Eur J Cancer Clin Oncol, Vol. 25, No. 1, pp. 123–128, 1989.
Printed in Great Britain

0277–5379/89$3.00 + 0.00

Neuron Specific Enolase, Carcinoembryonic Antigen and Lactate Dehydrogenase as Indicators of Disease Activity in Small Cell Lung Cancer

L.G.M. JØRGENSEN,* H.H. HANSEN* and E.H. COOPER†

**The Department of Oncology ONB, The Finsen Institute, Rigshospitalet, Copenhagen, Denmark and †The Unit for Cancer Research, University of Leeds, U.K.*

Abstract—*The clinical value of the three serum biomarkers neuron specific enolase (NSE), carcinoembryonic antigen (CEA) and lactate dehydrogenase (LDH) were evaluated prospectively in 86 patients with small cell lung cancer (SCLC) entered into randomized clinical trials. The patients were monitored clinically very closely and biomarkers were measured before each course of chemotherapy. The correlation between disease extent and biomarker was significant for both NSE (2P: 0.001) and LDH (2P: 0.05). Of those two biomarkers NSE was the most sensitive and was raised in 75% of all patients at diagnosis, in 67% of patients with limited disease, and in 86% of patients with extensive disease. All patients with three or more sites involved presented raised serum NSE levels but there was no significant correlation between definite number or specific sites known to have metastatic disease. There was a tendency towards a higher serum CEA level in extensive disease than in local disease. Only half the patients with metastatic disease had elevated (>5.0 ng/ml) levels of CEA, and values above 50.0 ng/ml were unusual. In patients initially seropositive for NSE a close correlation was found during follow up between serum NSE and response (98%) or progressive systemic disease (100%). During a major response, either complete or partial, serum NSE showed minor fluctuations (mean 8 ng/ml, S.D. 1.79, range 4.6–12.1).*

At present serum NSE seem to be the most sensitive and valuable biomarker in the management of SCLC, while the gain by adding CEA is small. Furthermore, NSE may be a useful tool in the estimation of disease extent and response to treatment in patients in whom clinical or radiological evaluation is difficult.

INTRODUCTION

THE ROLE of biomarkers in the management of small cell lung cancer (SCLC) is still unclear. Several markers have been advocated, including neuropeptides [1], calcitonin [2], carcinoembryonic antigen (CEA) [3], the acute phase reactant, alpha-1-acid glycoprotein (AGP) [4], and enzymes such as creatine kinase BB [5], deoxythymidine kinase, lactate dehydrogenase (LDH) [6] and neuron specific enolase (NSE) [7, 8].

Much attention has been focused on NSE in recent years, as it appears to have potential value for clinicians. The glycolytic enzyme enolase occurs in several isoforms, each of two sub-units. The gamma subunit has been shown by immunohistochemical staining to be present in differentiated neuronal tissue and in neuroendocrine tissue belonging to the cells of the amine precursor uptake and carboxylation system (APUD). Enolases containing this gamma subunit (alpha–gamma and gamma–gamma) have been called neuron specific enolase [9], and increased concentrations of NSE have been detected in tumors expressing APUD characteristics [10]. Small cell lung cancer is considered to be a tumor of neuroendocrine origin. An elevated pretreatment level of serum NSE has been reported in 40–70% of patients with local SCLC and in 83–98% of patients with extensive disease [7, 11–14]. These authors reported also that when the tumor responded to chemotherapy there was a considerable fall in the serum NSE level, which rose again at relapse. Enolase including NSE is an

Accepted 5 September 1988.
E.H.C. is supported by The Yorkshire Cancer Research Campaign. We wish to thank Ms. Lynda Rodgers and Ms. Kirsten Wolf for careful technical assistance.
Address reprint requests to Lone Jørgensen, Department of Oncology ONB, The Finsen Institute, Rigshospitalet, 49 Strandboulevarden, DK-2100 Copenhagen 0, Denmark.

intracellular enzyme concerned with energy production. Serum NSE may thus be a reflection of the turnover and cell death rate and release of enzyme in an NSE producing tumor.

To make a firm judgement about the clinical value of serum NSE measurements it seemed to be important to examine this marker prospectively in the context of a controlled trial and to compare NSE with two widely used markers, CEA and LDH which both have their advocates as aids to monitoring and assessing SCLC. In this paper we consider these three biomarkers in monitoring the disease, while in a following paper we will analyze their contribution as prognostic indicators.

MATERIALS AND METHODS

Patients

Eighty-six patients with histologically proven SCLC referred to the Finsen Institute for treatment between June 1985 and January 1987 were entered into the study. The follow up was closed on 31 March 1987. An additional 15 patients with SCLC were referred to the hospital during this time but they were excluded from the study, as no presentation serum samples were available. Pre-treatment staging procedures included clinical examination, chest X-ray, bronchoscopy, bilateral bone marrow biopsies and ultrasound or peritoncoscopy of the liver, including liver biopsy. The patients were defined as having local disease (LD) if the tumor was confined to one hemithorax including ipsilateral supraclavicular nodes or as extensive disease (ED) if the tumor had spread beyond these limits.

Treatment

All patients were treated according to protocol controlled trials chemotherapy using cyclic combinations [15, 16].

Clinical and laboratory assessment

Prior to commencing therapy the patients, except those who were critically ill, were staged as described above. Performance status (PS) was estimated according to the Eastern Cooperative Oncology Group (ECOG), and the serum levels of NSE, CEA and LDH were measured in all patients. At monthly intervals, before commencing the next chemotherapy cycle, the chest X-ray and the clinical condition were assessed and serum levels of NSE, CEA and LDH were measured.

All patients with a complete response (CR) based on clinical examination and chest X-ray following 3 months of chemotherapy were reevaluated by bronchoscopy and appropriate investigations of any other site that contained tumor at presentation. The patients were then reclassified according to the WHO criteria [17] as CR, partial response (PR) or no change (NC). Progressive disease (PD) was defined as an increase by more than 25% of existing lesions or the occurrence of metastases in new sites. Treatment was continued in accordance with the protocol until progression or for either 11 or 18 months. If the patients at that time at careful reevaluation were in CR, the treatment was discontinued. Twelve patients, who died within 1 month of presentation, were classified as early deaths.

Biochemical measurements

Serum NSE was measured by a radioimmunoassay NSE-RIA (Pharmacia Diagnostics AB, Uppsala, Sweden) [13] serum CEA by the Amerwell CEA-RIA (Amersham International plc, Amersham, Bucks, U.K.), serum LDH according to the Nordic recommendation [18].

The upper limits of normal values were as follows: NSE: 12.5 ng/ml, CE: 5.0 ng/ml and LDH: 450 U/l [19].

Statistics

As the biomarkers were not normally distributed in disease a nonparametric statistical test, the Wilcoxon signed-ranks test [20] was used to test the significance of disease related changes of the analyses. P values below 0.05 were accepted as significant.

RESULTS

Pretreatment levels, extent of disease and tumor burden

The pretreatment characteristics of the patients are listed in Table 1. Table 2 shows the median, mean and range of NSE, CEA and LDH levels at presentation according to extent of disease. It will be seen that the levels of NSE and LDH were significantly higher in ED compared to LD. On the other hand, whilst CEA tended to be lower in LD, there was no significant difference compared to ED.

Table 1. *Pretreatment characteristics in patients with SCLC*

	LD	ED
Number	49	37
Females (%)	19	12
Age (median)	62 (range 41–73)	61 (range 38–77)
Performance		
0	19	4
1	23	16
2	3	9
3	2	4
4	2	4
Bone marrow		
Metastases	0	16
Liver metastases	0	21
Cerebral involvement	0	6

LD: local disease, ED: extensive disease.

Table 2. Biomarker distribution at presentation

	Median	Range	Mean	*n*	2*P**
NSE(ng/ml)					
LD	19.4	3.3–96.7	25.2	49	0.001
ED	53.2	6.7–285.0	77.3	37	
CEA(ng/ml)					
LD	3.4	0.3–88.9	8.3	49	0.30
ED	6.1	0.1–121.0	19.6	37	
LDH(U/l)					
LD	387	218–929	430	47	0.05
ED	769	253–4640	898	37	

*Significance of difference between LD and ED. Description of the three biomarkers NSE, CEA and LDH in small cell lung cancer at presentation. LD = local disease, ED = extensive disease.

The distribution of NSE and CEA are compared in Fig. 1 and that of LDH is shown in Fig. 2.

Sensitivity

The percentage of raised biomarkers in patients with LD and ED is shown in Table 3. Only half the patients with metastatic disease showed an elevated CEA, and levels of CEA >50 ng/ml were unusual (7/86 = 8.0%). Six out of the 15 patients with normal levels of NSE and LDH and LD had an increase in CEA compared to four out of the five

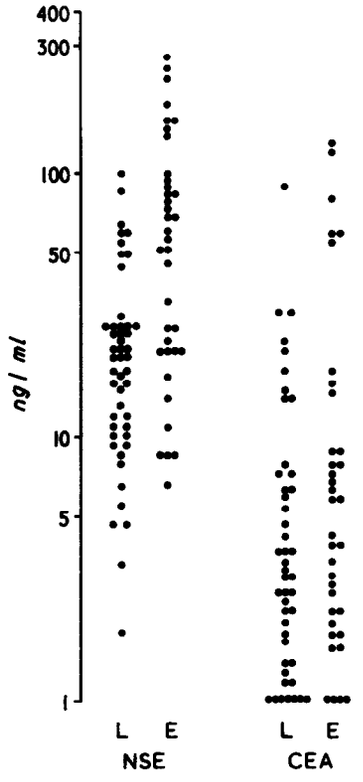

Fig. 1. Distribution of serum NSE and CEA in SCLC at presentation. L: local disease, E: extensive disease.

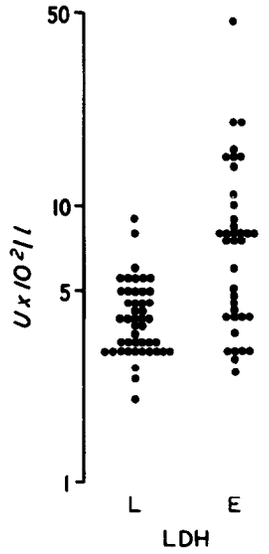

Fig. 2. Distribution of serum LDH levels in SCLC before starting chemotherapy. L: local disease, E: extensive disease.

Table 3. Frequency of elevated pretreatment levels of biomarkers

Test	LD	ED	LD + ED
NSE	34/49 (69)	32/37 (86)	66/86 (77)
CEA	18/49 (37)	20/37 (51)	38/86 (44)
LDH	18/47 (38)	25/37 (68)	43/84 (51)

Number elevated/total number tested (%). LD = local disease, ED = extensive disease.

patients with ED. A raised CEA, >5.0 ng/ml, with a normal NSE, <12.5 ng/ml, occurred in 5/47 (10.8%) cases of LD, and 4/36 (11.7%) in ED.

One out of 15 LD patients with normal NSE levels had increased serum LDH values. The corresponding values for ED were two of five patients. Normal levels of LDH, NSE and CEA were found in 9.3% of all cases, while NSE and CEA were both normal in 10.4% of all cases.

Distribution of metastatic disease

The percentage of patients with pretreatment elevated biomarkers according to the site and extent of metastases involved is shown in Table 4.

In ED there was no significant correlation between the number of sites of histologically proven metastases and the presentation level of NSE. However, 73% of patients with metastases involving a single site had a raised NSE at presentation, and all patients with two or more sites involved had a raised NSE at presentation.

Reevaluation

In 20 patients NSE was not increased at presentation (mean 8.4, S.D. = ± 2.5, range 3.3–12.1 ng/ml), but five of them later (median 253 days,

Table 4. Pretreatment increased levels of biomarkers by peripheral metastatic sites involved

	NSE	CEA	LDH
Liver	21/21 (100)	10/21 (48)	13/21 (62)
Bone marrow	15/16 (94)	9/16 (59)	14/16 (88)
One site	11/15 (73)	8/15 (53)	9/15 (60)
Two sites	12/12 (100)	6/12 (50)	7/12 (58)
Three sites or more	8/8 (100)	5/8 (63)	7/8 (88)

Number elevated/total number tested (%). All patients had extensive disease. Upper normal levels: see Biochemical Measurements. Two of the patients with ED had cerebral metastases only.

range 149–600) had progressive disease accompanied by an increased NSE level. Two patients developed isolated cerebral metastases without an increase in serum NSE. One patient died early. In one patient the disease status was classified as NC and no increase in NSE level was observed. In another patient the NSE level doubled during a pause in chemotherapy and returned to normal after chemotherapy was started again. In the 11 other patients NSE levels remained normal through an observation time of median 314 days (range 69–280). All achieved a major response CR or PR. Minor fluctations in serum NSE were observed during CR/PR and chemotherapy (mean 8 ng/ml, range 4.6–12.1).

The NSE levels in 52 patients presenting with a raised NSE had decreased to normal in 50 (96%) (mean 5.23, S.D. 2.12) at the time when they all with exception of two achieved a major clinical response, CR or PR. One was difficult to evaluate because of pleural effusion. The other failed to respond. One patient, who achieved PR, had a persistently raised NSE and 25 days later showed progressive disease. Of the 50 patients achieving CR or PR, 49 had normal levels of NSE (98%). Twenty-three patients achieved CR (mean 4.84, S.D. 2.18) and 27 patients PR (mean 6.24, S.D. 4.15). No significant difference was found in the NSE levels between CR and PR ($P < 0.05$).

In patients presenting with a raised CEA, 10 patients showed a fall to normal similar to the trend of NSE, but six showed persistently elevated CEA levels at a time when the NSE was normal, and this therefore appeared to be a better indicator of residual disease than NSE in this small number of patients. When the patients achieved a sustained CR or PR, the mean level of LDH in the whole group of ptients was 351 ± 177 U/l (range 195–1309).

Progression

Thirty-four patients having achieved CR or PR subsequently developed systemic metastases whilst receiving chemotherapy. The distribution of NSE levels in patients in CR or PR are compared to those at the time of diagnosis of PD and shown in Table 5. It will be seen that there was a significant increase of NSE associated with PD when the disease involved sites outside the CNS. Longitudinal studies indicated that the rise of NSE level preceded radiological PD in 64% of patients.

Following remission and during chemotherapy, cerebral metastases without evidence of systemic recurrence occurred in six patients. None of the biochemical indicators gave warning of this form of metastasis. In two patients localized recurrence with squamous cell carcinoma in the tumor was observed without change in the level of NSE or CEA.

CEA was an indicator of progression in only 53% of patients. In three of the patients with a rise of CEA associated with PD there was no coincidental rise of NSE. LDH rose in 59% of cases as the patients developed recurrent tumors but did not possess the sensitivity required to provide early warning of the recurrence.

DISCUSSION

The initial studies describing the use of NSE as a tumor marker in SCLC have used a mixture of prospective serum collection and retrospective samples from serum banks. These papers [7, 11–14, 19, 21] have clearly established the distribution of serum NSE at presentation, its fall in CR or PR and rise in progression. However, it still leaves open

Table 5. Serum NSE in progressive disease

	Clinical status	Mean (ng/ml)	Range (ng/ml)	Frequency elevated (%)
Systemic disease	CR/PR	6.1	02.6–019.5	1/34 (03)
	PD	74.2	14.1–363.0	34/34 (100)
Isolated cerebral metastases	CR/PR	7.9	2.7–024.4	1/6 (17)
	PD	8.7	5.9–011.2	0/6 (00)

Response to treatment was evaluated according to the WHO criteria and graded as complete response (CR), partial response (PR), no change (NC) or progressive disease (PD).

questions about the value of NSE in closely monitored patients, and whether it is really the test of choice compared to LDH and CEA. This study was designed to evaluate further these questions, in the context of a single institution committed to protocol controlled clinical trials of therapy for SCLC.

In our patients serum NSE was the biochemical parameter, which correlated best with untreated and recurrent disease. It was raised in 75% of all patients at diagnosis, in 67% of patients with LD, and in 86% of patients with ED. Moreover, all patients with metastatic tumors in two or more sites presented raised (>12.5 ng/ml) serum NSE levels. The median serum NSE in patients with LD was significantly lower than that in patients with ED. Both in case of peripheral CR/PR or PD a close correlation was found between the clinical findings and the response indicated from the serum NSE level. However, NSE is not sensitive enough to detect residual disease in PR; similar insensitivity has been observed in neuroblastoma [22]. In other studies [7, 23], no correlation was found between initial serum NSE level and response to treatment. Since our data suggests a close correlation between tumor burden and serum NSE levels, serum NSE measurements may be of value in assessment of tumor burden in SCLC at presentation and a helpful parameter during therapy. In this closely monitored group of patients the rise of NSE was generally the most reliable indicator of the advent of systemic recurrence.

The development of a pulmonary recurrence with a normal NSE, which histologically was found to be a squamous cell cancer, has been described by Splinter *et al.* [21], and we observed two such cases in our series. This is probably due to the suppression of a chemosensitive SCLC and the slow growth of a chemoresistant NSCLC in a tumor of mixed differentiation.

The combination of NSE and CEA in SCLC has previously been examined [2, 3, 24]. The precise inter-relationship of these studies is difficult to assess as they use different CEA assays. However, a number of general points emerge. In Havemann's multicenter study a fall of CEA after the first 2–3 courses of chemotherapy was a valuable sign of a treatment success, but it was not helpful as a marker of early progression. A similar conclusion is drawn by Sculier *et al.* [25] in their study of 180 patients with SCLC. Adewole and Newlands [24] advocate using both NSE and CEA to monitor the clinical course of the disease and to predict recurrence. By contrast, Aroney *et al.* considered the combination of CEA, NSE and LDH when used to monitor disease activity in SCLC were no more sensitive than standard clinical methods. Woo *et al.* [26] found a correlation between disease status and CEA level above a fixed level of CEA. This study did not, however, include an evaluation of NSE. Our experience favors the concept that NSE and CEA produces the best system for early warning of progression, although the gain by adding CEA is relatively small. In instances of progression, NSE was positive alone in 16/34, CEA and NSE in 18/34, CEA alone in none.

LDH levels essentially reflected a large tumor burden [4]. Nineteen of 32 known LDH values were increased in patients with progressive disease. In this respect LDH behaves like some of the other enzymes that tend to rise with increasing tumor burden such as thymidine kinase [6] and creatine kinase [5].

In summary this study indicated that NSE compared to the two other biomarkers was the marker of choice for the monitoring of SCLC, but it clearly lacks the sensitivity required to identify low volume residual disease, as the levels in CR and PR are similar. In SCLC the gain by adding CEA is small.

In practical terms whether to use one or two markers depends on the clinical question being investigated. The evaluation of the initial response to chemotherapy is one objective, the early detection of progression is another. No doubt the interest in the markers in SCLC will continue and increase as second line treatments improve.

REFERENCES

1. Hansen M, Pedersen AG. Tumor markers in patients with lung cancer. *Chest* 1986, **89**, 2219–2245.
2. Havemann K, Holle R, Gropp C. Prospective multicenter study of hormone markers in small cell lung cancer. *Recent Results Cancer Res* 1985, **99**, 194–208.
3. Aroney RS, Dermody WC, Aldenderfer P *et al.* Multiple sequential biomarkers in monitoring patients with carcinoma of the lung. *Cancer Treat Rep* 1984, **68**, 859–866.
4. Ganz PA, Ma PY, Wang H-J, Elashoff RM. Evaluation of three biochemical markers for serially monitoring the therapy of small cell lung cancer. *J Clin Oncol* 1987, **5**, 472–479.
5. Carney DN, Zweig MH, Ihde DC, Cohen MH, Makuch RW, Gazdar AF. Elevated serum creatine kinase BB levels in patients with small cell lung cancer. *Cancer Res* 1984, **44**, 5344–5403.
6. Gronowitz JS, Steinholtz L, Kallander CFR, Hagberg H, Bergh J. Serum deoxythymidine kinase in small cell carcinoma of the lung. *Cancer* 1986, **58**, 111–118.
7. Carney DN, Marangos PJ, Ihde DC *et al.* Serum neuron-specific enolase: a marker for disease extent and response to therapy of small cell lung cancer. *Lancet* 1982, **1**, 583–585.

8. Ariyoshi Y, Kato K, Ishiguro Y, Ota K, Sato T, Suchi T. Immunoassay of three enolase isoenzymes in human serum and in blood cells. *Clin Chim Acta* 1983, **127**, 353–363.
9. Schmechel D', Marangos PJ, Zis AP, Brightman M, Goodwin FK. The brain enolases as specific markers of neuronal and glial cells. *Science* 1978, **199**, 313–315.
10. Tapia FJ, Polak JM, Barbosa AJA *et al.* Neuron-specific enolase is produced by neuroendocrine tumours. *Lancet* 1981, **1**, 808–811.
11. Ariyoshi Y, Kato K, Ishiguro Y, Ota K, Sato T, Suchi T. Evaluation of serum neuron-specific enolase as a tumor marker for carcinoma of the lung. *Gann* 1983, **74**, 219–225.
12. Johnson DH, Marangos PJ, Forbes JT *et al.* Potential utility of serum neuron-specific enolase levels in small cell carcinoma of the lung. *Cancer Res* 1984, **44**, 5409–5414.
13. Cooper EH, Splinter TAW, Brown DA, Muers M, Peake M, Pearson S. Evaluation of a radioimmunoassay for neuron specific enolase in small cell lung cancer. *Br J Cancer* 1985, **52**, 333–338.
14. Esscher T, Steinholtz L, Bergh J, Nou E, Nilsson K, Påhlman S. Neuron specific enolase: a useful diagnostic serum marker for small cell carcinoma of the lung. *Thorax* 1985, **40**, 85–90.
15. Østerlind K, Pedersen AG, Vindeløv L *et al.* Alternating or continuous chemotherapy of extensive stage small cell lung cancer (SCC). *Lung Cancer* 1986, **2**, 127–128.
16. Pedersen AG, Østerlind K, Vindeløv L *et al.* Alternating or continuous chemotherapy of extensive stage small cell lung cancer (SCC). *Lung Cancer* 1986, **2**, 127–128.
17. World Health Organization. *WHO Handbook for Reporting Results of Cancer Treatment.* Geneva, 1979.
18. The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology. Recommended methods for the determination of four enzymes in blood. *Scand J Clin Lab Invest* 1973, **2**, 291–306.
19. Gerbitz KD, Summer J, Schumacher I. Enolase isoenzymes as tumour markers. *Clin Chem Clin Biochem* 1986, **24**, 1009–1016.
20. Siegel S. *Nonparametric Statistics.* New York, McGraw-Hill, 1956.
21. Splinter TAW, Cooper EH, Kho GS, Osterom R, Peake MD. Neuron specific enolase as a guide to the treatment of small cell lung cancer. *Eur J Cancer Clin Oncol* 1987, **23**, 171–176.
22. Cooper EH, Pitchard J, Bailey CC, Ninane J. Serum neuron specific enolase in children's cancer. *Br J Cancer* 1987, **56**, 65–67.
23. Akoun GM, Scarna HM, Milleron BJ, Benichou MP, Herman DP. Serum neuron specific enolase. A marker for disease extent and response to therapy for small-cell lung cancer. *Chest* 1985, **1**, 39–43.
24. Adewole IF, Newlands ES. Neuron specific enolase (NSE) as a tumour marker and comparative evaluation with carcinoembryonic antigen (CEA) in small-cell lung cancer. *Med Oncol Tumor Pharmacother* 1987, **4**, 11–15.
25. Sculier JP, Feld R, Evans WK *et al.* Carcinoembryonic antigen: a useful diagnostic marker in small cell lung cancer. *J Clin Oncol* 1986, **3**, 1349–1354.
26. Woo KB, Waalkes P, Abeloff MD, Ettinger DS, McNitt K, Gehrke CW. Multiple biologic markers in the monitoring of treatment for patients with small cell carcinoma of the lung. *Cancer* 1981, **48**, 1633–1642.