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Neuron-specific Enolase as a Prognostic Factor in Metastatic Malignant Melanoma

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Serum neuron-specific enolase (NSE) was measured in 63 patients with metastatic malignant melanoma. 20 patients (32%) had elevated serum NSE (> 10 μg/l) before the start of treatment. Another 13 patients (21%) developed pathological NSE values during the course of the disease. In many patients, elevated NSE was related to a large tumour burden, and a gradual rise in serum NSE indicated disease progression. Patients with elevated pretreatment NSE had a median survival time of 3 months compared with 12 months for those with normal pretreatment NSE values. NSE thus proved to be a useful prognostic factor in metastatic malignant melanoma.
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INTRODUCTION

CHEMOTHERAPY HAS limited success in patients with malignant melanoma. Some patients with advanced disease have extremely poor prognosis and should not be subjected to toxic treatments. It is, however, difficult to determine the prognosis of the individual patient. A reliable and specific serum marker could possibly be useful to select patients that may benefit from systemic therapy.

The enzyme enolase exists as several dimeric isoenzymes, of which the αγ and γγ dimers are known as neuron-specific enolase (NSE). Enolase catalyses the interconversion of 2-phosphoglycerate and phosphoenolpyruvate in the glycolytic pathway, and thus participates in the formation of a high-energy phosphate bond. We have shown in a previous study that approximately half the patients with metastatic malignant melanoma have elevated levels of serum NSE during the course of the disease [1]. In small cell lung cancer, serum NSE has proved to be a useful prognostic factor [2]. In the present study the prognostic significance of elevated serum NSE in patients with malignant melanoma is evaluated.

PATIENTS AND METHODS

All patients with metastatic malignant melanoma admitted to the Norwegian Radium Hospital in the period January 1988 to April 1989 entered the present study. For these 63 patients the time interval between treatment for primary melanoma and diagnosis of first metastases varied from being contemporary events for some patients to more than 10 years for others. The mean time interval was 41 months, while the median value was 15 months. Serum NSE levels were measured at each admittance to the hospital, and the patients were followed for at least 20 months from the first admittance with metastatic disease.

A newly developed and highly sensitive immunoradiometric assay based on monoclonal antibodies with monodisperse magnetisable particles as the solid phase [3] was used to measure serum NSE. Values exceeding 10 μg/l were considered elevated [1].

We also measured serum lactate dehydrogenase (LDH), alkaline phosphatase (AP), erythrocyte sedimentation rate (ESR) and the leucocyte count. Furthermore, the number and size of all detectable tumour manifestations were recorded. To quantify the extent of disease, we examined 11 specific sites/organs for tumour manifestations (Table 1). Other tumour sites were

*Table 1. Quantification of extent of disease**

Cutaneous/subcutaneous lesions
1: ≤ 2 lesions, ≤ 5 cm
2: > 2 lesions and/or > 5 cm
Superficial lymph nodes
1: only 1 region, ≤ 5 cm
2: ≥ 2 regions and/or > 5 cm
Mediastinal lymph nodes
1: ≤ 2 lesions, ≤ 5 cm
2: > 2 lesions and/or > 5 cm
Abdominal lymph nodes
1: ≤ 2 lesions, ≤ 5 cm
2: > 2 lesions and/or > 5 cm
Lung
1: ≤ 2 lesions, ≤ 5 cm
2: > 2 lesions and/or > 5 cm
Liver
1: ≤ 2 lesions, ≤ 5 cm
2: > 2 lesions and/or > 5 cm
Brain
1: ≤ 2 lesions, ≤ 5 cm
2: > 2 lesions and/or > 5 cm
Bone
1: ≤ 2 lesions
2: > 2 lesions
Adrenal glands
1: unilateral
2: bilateral
Pleural fluid
1: unilateral blunting of costovertebral angle
2: bilateral and/or > blunting of costovertebral angle
Ascites
2: always if present
Miscellaneous
1: ≤ 2 lesions, ≤ 5 cm
2: > 2 lesions and/or > 5 cm

*1: Limited disease; 2: extensive disease.

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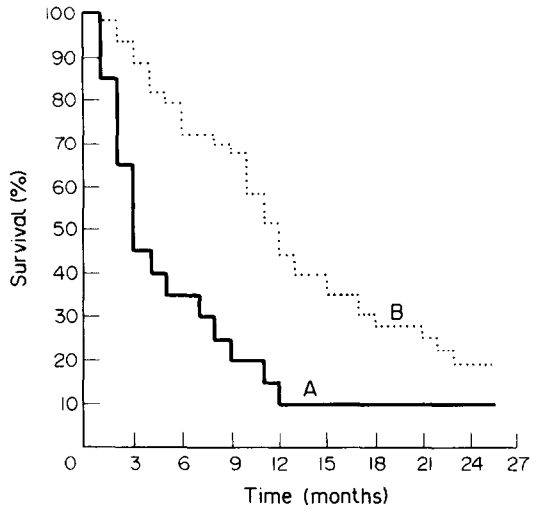

Fig. 1. Kaplan–Meier plots for patients with initially elevated serum NSE (curve A, $n = 20$) and normal serum NSE (curve B, $n = 43$), respectively.

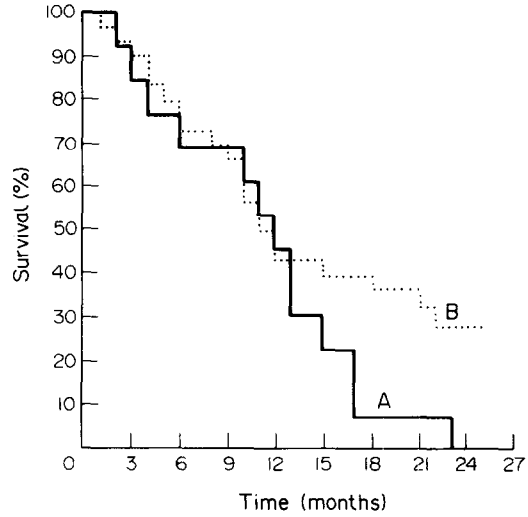

Fig. 2. Kaplan–Meier plots for patients who developed pathological NSE values during the course of the disease (curve A, $n = 13$) compared with plots for patients with persistingly normal NSE values (curve B, $n = 30$).

recorded in the miscellaneous group which was defined as a twelfth site. As described in Table 1, limited disease at 1 site/organ was scored as '1', while extensive disease was scored as '2'. By adding the scores from the 12 different sites the total extent of disease could be registered for each patient on a scale from 1 to 24. This is a modification of a scoring system used earlier for metastatic breast cancer disease [4, 5].

Drugs employed for systemic therapy were: cisplatin, vindesine, dacarbazine, lomustine, fotemustine, interferon and interleukin-2. The first-line regimen usually consisted of two or three of these drugs in combination. Some patients also received palliative radiotherapy.

The survival time was estimated from the first serum NSE measurement, i.e. from the first arrival at the hospital with metastatic disease. All deaths were considered due to malignant melanoma, and no patients were excluded from the material. The survival curves were calculated according to the Kaplan–Meier method [6]. The log rank test [7] was used to test differences between survival curves. Cox proportional hazards model [8] was used to simultaneously analyse the importance of several prognostic factors. Statistical analyses were performed with the BMDPC package [9].

The cut-off points used in survival analysis for LDH (450 U/l), ESR (15 mm/h) and leucocyte count (10.0×10^9/l) were the same as in an earlier report from our department [10]. For AP, the normal range limit (270 U/l) was used as cut-off point. The parameter 'extent of disease' was divided into two groups: 1–4, and more than 4.

RESULTS

Figure 1 shows the survival curve for patients with elevated serum NSE (curve A) compared with those with a normal pretreatment NSE value (curve B). Only 2 of the 20 patients (10%) with initially elevated NSE were alive at the end of observation, whereas 9 of the 43 patients (21%) with initially normal NSE were still alive. The median survival times were 3 and 12 months, respectively.

Of the 43 patients with initially normal serum NSE, 13 (30%) developed pathological NSE values during the course of the disease. Figure 2 shows the survival curve for the 13 patients with serum NSE that converted from negative to positive values (curve A) compared with the curve for the 30 patients (70%) with persistingly negative NSE values (curve B). In the latter group, 9 patients (30%) were still alive at the end of observation; estimated survival at 25 months was 28%. All the patients converting to positive NSE values had died by the end of observation. The median survival time was 12 months in both groups. However, for the 13 patients who converted from normal to pathological NSE values, the median survival time was only 4 months when measured from the time point of NSE conversion (curve not shown).

In addition to NSE values > 10 μg/l before treatment, the presence of brain metastases, extent of disease > 4, LDH > 450 U/l, leucocyte count > 10.0×10^9/l and ESR > 15 mm/h were associated with short survival. The results of the univariable analysis are presented in Table 2. For patients with elevated AP (> 270 U/l) before treatment, the apparent reduction in survival time was not statistically significant. In Table 2 the right-hand column shows the number of patients above the cut-off value (denominator), and how many of these patients lived for 3 months or less (numerator).

Table 2. Survival in different prognostic groups

Prognostic factor	Median survival (months) (95% confidence limits) Yes	No	Patients living ≤ 3 months/patients above cut-off values
NSE > 10μg/l	3 (2–7)	12 (10–13)	11/20
Brain metastases	3 (2–4)	11 (8–12)	5/8
Leucocyte count > 10.0×10^9/l	3 (2–5)	11 (8–13)	6/12*
Extent of disease > 4	4 (3–8)	15 (10–21)	12/30
LDH > 450 U/l	6 (3–9)	17 (10–21)	14/36**
ESR > 15 mm/h	6 (4–9)	13 (10–18)	12/41***
AP > 270 U/l	5 (3–8)	10 (7–11)	6/15**

NSE = Neuron-specific endase; LDH = lactate dehydrogenase; ESR = erythrocyte sedimentation rate; AP = alkaline phosphatase. *1 missing value. **2 missing values. ***3 missing values.

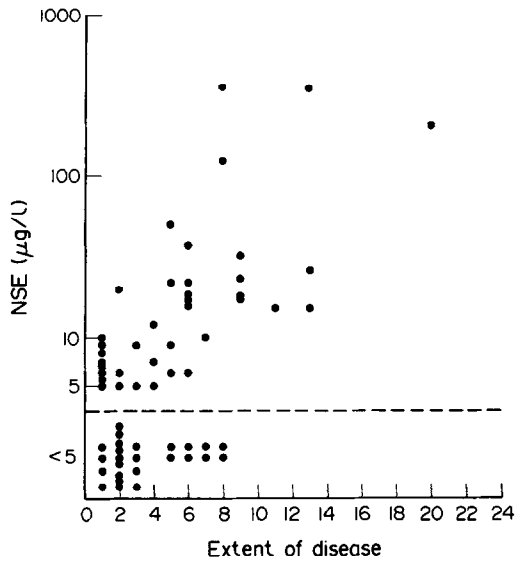

Fig. 3. Initial serum NSE (log scale) plotted against extent of disease. 25 patients had NSE values < 5 μg/l as indicated in the bottom of the figure.

The univariable survival analyses demonstrated no clear superiority of any organs involved except for central nervous system (CNS) metastases. As the measurements of disease extent are rather investigation-intensive, multivariable analyses were first performed without extent of disease as a variable. CNS metastases, elevated LDH and leucocyte count were then selected as significant variables in a stepwise analysis. NSE was closely correlated to LDH ($r = 0.51$) and only one of them was selected in the regression analyses. As NSE is more tumour-specific than the other blood analyses tested, a final regression analysis was performed with the condition that NSE should be one of the preferred variables. Then only CNS metastases ($P < 0.001$) was selected in addition to NSE ($P < 0.05$).

Figure 3 shows the different initial serum NSE values plotted against the extent of the disease, which was estimated by means of the scoring system shown in Table 1. Though considered as being in the normal range, NSE values between 5 and 10 μg/l are included in this plot. NSE values less than 5 μg/l were classified as not detectable.

The data in Fig. 3 suggest an association between tumour burden (extent of disease) and the amount of NSE in serum. Statistical analysis confirmed a correlation ($r = 0.51$) between extent of disease and serum NSE at the first admittance to the hospital. In fact, all patients with 'extent of disease' of more than 8, had elevated serum NSE values. There is, however, a large spread in the NSE values, and several patients with a considerable tumour burden had normal serum NSE.

DISCUSSION

In 1982, Dhillon *et al.* demonstrated that immunohistochemical staining of paraffin-processed tumour material for NSE could be useful for the diagnosis of malignant melanoma [11]. Our finding of elevated serum NSE in patients with disseminated malignant melanoma, confirms earlier reports from small clinical materials [12, 13]. Royds *et al.* [12] measured serum NSE in 8 patients and found pathological values in all of them, while Lorenz and Dippold [13] found elevated NSE in 14 out of 22 patients.

To our knowledge, the present study is the first to demonstrate the prognostic significance of elevated serum NSE in patients with metastatic malignant melanoma. As single prognostic factor, elevated serum NSE indicated a median survival time of only 3 months, while patients with normal serum NSE values had a median survival time of 12 months.

The αγ-enolase and γγ-enolase isoenzymes are known as neuron-specific enolase because initially they were found in neurons, neuroendocrine cells, and in neoplasms derived from these cells [14]. Later it has been shown that NSE is not a specific marker of neurons and neuroendocrine cells; it can also be found in some adenocarcinomas, squamous cell carcinomas and sarcomas [15]. Dhillon *et al.* [11] showed that there is a considerable variation in the amount of NSE within each melanoma tumour. Moreover, the NSE staining intensity was significantly higher in malignant melanomas than in benign naevi [11, 16]. In accordance with these findings, one interpretation of our results could be that the NSE-producing fraction of the melanoma cells grows more aggressively than the other cells, and gradually becomes an increasing part of the tumour as the disease progresses. This hypothesis is supported by the fact that the change from normal to pathological serum NSE values in the individual patient was a reliable indicator of rapidly progressing disease. All patients converting from negative to positive NSE values died before the end of observation (Fig. 2). Thus, NSE proved to be a useful serum marker even in patients with initially normal NSE values.

The amount of NSE in serum also seemed to be related to the tumour burden. However, the amount of NSE in serum should not be regarded as a general measure of the number of tumour cells in a patient with malignant melanoma. The variation from one patient to another in the ability of the melanoma cells to produce NSE is demonstrated by the fact that 8 patients with a large initial tumour burden (extent of disease > 4), had serum NSE values less than 5 μg/l (Fig. 3).

The present results support the formerly published data from our hospital where elevated LDH, leucocyte count and ESR, as well as the presence of brain metastases, were found to be indicators of short survival in melanoma patients [10]. LDH, the leucocyte count and ESR, are, however, non-specific markers that probably reflect the reaction of the organism to the tumour cells. Moreover, pathological values of these parameters can appear from several other reasons than cancer, while NSE is a product of the tumour cells. We suggest that special attention is paid to the serum NSE value when estimating the prognosis of patients with metastatic melanoma.

In the present study the estimation of extent of disease was necessary to see if the amount of NSE was correlated with the tumour burden (Fig. 3). The estimation of the total tumour burden demands several diagnostic procedures which usually entail hospitalisation. The estimation of serum NSE requires only a blood test and seems to add valuable prognostic information.

The questions should be raised as to whether elevated serum NSE values should also be an exclusion criterion in phase II trials, and whether patients with elevated values should not be subjected to toxic treatments. To answer these questions, however, larger studies are needed.

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Prognostic Significance of Pretreatment Serum Levels of Squamous Cell Carcinoma Antigen and CA 125 in Cervical Carcinoma

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Serum levels of squamous cell carcinoma antigen SCC, carcinoembryonic antigen CA 125, and tissue polypeptide antigen were determined in 142 patients with primary cervical carcinoma, 60 patients with precancerous lesions and in 129 healthy women. With regard to elevated tumour marker levels, specificity ranged from 94.6% to 97.7%. Sensitivity was highest (44.4%) for SCC. A stage relation was found for all tumour markers except for carcinoembryonic antigen. In stage Ib, SCC levels increased according to tumour volume. SCC, CA 125 or both markers were elevated in 7 of 8 patients with pelvic lymph node metastases compared with only 17 of 58 patients with negative nodes ($P = 0.005$). In a multivariate analysis, pretreatment serum levels of SCC and CA 125 were found to be significantly related to patient survival, in addition to stage. In cervical SCC, the risk of a fatal outcome increased 16 times with SCC levels ≥ 4.5 ng/ml, compared with SCC levels ≤ 1.3 ng/ml. We conclude that pretreatment serum levels of SCC may be of value as an adjunct to clinical staging. In addition, serum determinations of SCC and CA 125 seem to be useful in predicting the risk of pelvic lymph node metastases and as prognostic risk factors for disease outcome.
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INTRODUCTION

THE OVERALL incidence of cervical carcinoma has declined in Sweden during the last decades, constituting 2–3% of newly diagnosed cancers in women today [1]. Although many of these women present clinically in early stages of cervical carcinoma, which indicate a favourable prognosis, 10–20% of them will die of their disease [2].

The extension of disease, estimated by clinical staging according to the International Federation of Gynaecology and Obstetrics (FIGO), is usually the factor which determines the mode of treatment and the disease outcome. Several authors have suggested that the volume of the tumour is of prognostic value [3]. However, treatment is more or less identical for a given stage (especially in the early stages) despite sometimes significant variations in tumour volume.

The discrepancies between clinical and surgical staging, which

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