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Value of Neuron Specific Enolase in Early Detection of Relapse in Small Cell Lung Carcinoma

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Abstract—*Serum levels of NSE were monitored in 20 SCLC patients who completely responded to combination chemotherapy. An elevation of NSE was observed in five of nine patients with recurrent disease, but predated a relapse in only one. No elevation in NSE level was noted in nine patients who remained in complete remission. The addition of serial assays of LDH to NSE monitoring did not result in any gain in early warning of relapse. This study suggests that the value of serial NSE measurements for predicting a relapse of disease is limited.*

INTRODUCTION

SERUM neuron specific enolase (NSE) is a sensitive and specific tumor marker for small cell lung cancer (SCLC) [1–5]. It correlates with tumor burden, prognosis and response. The potential value of NSE in monitoring the disease activity in an individual patient is a subject of controversy, however, as there is scarcely any evidence that this marker is more sensitive than standard clinical methods of investigation. Of particular importance is the question whether subclinical relapse in SCLC patients in remission following chemotherapy may be detected by an increase in serum NSE. The present study was designed in order to answer this problem. Serial assays of lactate dehydrogenase (LDH), an enzyme that has been suggested to have a value as a marker of tumor burden and prognosis in SCLC [6–8] were conducted concurrently with NSE determinations to delineate the gain obtained by adding LDH to NSE in monitoring response in SCLC patients.

MATERIALS AND METHODS

Patients

Out of 81 consecutive patients with histologically proven SCLC referred to The Netherlands Cancer Institute between 1982 and 1988, only those patients were included in the study who achieved complete response after chemotherapy and for whom serial NSE measurements were obtainable. Apart from standard clinical and laboratory examination, all patients underwent extensive evaluation of the chest by CT scan and fiberoptic endoscopy, ultrasound of the abdomen, bone scintigraphy and bone marrow aspiration and biopsy. Limited stage disease was defined as lesions confined to one hemithorax and the supraclavicular lymph nodes. Patients with documented tumor spread beyond these limits were classified as extensive disease.

Treatment and clinical assessment

All patients were treated within the EORTC protocols 08825 and 08862 with combination chemotherapy regimens consisting of cyclophosphamide, doxorubicin and etopside or carboplatin, ifosfamide and vincristine. Patients with limited disease who were in complete response after five courses of chemotherapy also received prophylactic cranial irradiation. Restaging was performed routinely after five courses and consisted of clinical, radiological and fiberoptic bronchoscopic examinations.

Evaluation of treatment response was based on a repetition of all initially abnormal studies. A complete remission (CR) was defined as a complete disappearance of all known disease and was confirmed by bronchoscopy. A reduction of 50% or more of all measurable lesions was defined as a partial remission (PR). Following completion of treatment patients were evaluated every 6 weeks in the outpatient clinic with a physical examination,

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chest radiograph, complete blood count and clinical chemistry.

Tumor marker assays

Blood samples for NSE determination were obtained from patients at the time of routine blood evaluation during treatment and in the follow-up period usually at 3–12 week intervals (median 6 weeks). In most patients the first sample was obtained at the time the response was first recorded. Serum samples were stored at −20°C until assayed.

Serum NSE was determined by a double antibody radioimmunoassay (NSE-RIA, Pharmacia Diagnostics, AB, Uppsala, Sweden) [9]. NSE in the sample competes with a fixed amount of ^{125}I-labelled NSE for binding sites of the polyclonal antibodies against αγ and γγ isoenzymes of the enolase enzyme (EC 4.2.1.11). Bound and free NSE are separated by use of a second antibody bound to agarose. Definite separation is achieved by centrifugation. The amount of radioactivity in the pellet is inversely proportional to the quantity of NSE in the sample. All analyses were carried out in duplicate. Haemolysed samples were rejected. A cut-off value of <12.5 ng/ml of NSE was regarded as normal.

Blood samples for LDH were collected concurrently with NSE and were determined with the use of automated analysis together with other routine chemistry studies. A value of 215 U/l at 25°C was considered as the upper limit of normal.

RESULTS

Twenty SCLC patients (14 males and six females, 17 limited disease and three extensive disease, with median age of 60 years, range 44–78) who responded to combination chemotherapy entered into this study from September 1982 to August 1988. The median duration of follow-up was 108 weeks (range 25–237 weeks). Currently eight patients have died with progressive disease and of the 12 surviving patients nine have remained in complete remission for 35–237 weeks (median 174 weeks).

A total 174 NSE assays (median seven assays, range 1–22 assays) were performed. Pretreatment values of NSE were only obtained in three patients and were elevated in two. In all patients the serum NSE level was within normal limits when remission was documented and the median NSE level at that time was 5.1 ng/ml (3.2–9.8). Of 11 patients in whom the disease recurred, nine had sufficient NSE measurements to evaluate the pattern of NSE changes. Relapse sites in this group included: lung in five patients, brain in one, regional lymph nodes in one and multiple organs in two. An elevation of NSE levels accompanied the relapse detection in five patients (Fig. 1). In another two patients NSE serum level increased above the normal value 5 and 6 weeks, respectively, after the progression had been detected. The median serum level at the time of first clinical detection of disease relapse was 15.6 ng/ml (6.3–83.5). In 10 patients NSE levels were measured 3–12 weeks (median 6 weeks) before the detection of relapse. At that time the median NSE level was 5.9 mg/ml (4.7–15.0) and only in one patient was an elevated value noted. In nine patients clinical signs of relapse did not occur and in none of them was a rise of NSE level noted within the whole disease-free period of 35 to 237 weeks (Fig. 2).

The median pretreatment value of LDH assessed in 18 patients was 168 U/l (106–286) and was elevated in four patients (22%). At the first measurement after completion of treatment the median LDH level was 162 U/l (97–233) and was slightly elevated in one patient. No early increase of LDH was noted in 12 patients who subsequently developed progression at the last measurement performed 6–12 weeks (median 6 weeks) before a relapse was detected (median 170 U/l, range 127–212 U/l). At the time progression was detected the median LDH level was 185 U/l (range 125–398 U/l) and was elevated in three of nine evaluated patients (33%). Of the three patients with an elevated LDH level, two had also an elevated NSE level. Of the eight patients who remained free of relapse a transient rise of LDH level was seen in one. No clear relationship could be detected between NSE and LDH levels.

Table 1. *Patient characteristics* (n = 20)

Median age (range)	60 (44–78)
Sex	
Males	14
Females	6
Extent of disease	
Limited	17 (85%)
Extensive	3 (15%)

DISCUSSION

An ideal tumor marker should be highly specific and sensitive, should closely correlate with the

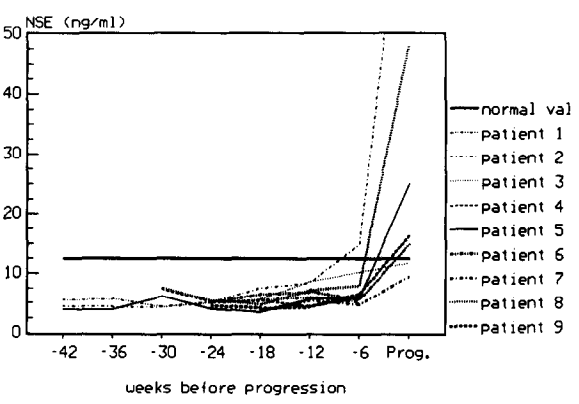

Fig. 1. *NSE levels for patients with progression.*

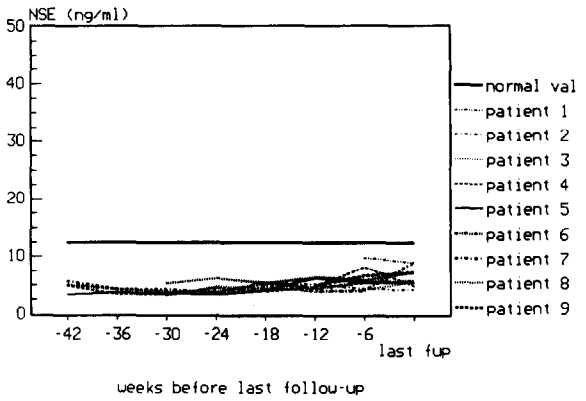

Fig. 2. NSE levels for patients without progression.

tumor burden and with response to treatment and should give early warning of recurrence of disease. NSE meets some of these criteria. Raised serum levels are present in a majority of SCLC patients [2–7, 10], are significantly less frequent in other histological types of lung cancer [3, 6, 11, 12] and are very rarely seen in healthy adult subjects [2, 4, 12, 13]. The correlation with tumor burden is reflected by the significant difference in NSE values between SCLC patients with extensive and with limited disease [1–4, 7, 10, 11, 13]. NSE values decline after successful chemotherapy and increase with relapse [2–4, 12, 13].

The important clinical question whether this marker may be useful in early detection of subclinical relapse in routine management of SCLC still remains open. In a study of Johnson *et al.* [4] an elevation of NSE predated by 4–12 weeks the clinical recognition of relapse in 65% of patients. In seven of 20 responders in that study NSE levels remained above the normal values when remission was recorded, suggesting a high risk of subsequent relapse. The important finding of that study was that the increase of NSE in patients who previously achieved a partial or complete response was always accompanied by subsequent relapse, i.e. there were no 'false positive' increases in NSE levels.

Splinter *et al.* [10] noted an exponential rise of NSE, although not always to values above 'normal' in a period preceding the clinical detection of progression. The number of responders who subsequently developed relapse was not reported, however. The doubling time of NSE levels in that series varied from 10 to 94 days and correlated with survival.

The rise of NSE preceded radiological progressive disease in 64% of patients in a series of Jorgensen *et al.* [7], but no detailed data for patients who had earlier achieved a clinical remission were presented in this study.

Our study failed to confirm earlier promising reports, suggesting the potential value of NSE in predicting recurrent disease. Although no 'false positive' increases of NSE were noted in patients remaining in remission consistent with an observation of Johnson *et al.* [4], the sensitivity of this test in warning of early relapse was low.

There are several factors that could contribute to this negative finding. A correlation of NSE levels and tumor bulk seems to be confined to the patients with relatively large tumor burden, especially in pretreatment measurements. Normal values of NSE were observed in 37–86% of patients with limited disease patients [1, 2, 10, 12, 14]. A significant fall in NSE level is commonly observed in responders, but the values in patients who experience complete and partial remission are similar [7, 10]. Our study included patients with minimal, if any, tumor burden. Most of the patients were at limited stage of disease at presentation and all were in complete remission at the time NSE monitoring was started. It may therefore be unjustified to expect the detection of subclinical tumor burden while NSE is on frequent occasions not sensitive enough to detect apparent disease.

Moreover, most of the SCLC patients relapse with a chemoresistant variant of SCLC or even with non-small cell lung cancer types and these subsets of the tumor usually have lost neuroendocrine properties [7, 10, 12] In the present study the elevation in NSE levels accompanied the tumor progression in five out of nine patients. Of these five patients, a clear rise of NSE level preceded tumor progression in only one and showed an exponential increase within the normal value limits in another one. We do not know at what time the NSE level increased in the remaining patients. It is therefore possible that NSE monitoring at intervals shorter than 6 weeks would increase the predictive value of this tumor marker. On the other hand, regarding the present therapeutic possibilities, the potential gain in early detection would not justify the additional visits to the clinic required for NSE assays at intervals shorter than 6 weeks. The current measurements of LDH are easy and commonly performed during follow up of SCLC patients. This marker correlates well with disease extent [8], disease activity [6] and prognosis [15]. Our study has shown, however, that LDH, either considered individually, or in addition to NSE is also of limited value in monitoring patients with minimal tumor burden.

In conclusion, this study failed to demonstrate an advantage of serial NSE measurements for the early prediction of relapse in SCLC patients. Our data suggest that the utility of this marker in routine monitoring of patients with minimal tumor burden after chemotherapy is limited.

REFERENCES

1. Bork E, Hansen M, Urdal P *et al.* Early detection of response in small cell bronchogenic carcinoma by changes in serum concentrations of creatinine kinase, neuron specific enolase, calcitonin, ACTH, serotonin and gastrin releasing peptide. *Eur J Cancer Clin Oncol* 1988, **24**, 1033–1038.
2. Carney DN, Marangos PJ, Ihde DC, Bunn PA Jr, Cohen MH, Minna JD. Serum neuron-specific enolase: a marker for disease extent and response to therapy of small cell lung cancer. *Lancet* 1982, **3**, 583–585.
3. Fischbach W, Schwarz-Wallrauch C, Jany B. Neuron-specific enolase and thymidine kinase as an aid to the diagnosis and treatment monitoring of small cell lung cancer. *Cancer* 1989, **63**, 1143.
4. 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.
5. Jorgensen LGM, Osterlind K, Hansen HH, Cooper EH. The prognostic influence of serum neuron specific enolase in small cell lung cancer. *Br J Cancer* 1988, **58**, 805–807.
6. 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.
7. Jorgensen LGM, Hansen HH, Cooper EH. Neuron specific enolase, carcinoembryonic antigen and lactate dehydrogenase as indicators of disease activity in small cell lung cancer. *Eur J Cancer Clin Oncol* 1989, **25**, 123–128.
8. Cohen MH, Makuch R, Johnston-Early A *et al.* Laboratory parameters as an alternative to performance status in prognostic stratification of patients with small cell lung cancer. *Cancer Treat Rep* 1981, **65**, 187–195.
9. Pahlman S, Esscher T, Bergvall P, Odelstad L. Purification and characterization of human neuron-specific enolase: radioimmunoassay development. *Tumor Biol* 1984, **5**, 127–139.
10. Splinter TAW, Cooper EH, Kho GS, Oosterom 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.
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. Cooper EH, Splinter TAW, Brown DA, Muers MF, Peake MD, Pearson SL. Evaluation of radioimmunoassay for neuron specific enolase in small cell lung cancer. *Br J Cancer* 1985, **52**, 333–338.
13. Paus E, Risberg T. Establishment and evaluation of radioimmunoassay for neuron specific enolase. *Tumor Biol* 1989, **10**, 23–30.
14. Gomm SA, Keevil BG, Thatcher N, Hasleton PS, Swindell RS. The value of tumor markers in lung cancer. *Br J Cancer* 1988, **58**, 797–804.
15. Ganz PA, Yeung Ma P, Wang H, Elashoff RM. Evaluation of three biochemical markers for serially monitoring the therapy of small cell lung cancer. *J Clin Oncol* 1987, **5**, 472–479.