity. If sampling techniques are optimally utilised, bronchoscopy has a potential diagnostic sensitivity of > 80% [22]. In clinical practice, however, these results are difficult to achieve [23]. Furthermore, not all patients are eligible for a bronchoscopy, e.g. due to age or a poor performance status. TTNA is used preferably in cases of peripheral tumours and negative bronchoscopy, or in patients that are not eligible for bronchoscopy. In the latter patient category, high rates of complications following TTNA have been reported [24].

The major advantage of tumour marker analysis is the simplicity of the sampling technique (only a blood sample is required), which makes it applicable in all patients. For diagnostic use in the investigation of lung cancer, tumour marker analysis is potentially useful as a complementary tool if standard diagnostic techniques are not applicable or have failed to confirm a cancer diagnosis, provided a high predictive value for lung cancer could be achieved without losing too much of the diagnostic sensitivity of the test. However, the diagnostic accuracy of single markers has hitherto not fulfilled this requirement.

In general, our data are consistent with the results from previous studies of NSE and CEA in lung cancer. Neither of these markers detected more than one-third of the cases if a PPV for lung cancer ≥ 95% was required. The diagnostic accuracy of CA-50 in the current clinical setting was inferior to that of NSE and CEA, and as a sole marker for diagnostic purposes in lung cancer CA-50 probably has very limited clinical value. In the multivariate analysis, however, the diagnostic sensitivity of all three markers combined was substantially enhanced and comparable to that of sputum cytology with a consistently high PPV, which lends support for its potential use as a complementary tool in the diagnostic evaluation of lung cancer.

The positive correlation between tumour stage and the diagnostic sensitivity of the tumour markers probably affects the utility of the marker analysis in cases where the primary routine investigation has failed to establish a cancer diagnosis, since in these cases, stage 1 and 2 tumours are likely to be overrepres-

ented. On the other hand, stage 3 and 4 tumours probably occur more frequently in patients that are not eligible for extensive investigations, but who are likely to be detected with multiple marker analysis. In the present study, the results from multiple marker analysis did not vary significantly with respect to the time span from admission to diagnosis, implying that tumour marker analysis may also be informative in patients that are not amenable to standard diagnostic procedures or in patients where other diagnostic procedures have failed to confirm a lung cancer diagnosis. Overall, 22% of lung cancer diagnoses were confirmed more than a month after hospital admission. In 52% of these patients, a cancer diagnosis was implied from the initial combined analysis of NSE, CEA and CA-50. On the other hand, this means that in approximately 90% of all lung cancer patients, serum marker analysis either failed to detect a cancer diagnosis, or did not add any significant diagnostic information compared with standard procedures. Thus, the potential diagnostic utility is confined to a subgroup of patients as previously defined.

Since heightened serum levels of both CA-50 and CEA have been more frequently reported in non-malignant chest diseases in comparison with healthy subjects, the selection of controls may influence the diagnostic accuracy of the tests. In the present study, the diagnostic properties of NSE, CEA and CA-50 were investigated in a consecutive series of lung cancer patients vs. 'real' control patients (i.e. patients with benign chest diseases who were initially referred for investigation of suspected lung cancer), which facilitates clinical application of the data. The comparably large number of lung cancer patients and representative distribution across histological cancer types in our study enhances the validity of the results. The multivariate model used does not require special data equipment and is simple enough for clinical application.

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