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Neuron-Specific Enolase as a Guide to the Treatment of Small Cell Lung Cancer

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Abstract—*A retrospective evaluation of serial measurements of neuron-specific enolase (NSE) has been performed in 58 patients with small cell lung cancer (SCLC). All 58 patients received first-line chemotherapy and 11 patients received also second-line treatment after relapse. Samples were obtained every 3–4 weeks during treatment before each cycle of chemotherapy and every 6 or 12 weeks during follow-up. NSE values were depicted on semi-logarithmic paper. Fifty-one times a major response (complete or partial remission) was observed and 49 times the NSE level reached a plateau between 3.5–10 ng/ml. The NSE level did not discriminate between a complete or a partial remission. Seven times stable disease was obtained and the NSE level declined but remained above the normal plateau of 3.5–10 ng/ml. On 50 occasions progressive disease was found. In 3 cases progressive disease was due to a histologically-proven non-small cell lung cancer and NSE levels did not change. In only 5 out of the remaining 47 occasions NSE levels were normal at the time of relapse but rose later in 4. On 42 occasions of progressive SCLC an exponential rise of NSE was found, often within the range of 3.5–20 ng/ml. None of 6 patients, who are still incomplete remission for 1–5 years, showed a consistent rise of NSE. Serial measurements of serum NSE, can predict the occurrence of a major response, stable disease and progressive disease outside the brain with a very high accuracy and seem to be at least a useful addition to standard investigational methods to guide the treatment of SCLC.*

INTRODUCTION

NEURON-Specific Enolase (NSE) has been shown histologically to be present in tissues of neural or neuroendocrine origin, so-called APUD cells [1]. However it has also been found in blood cells [2, 3]. Small cell lung cancer (SCLC) is the commonest type of tumour showing neuroendocrine differentiation. Raised pretreatment serum levels of NSE have been found in 40–70% of patients with limited disease and in 83–98% of patients with extensive disease [4–7]. The measurement of serum NSE has shown to be of value in monitoring SCLC [4–7]. Recently however, Aroney *et al.* [8] investigated whether measurement of biomarkers such as NSE could be used to monitor disease activity and came to the conclusion that biomarkers were no more sensitive than standard clinical investigational methods. The conclusion of their studies is almost exclusively based on 2 or 3 measurements during the course of the disease, i.e. before the start of treatment, at the evaluation of response and at progression of the disease.

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NSE is released into the interstitial fluid from dying or dead cells. Therefore, one may not expect serum NSE to be a sensitive marker, but it may be a specific marker in the individual patient to monitor the disease. In the present study we have evaluated retrospectively serial measurements of NSE in SCLC patients during treatment and follow-up in order to find out whether NSE might be a cheap and easy method to guide the treatment of SCLC either as a useful addition to, or as a replacement of, standard investigational methods.

MATERIALS AND METHODS

Patients

Fifty-eight patients with SCLC were staged as having limited (LD) or extensive disease (ED) by physical examination, chest X-ray, CT-scan of the thorax and upper abdomen, including liver, pancreas, retroperitoneal lymph nodes and adrenals, nucleide bone scan and one-sided bone-marrow biopsy. All patients were treated with chemotherapy according to the EORTC protocol 08825 or 08862 or a local protocol. After two or five courses of induction chemotherapy, evaluation of

response was done according to the WHO [9]. The patients then received either no treatment or additional chemotherapy courses. Some patients received radiotherapy to the primary tumour. At the time of progression of the disease a bronchoscopy and biopsy were repeated in order to verify the histological nature of the relapse. A CT-scan of the brain was made if indicated by clinical symptoms. From July 1982 all patients with SCLC, who had been treated, were being treated or were new patients, admitted for treatment, were entered into the study. Only those patients, who were evaluable for response after induction chemotherapy or showed progressive disease or were disease-free for more than 1 year, were used for this evaluation. Fifty-eight patients fulfilled these criteria. Twenty-nine patients had LD and 29 ED. The response after two or five courses of induction chemotherapy was evaluated as progressive disease (PD) in 5, as stable disease (SD) in 5, as a partial remission (PR) in 29 and as a complete remission (CR) in 19 patients. Eleven patients were treated at progression by second- or third-line chemotherapy or radiotherapy. The response was evaluated after every two courses of chemotherapy or after the end of radiotherapy. During chemotherapy blood samples were obtained at least every 3–4 weeks before the start of each cycle and during follow-up every 6 weeks for the first 2 years and every 12 weeks thereafter. During the first part of the study, from July 1982 till September 1984, heparin plasma and later only serum was obtained. All samples were stored at −20° C prior to analysis. Haemolysed samples were not used.

Effect of haemolysis

Erythrocytes from EDTA-blood, obtained from healthy individuals and 1 SCLC patient with an elevated serum NSE level, were washed three times with saline and were then frozen. After thawing the haemolysate was centrifuged (10 min, 2000 × ***g***) and haemoglobin (Hb) was measured in the cell-free supernatant. Dilutions of the haemolysates were made with saline and fixed aliquots were added to the serum of a healthy individual and a patient. To the control serum samples the same aliquot of only saline was added.

Methods

NSE was measured with the Pharmacia NSE RIA test (Pharmacia AB, Uppsala, Sweden) as described previously [10]. The NSE values were depicted on semi-logarithmic paper. A consistent change in NSE level was defined as two or more subsequent values in the same direction.

RESULTS

Mean NSE levels, measured in EDTA plasma, heparin plasma and serum, obtained from 4 healthy individuals were 5.2, 5.7 and 5.8 ng/ml respectively. After storage of serum with an elevated NSE level (140 ng/ml) at 4° C and at room temperature for 72 hr no decrease of the NSE level was observed at 4° C and a small decrease of 15% at room temperature.

The addition of a haemolysate, prepared from erythrocytes of both healthy individuals and an SCLC-patient with a raised serum NSE level (30 ng/ml) to control serum or patient serum increased the NSE level in a linear fashion. However, the slope of the curve (Y-axis: NSE ng/ml, X-axis: Hb nmol/ml) after addition of the patient-derived haemolysate was steeper ($Y = 0.151\,X$) than after addition of the control-derived haemolysate ($Y = 0.112\,X$). The slope of the curves was independent of the source of the serum, to which the haemolysate was added. Storage of whole blood with or without anticoagulants for 72 hr at 4° C or room temperature did not result in increased plasma- or serum NSE levels, as long as no visible haemolysis was present. Haemolysis, which was just visible, correlated with a serum haemoglobin content of 20–25 nmol/ml and an increase of NSE of 3–5 ng/ml. The median pretreatment NSE levels in the patients with LD were 25 ng/ml (range 3.5–132.4, $n = 22$) and with ED 51 ng/ml (range 18.4–1142, $n = 24$).

On 51 occasions a major response (CR or PR) was observed and the NSE levels declined to reach a plateau between 3.5 and 10 ng/ml after 1–4 courses on all occasions except 2. The velocity with which the plateau was reached, and the level of the plateau, was similar in patients who reached a CR or a PR. An example of the decline of NSE in a responding patient is shown in Fig. 1.

Seven times SD was achieved. The NSE levels showed a decrease but clearly remained above 10 ng/ml ranging from 14.6 to 145 ng/ml. An example is shown in Fig. 2.

On 50 occasions PD outside the central nervous system (CNS) was observed, 23 times during treatment and 27 times during follow-up. Five patients showed early PD after the first 1–2 courses of chemotherapy. An example is shown in Fig. 3. Overall on 42 occasions of PD outside the CNS a consistent rise of NSE, i.e. measured in 2 or more consecutive samples, was observed. The rise of serum NSE preceded the clinical detection of PD by 0–112 days (median 42). When depicted on semi-logarithmic paper, it was found that NSE rose exponentially. It is important to note that the exponential rise also at levels below 20 ng/ml and not the absolute value of NSE was predictive of PD! The doubling time of the NSE level varied from 10 to 94 days. The lowest doubling time of 10 days correlated with a survival of 37 days and the highest of 94 days with a survival of 290 days,

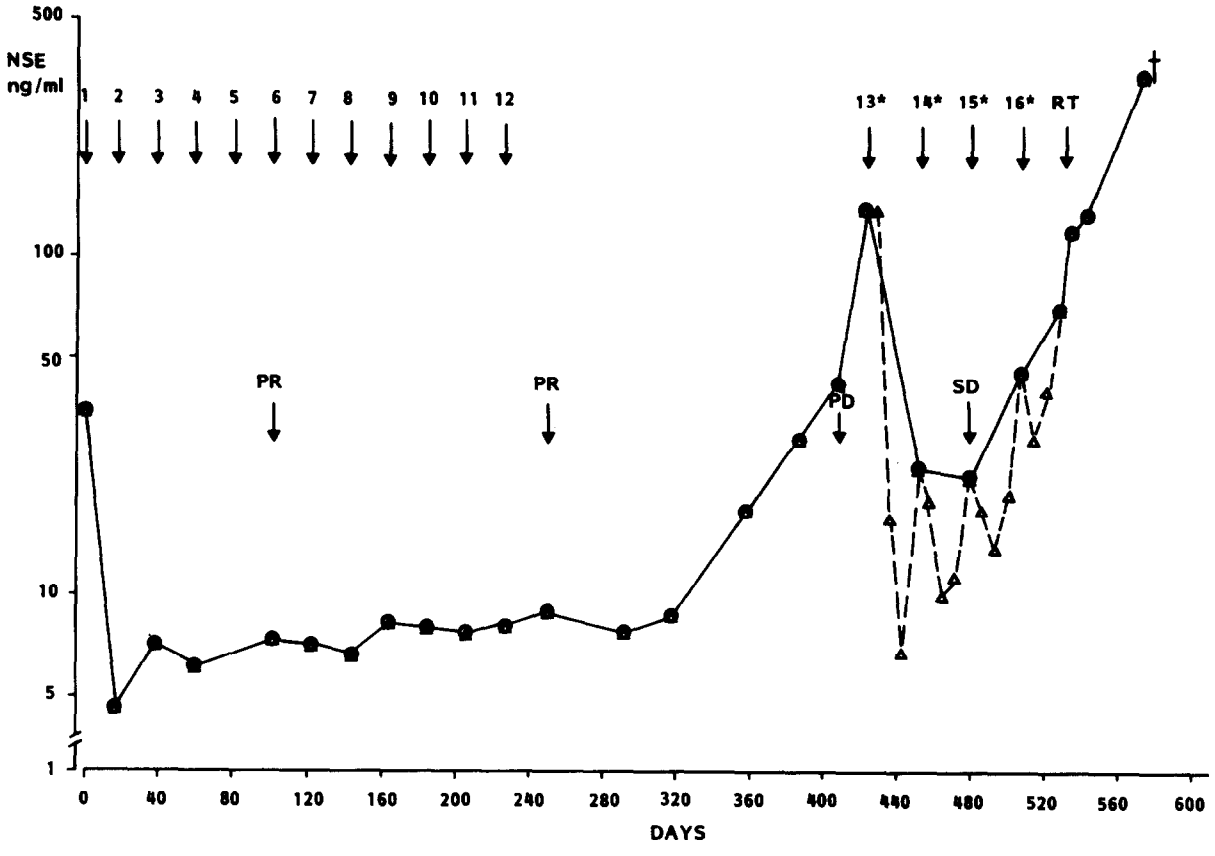

*Fig. 1. A 36-year-old male with extensive disease. After 5 and 12 courses of chemotherapy vital tumour cells were found at bronchoscopy as the only remaining abnormality. At relapse the patient was treated with Carboplatin (Jm8). ↓ : course of first-line chemotherapy. ↓ *: course of second-line chemotherapy. PR: partial remission. PD: progressive disease. SD: stable disease. RT: radiotherapy. Solid line: NSE measurements at 3–6 wk intervals. Dotted line: NSE measurements between courses of chemotherapy.*

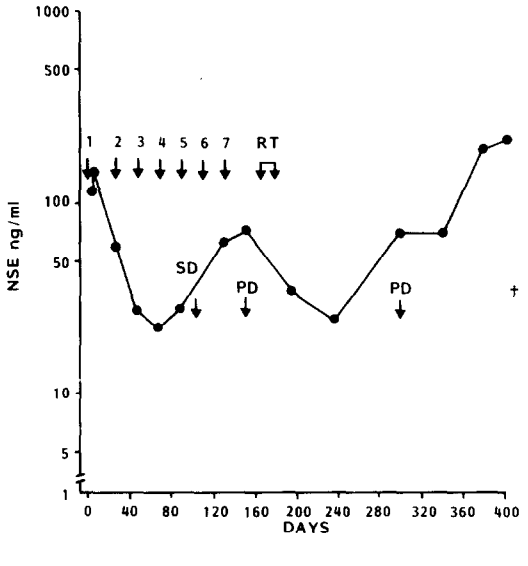

Fig. 2. A 70-year-old male with extensive disease. After 5 courses of chemotherapy stable disease (SD) was obtained. PD: progressive disease. RT: radiotherapy. ↓ : course of first-line chemotherapy.

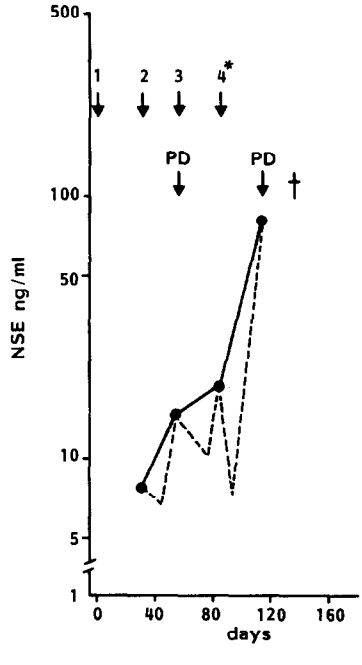

*Fig. 3. A 61-year-old man with extensive disease. After 3 courses of chemotherapy progressive disease (PD) was observed, which remained unaltered after second-line chemotherapy with Carboplatin (Jm8). ↓ : course of first-line chemotherapy. ↓ *:course of second-line chemotherapy. Solid line: NSE measurements at 3–4 wk intervals. Dotted line: NSE measurements between courses of chemotherapy.*

Table 1. Predictive value of serial NSE measurements for the evaluation of response and PD during follow-up

	CR + PR	SD	PD	PD during follow up
True positive*	49	7	20	22
True negative*			1	8
False positive*				
False negative*	2		2	3
Accuracy		95%		91%

*For an explanation of the definitions see results.

calculated from the time NSE was seen to rise.

On eight occasions of clinical PD no rise of serum NSE was observed. Three patients had a histologically-proven relapse of non-small cell lung cancer (NSCL), which was treated by surgical resection in one of them, as recently described [11]. All had normal NSE levels till death. Of the remaining 5 patients 4 showed a delayed exponential rise of NSE 3–6 weeks after the clinical detection of PD and 1 patient died within 3 weeks. The exponential rise of NSE at PD did not seem to be dependent on the NSE level before treatment, since all 4 patients with the lowest pretreatment levels of 5–14 ng/ml showed an exponential rise at PD, whereas 3 of the patients with a delayed rise of NSE had initial values of 18.5, 30 and 130 ng/ml respectively. Six patients are still in CR for more than 1 year (range 1–5 years) and no NSE level above 10 ng/ml has been measured during the whole disease-free period.

In most patients, who had reached a CR or PR small fluctuations of NSE levels between 3.5 and 10 ng/ml were observed especially during treatment. In only 2 patients, once, an NSE level between 10 and 20 ng/ml was measured, which was not due to haemolysis.

In Table 1 the predictive value of serial NSE measurements for the evaluation of response (CR + PR and SD) and the occurrence of PD outside the CNS is summarized and the accuracy is calculated. The definition of the "true positive NSE value" in the case of a major response or SD is a raised pretreatment value, which declined during therapy to a plateau between 5 and 10 ng/ml or to a value above the plateau respectively. The definition of "true positive NSE value" in case of PD is an exponential rise of 2 subsequent measurements to a level above 10 ng/ml. Accuracy is defined as the number of true positive plus true negative evaluations divided by the total number of evaluations.

From 9 patients blood samples were obtained during treatment at weekly intervals to investigate which sampling-frequency was optimal to monitor the effect of treatment. Examples are shown in Figs 1, 3 and 4. Between 2 chemotherapy cycles oscillating levels of NSE (dotted lines) were observed, which could both increase, followed by a decrease, and *vice versa*.

Eight patients developed symptomatic brain metastases, proven by CT-scan. In only one of them, who had PD outside the CNS at the same time, a rise of NSE was observed.

In 2 patients NSE was measured in cytologically proven malignant pleural effusion and ascites respectively. The NSE values in the effusion and seum were 84 and 184 ng/ml respectively and in the ascites and serum 26 and 350 ng/ml.

DISCUSSION

Can NSE be used to monitor the disease in the individual patient either as a useful addition to or a replacement of clinical investigational methods, especially expensive imaging techniques? Data from the literature have shown that NSE may have some value in monitoring SCLC but in the majority of patients, levels have been measured only before treatment, at the evaluation of response and at progression [4–7]. In very few patients serial measurements of NSE during the whole course of the disease have been performed.

From July 1982 blood samples were obtained from all SCLC-patients at 3-weekly intervals during treatment and at 6- or 12-weekly intervals during follow up. Sufficient clinical data and NSE measurements to evaluate the significance of NSE during treatment and/or follow-up were available for 58 patients. The results suggest that serial measurements of NSE, when depicted on semi-logarithmic paper, can predict the occurrence of a major response (CR + PR), stable disease and progressive disease outside the CNS with a very high accuracy. NSE is not sensitive enough to distinguish between CR and PR, which means that evaluation of tumour response has still to be done by clinical restaging methods. Furthermore, since 70–80% of the patients achieve a major response after first-line combination chemotherapy [12], the prediction of a major response, by NSE measurements at that time, is not very interesting.

However, if patients relapse, most second-line therapies are experimental and very often ineffective. In such a situation NSE measurements may be very helpful to predict a major response, SD or PD and to prevent further administration of ineffective treatment. It is important to realize that these conclusions are based on NSE levels, measured just before the start of each chemotherapy cycle in non-haemolysed serum. Measurements of NSE between two chemotherapy cycles may show either a transient increase (Fig. 4), possibly due to a release of the enzyme as described

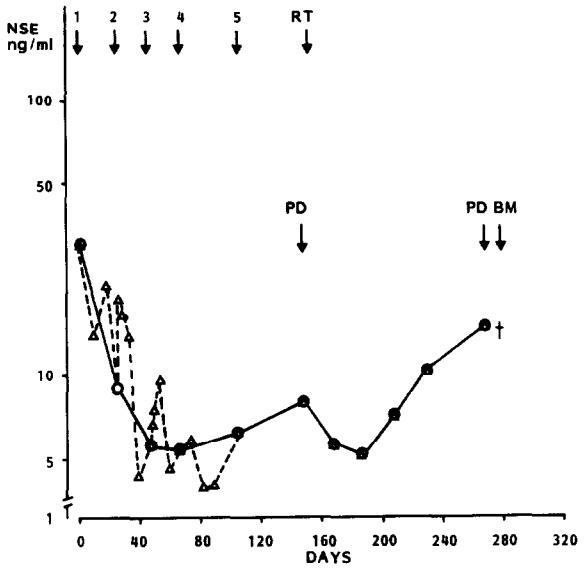

Fig. 4. A 56-year-old male with limited disease. After 5 courses of chemotherapy a partial remission was achieved. At relapse the primary tumour was irradiated (RT). Soon afterwards progressive liver metastases (PD) and brain metastases (BM) were observed. ↓ : course of first-line chemotherapy. Solid line: NSE measurements at 3–6 wk intervals. Dotted line: NSE measurements between courses of chemotherapy.

by Akoun *et al.* [13] or a transient decrease (Figs 1 and 3). Not enough data are available yet to interpret these oscillations of NSE levels, but both phenomena and especially the predictive value of NSE release after chemotherapy are now subject of investigations *in vivo* and *in vitro*.

Another real advantage of serial NSE measurements seems to be the highly accurate prediction of PD either at the same time as or preceding clinical detection as long as 112 days. This may save the patients expensive and burdensome investigations and at the same time a relapse is diagnosed early enough to enter the patient into a study of second-line treatment. However, since a relapse of NSCLC is not detected by NSE measurements, serial chest X-rays cannot be omitted during follow-up.

Serum NSE levels seem to be useless for the detection of CNS metastases, since in all 7 patients without PD outside the CNS, no rise of NSE levels could be detected. Recently it has been described how the measurement of NSE in cerebrospinal fluid (CSF) is a sensitive method to detect CNS metastases [14]. These data suggest that NSE is not permeating from the CSF into the blood and *vice versa* as other tumor markers such as alpha-foetoprotein and beta-HCG do. The fact that NSE may not easily permeate from one body-compartment to the other is supported by the ratio between pleural effusion or ascites and serum respectively, as observed in this study.

In agreement with other investigators [6, 7] it was found that haemolysis may give rise to falsely elevated NSE levels, which are the more important when even changes of NSE levels within the normal range can be used to predict PD. Fortunately, NSE seems to be a relatively stable enzyme and levels in whole blood, plasma or serum remain constant, when no haemolysis occurs for at least 72 hr at 4° C, which facilitates blood sampling and storage during the night and weekends and shipment.

In conclusion, this retrospective evaluation indicates that serial NSE measurements in SCLC-patients are at least a useful addition to standard investigational methods, especially to detect PD and to evaluate the tumour response after second-line treatment. To investigate whether and which clinical investigations can be replaced by measurements of NSE a prospective single-blind study has been started, in which the response to treatment and the course of the disease will be predicted on NSE levels only and retrospectively compared with the clinical findings.

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