Neurone specific enolase: a useful diagnostic serum marker for small cell carcinoma of the lung

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ABSTRACT Among lung cancers small cell carcinoma is the most sensitive to chemotherapy and radiation. This has emphasised the importance of an accurate diagnosis of this cell type, and the present study examined the use of serum neurone specific enolase (NSE) as a diagnostic marker for small cell carcinoma. NSE was measured in pretreatment sera from 103 patients with small cell carcinoma and in sera from relevant controls, including patients with other lung cancers, non-malignant lung diseases, and healthy adults. Serum NSE concentration was raised (> 25 ng/ml) in 72% of patients with small cell carcinoma. Ninety one per cent of patients with extensive disease and 50% of patients with limited disease were serum NSE positive. Patients with extensive disease in general had higher serum NSE concentrations than patients with limited disease. No definite difference in serum NSE positivity could be shown between oat cell and intermediate cell subtypes. Out of 51 patients with other lung cancers, four (8%) had a raised serum concentration, whereas all patients with non-malignant diseases and healthy individuals had normal serum NSE concentrations. Serum NSE determination seems to be a valuable tool for the diagnosis of small cell carcinoma.

Small cell carcinoma of the lung, accounting for about 20% of lung cancers,¹ has proved to be the lung cancer most sensitive to combination chemotherapy and radiation. This has resulted in a considerable improvement of survival time and, in a small group of patients with small cell carcinoma, a complete cure has even been documented.² A correct diagnosis of cancer cell type is therefore important and a serum marker would be of great value for identifying cases of small cell carcinoma. Several serum markers have been suggested but found to be of limited value, mainly because of insufficient specificity.³ Some promising reports on neurone specific enolase as a serum marker for small cell carcinoma have, however, been published recently.^{4,5}

Enolases (EC 4.2.1.11) are glycolytic enzymes, present in all human cells, catalysing the conversion of 2-phosphoglycerate to phosphoenolpyruvate. There are three dimeric isoenzymes, one of which is found in neurones and cells of neural origin.^{6,7} This form has been called neurone specific enolase (NSE) and is biochemically and immunologically distinct from the other two enolases.⁸⁻¹¹ Small cell carcinoma frequently shows neuroendocrine properties.^{1,12} Carney *et al*,⁴ in a series of 94 patients with small cell carcinoma, showed a positive correlation between serum NSE concentration and extent of disease and showed the value of serial NSE determinations in monitoring response to treatment. These authors did not include other types of lung cancers in their study. Ariyoshi *et al*⁵ found raised serum NSE concentrations in 13 out of 20 patients with small cell carcinoma before treatment but also in six patients with large cell carcinoma of the lung.

The aim of this study was to evaluate serum NSE as a marker for small cell carcinoma by the use of our anti-NSE antiserum in a newly developed radioimmunoassay.¹¹

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Methods

During 40 months 109 patients were diagnosed as suffering from small cell carcinoma at the department of pulmonary medicine, University Hospital, Uppsala. Apart from cases referred from other hospitals (76 patients), where a minor degree of selection might have operated, the material was unselected and irrespective of age and general physical condition all patients with the initial diagnosis of small cell carcinoma were included. In 105 cases the diagnosis was verified by biopsy specimens from one or several locations; in the remaining four cases the diagnosis was confirmed by cytology only. A histopathological review and classification of the biopsy material was made by one pathologist, who was unaware of the results of earlier classification. In the present study three patients were excluded from the consecutive series, one because of a revised histopathological diagnosis and two because of uncertainty of diagnosis. In all patients except three, frozen pretreatment serum samples were available for NSE analysis. Thus 103 patients with a verified diagnosis of small cell carcinoma and a serum sample drawn before treatment formed the basis of the study. There were 72 men and 31 women. The age of the patients at diagnosis ranged from 34 to 80 years with a median of 62 years. Fifty patients had oat cell carcinoma, 30 an intermediate cell type, and one a mixed cell type of small cell carcinoma. In 22 cases there were small or distorted biopsy specimens that did not allow a definite subclassification. For clinical tumour staging limited disease was defined as a tumour confined to one hemithorax and ipsilateral supraclavicular, hilar, or mediastinal lymph nodes. According to this definition 48 patients had limited disease and 55 had extensive disease.

Pretreatment serum samples from 51 patients with lung tumours other than small cell carcinoma and 169 patients with non-malignant diseases of the lung or mediastinum were used as control material. The histopathological diagnosis of these lung tumours was reviewed by the same pathologist, and

Serum neurone specific enolase (NSE) in haemolytic sera from 11 patients with small cell carcinoma. The degree of haemolysis in each specimen of serum was compared visually with the haemolysis in a set of sera with known haemoglobin contents. The first group (+) represents sera with barely visible haemolysis. Sera in the groups marked ++ and ++++ had haemoglobin concentrations of about 0.5 and 1.25 A_{500} absorption units respectively, while sera in the group marked (+++) had intermediate haemolysis. The dashed lines refer to the NSE concentration interval limits used in table 1. Six of the 11 sera were referred to lower NSE concentration intervals as indicated on the figure.

again he was unaware of earlier histological classification. Blood from 110 blood donors and 42 healthy hospital employees was used for control purposes. All serum samples were kept frozen until assayed for neurone specific enolase. Some control sera in the group with non-malignant diseases had been kept frozen for several years before analysis. The small cell carcinoma sera were kept frozen up to

Table 1 Results of serum neurone specific enolase (NSE) determinations. Number of patients in each serum NSE concentration interval

NSE (ng/ml serum)		≤12	13-25	26-50	51-100	101-150	>151
Small cell carcinoma before treatment	(n = 103)	15	14	30	25	7	12
extensive disease	(n = 55)	1	4	17	16	5	12
limited disease	(n = 48)	14	10	13	9	2	
Other malignant diseases of the lung	(n = 51)	38	9	3	1		
Non-malignant diseases of the lung and mediastinum	(n = 169)	138	31				
Healthy controls	(n = 152)	151	1				

Healthy controls (n = 152): $\bar{X}$ (= 7 ng/ml) + 3 SD = 12 ng/ml.

Table 2 Mean, median and range of serum neurone specific enolase (NSE) concentrations in pretreatment sera and numbers of patients with a concentration greater than 25 ng/ml in patients with small cell carcinoma of the lung

Patients	Serum NSE (ng/ml)			No (%) of patients with serum NSE > 25 ng/ml
	Mean	Median	Range	
Total series (n = 103)	69	46	6-372	74 (72)
Extensive disease (n = 55)	99	62	12-372	50 (91)
Limited disease (n = 48)	34	26	6-138	24 (50)
Oat cell subtype (n = 50)	81	49	8-372	39 (78)
Extensive disease (n = 28)	113	70	12-372	26 (93)
Limited disease (n = 22)	40	27	8-138	13 (59)
Intermediate cell subtype (n = 30)	48	40	9-216	20 (67)
Extensive disease (n = 16)	62	45	15-216	13 (81)
Limited disease (n = 14)	32	21	9-120	7 (50)
Mixed cell subtype (extensive disease) (n = 1)	62	62	—	1 —
Non-classifiable (n = 22)	70	42	6-270	14 (64)
Extensive disease (n = 10)	123	110	34-270	10 (100)
Limited disease (n = 12)	26	17	6-85	4 (33)

40 months and sera from patients with other lung tumours and healthy controls for a few months.

DETERMINATION OF SERUM NEURONE SPECIFIC ENOLASE

We have developed a radioimmunoassay for NSE utilizing specific antibodies to human NSE raised in rabbits.¹¹ Purified human NSE was used as the standard. Serum samples (100 µl) were analyzed in duplicates. Within one assay, the mean interduplicate variation of cpm readings was 3.7%. Between assays, the variation of NSE readings was 2.4 ng/ml (20 healthy controls). Proteolytic inhibitors were added to the reagent solutions. The assay was performed according to our earlier reported procedures.¹¹ Serum samples containing visible haemolysis were dealt with separately (see Results).

Results

EVALUATION OF SERUM SAMPLES WITH HAEMOLYSIS

As haemolysis is known to affect the serum NSE concentration, the thawed small cell carcinoma sera were examined against a series of sera to which increasing amounts of lysed blood cells had been added and where the concentrations of free haemoglobin had been measured spectrophotometrically. Eleven out of the 103 small cell carcinoma sera showed varying degrees of visible haemolysis. Because of the unique character of the material from patients with small cell carcinoma the corresponding patients were not excluded from the study. The NSE results for these patients were, however, subjected to separate evaluation. In the figure the serum NSE for each of the 11 patients was plotted

against the degree of haemolysis. Six patients had serum NSE concentrations just above the limits of positivity used in table 1 and were referred to lower categories, as shown in the figure. These adjusted results are included in tables 1 and 2 and referred to below.

SERUM NEURONE SPECIFIC ENOLASE IN PATIENTS WITH SMALL CELL CARCINOMA

The results of NSE determinations are summarised in table 1. The upper limit of the normal interval for serum NSE (12 ng/ml serum) is defined as the mean value for the 152 healthy controls (7 ng/ml) plus three standard deviations. The serum NSE concentrations of the healthy individuals showed no significant correlation with the age or sex of the donors. When the upper normal limit for serum NSE was taken to be 12 ng/ml, 85% of the patients with small cell carcinoma were found to have raised NSE concentrations compared with 25% of patients

Table 3 Serum neurone specific enolase (NSE) concentration in 51 patients with malignant disease of the lung other than small cell carcinoma (figures indicate the number of patients in each serum NSE concentration interval)

NSE (ng/ml serum):	≤12	13-25	26-50	51-100
Squamous cell carcinoma (n = 23)	19	3	1	—
Adenocarcinoma (n = 17)	12	5	—	—
Large cell carcinoma (n = 4)	2	1	—	1
Carcinoid tumour (n = 2)	2	—	—	—
Mesothelioma (n = 1)	—	—	1	—
Metastatic carcinoma from the ovary (n = 1)	1	—	—	—
Not classifiable (n = 3)	2	—	1	—
Total (n = 51)	38	9	3	1

Table 4 Serum neurone specific enolase (NSE) in 169 patients with non-malignant diseases of the lung and mediastinum (figures indicate the number of patients in each serum NSE concentration interval)

NSE (ng/ml serum):	≤12	13-25
Obstructive pulmonary disease (n = 62)	52	10
Restrictive pulmonary disease (n = 27)	20	7
Infective diseases (n = 58)	48	10
Benign tumours (n = 9)	8	1
Other diseases (n = 13)	10	3
Total (n = 169)	138	31

with other lung carcinomas and 18% of those with non-malignant diseases. When an arbitrary upper limit for normal values of 25 ng NSE/ml serum was used the specificity of a raised serum NSE concentration was substantially increased. NSE concentrations of 26 ng/ml serum or more were therefore denoted positive. With this criterion, raised NSE concentrations were found in 74 (72%) of the 103 patients with small cell carcinoma, in four out of 51 (8%) patients with other lung carcinomas, in none of those with non-malignant diseases, and in none of the healthy controls.

Fifty out of 55 (91%) of the patients with extensive disease had raised serum NSE concentrations. NSE levels were in general higher in extensive disease than in limited disease (table 2). Although the distributions of serum NSE concentrations in extensive and limited disease did overlap considerably, the difference was significant ($p < 0.001$, Student's *t* test). A serum NSE concentration above 150 ng/ml was found exclusively in patients with extensive disease (table 1). The mean and median serum NSE, like the frequency of NSE positivity in patients with oat cell type carcinoma was higher than for those with an intermediate cell type (table 2). Because of the relatively large number of patients in which a subclassification was not possible, these differences could not be considered significant.

SERUM NSE IN CONTROLS

Tables 3 and 4 show the composition and NSE concentrations of the control patients. Among the 51 patients with non-small cell carcinoma four were serum NSE positive. The mean serum NSE for this group was 12 ng/ml. The 169 patients with non-malignant diseases had a mean serum NSE concentration of 9.6 ng/ml and none had a value above 25 ng/ml.

Discussion

The presence of NSE in small cell carcinoma cells has been verified biochemically, by means of

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chromatographic separation of the different enolase forms from tumour specimens (Bergh *et al*, unpublished findings), and in small cell carcinoma cell lines.⁴ With an immunocytochemical technique NSE can be demonstrated in most small cell carcinoma specimens.¹³ NSE is a cytoplasmic enzyme and a significant increase in serum NSE is therefore most likely to be the result of an increased turnover of NSE containing cells. This is in line with our previous experience that there is a definite association between malignancy *per se* and raised serum NSE levels. This is in particular true for neuroblastoma, where in the malignant forms the NSE content of the tumour is lower than in the benign ganglioneuromas but where serum NSE is raised only in patients with malignant tumours^{14, 15} (also Esscher *et al*, unpublished findings). Moreover, raised serum NSE levels have been reported by others in patients with malignant but not with benign tumours.^{4, 5, 15, 16}

The present report describes a consecutive series of patients with a diagnosis of small cell carcinoma, who were staged according to routine clinical diagnostic procedures in one institution. Only 6% of the patients had to be excluded, and no selection was made. In 96% of the cases the diagnosis was based on tissue biopsy specimens giving a high degree of diagnostic precision. The control material represented a wide variety of diseases, covering most lung disorders that might be included in the differential diagnosis of small cell carcinoma.

Our upper limit for the normal serum NSE concentration (12 ng/ml) is the same as that determined by Carney *et al*⁴ for human NSE. Using rat NSE, Ariyoshi *et al*⁵ defined a lower normal limit. In the series of Carney *et al*⁴ 69% of patients with small cell carcinoma had a raised serum NSE concentration. The corresponding figure for the series of Ariyoshi *et al*⁵ was 65%. Choosing the more discriminative limit for elevated serum NSE concentration (>25 ng NSE/ml), we found that the resulting drawback of a decreased sensitivity was outweighed by a higher specificity. Even with this limit the frequency of serum NSE positive patients in the present series of patients with small cell carcinoma was much the same as that reported by Carney *et al*⁴ and by Ariyoshi *et al*⁵.

The presence of visible haemolysis in 11 sera from patients with small cell carcinoma created a problem of interpretation in these patients. We have previously shown¹¹ a covariation of haemolysis, measured as haemoglobin concentration, with serum NSE. In the individual case, however, the relationship is unpredictable. This is illustrated by the two patients with moderately haemolysed sera and very low serum NSE levels (fig). Our evaluation of the haemolytic sera was guided by earlier investigations

and we were careful not to overestimate the results. For practical use in clinical work sera with visible haemolysis should be avoided.

Like Ariyoshi *et al*⁵ and Carney *et al*,⁴ we found that patients with extensive small cell carcinoma generally displayed higher serum NSE levels than patients with limited disease. The pattern is, however, not fully consistent. Limited versus extensive disease is a clinical concept of some prognostic importance but, as defined, the concept does not refer to the tumour burden, which is most likely to influence the serum NSE concentration. The tumour mass in the individual patients with small cell carcinoma could not be estimated well enough to justify a detailed presentation. In patients with limited disease, however, where the tumour size could be calculated from chest radiographs, there was no strict association between serum NSE and tumour mass.

According to our results and those of others, some 30% of patients with small cell carcinoma do not have a raised serum NSE level. The explanation for this could be that these tumours contain no NSE or low amounts of NSE, or that they were small or had a slow cell turnover. Carney *et al*⁴ found a high NSE content in all of 10 cultured small cell carcinoma cell lines, but no significant correlation between the NSE content of the cultured cells and the serum NSE concentrations of the corresponding patients. In our series there was no definite correlation in the individual patients, between serum NSE and intensity of immunocytochemical staining for NSE (Esscher *et al*, unpublished findings).

In the series of Ariyoshi *et al*⁵ serum NSE positivity was more frequent in patients with tumours of oat cell subtype (nine out of 10) than in those with tumours of intermediate subtype (four out of 10). Our results suggest the same pattern, although the relatively large group of unclassifiable tumours makes such a conclusion uncertain.

Ariyoshi *et al*⁵ found raised serum NSE concentrations in six cases of large cell carcinoma (one with some morphological resemblance to adenocarcinoma) and a positive immunocytochemical staining for NSE in two corresponding tumours that were investigated. In the present study we found four serum NSE positive patients with non-small cell carcinoma tumours (a large cell carcinoma, a squamous cell carcinoma, a mesothelioma, and a non-classifiable tumour). These tumours thus seem to present a problem and interfere with the use of serum NSE concentration as a completely reliable marker for small cell carcinoma.

Taken together, the present study shows that 72% of patients with untreated small cell carcinoma had a raised serum NSE concentration (91% in the case of extensive disease and 50% in limited disease). Four

out of 51 (8%) patients with non-small cell carcinoma lung cancer had raised serum NSE. We think that the results indicate that NSE is useful diagnostic serum marker for small cell carcinoma, combining an acceptable sensitivity with a high degree of specificity.

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