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Neuron specific enolase - selective marker for small-cell lung cancer

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Background. *Neuron specific enolase (NSE) is an isomer of the glycolytic enzyme enolase, which was first found in extracts of brain tissue, and was later shown to be present in neuroendocrine cells and neuroendocrine tumours. The aim of the study was to confirm the importance of serum NSE as a tumour marker in patients with small-cell lung cancer.*

Patients and methods. *Serum levels of NSE were measured by the radioimmunoassay in 71 patients with lung cancer (LC), in 24 patients with non malignant lung diseases and in 28 healthy adults.*

Results. *According to the serum values in the group of healthy adults, 16.6 ng/ml was determined as a cut of level of NSE. By the specificity of 88.13 % in the group of non malignant lung diseases, the sensitivity of 47.82 % was obtained in patients with LC, which increased to 72.72 % in the patients with SCLC. In patients with non-small-cell lung cancer (NSCLC) the sensitivity of NSE test was 38.89 %. The patients with SCLC-extensive disease had a significantly higher mean NSE level (290.48 ng/ml) than patients with the limited stage disease (46.94 ng/ml). Serial measurements in 16 patients receiving combined chemotherapy and/or radiotherapy showed an excellent correlation between serum NSE level and clinical response.*

Conclusions. *These results indicate that serum NSE may be a useful marker for diagnosis, staging and for monitoring response to the therapy in patients with SCLC.*

Key words: lung neoplasms; carcinoma small cell; tumor markers; biological; neuron specific enolase

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Introduction

Enolase is a glycolytic cytoplasmic enzyme, present in all human cells, catalysing the conversion of 2-phosphoglycerate to 2-phosphoenolpyruvate.[1] The enzyme consists of thrree dimeric isoenzymes, called as α, β and γ. Neuron specific enolase (NSE) is γ-γ dimer and presents the neuronal form of the enolase.[2] Originally extracted from the bovine

brain tissue, it was first considered that the gene coding for NSE was restricted to neurons, and that it was only present in the central nervous system. In 1978 Schmechel *et al.* have shown that NSE is also present in all peripheral and central neuroendocrine cells, named APUD (amine precursor uptake and decarboxylation) cells.[3] Tapia *et al.* have extended this work with immunohistochemical and extraction techniques and showed that NSE is present in a wide variety of APUD neoplasms or APUDomas including: islet tumours of the pancreas, gastrinomas, VIPomas, medullary carcinoma of the thyroid, pheochromocytoma, and small-cell carcinoma of the lung (SCLC) among others.[4] In contrast, they could not find NSE presence in any non-neuroendocrine tumours.[4,5] High pre-treatment levels of NSE have been detected by the radioimmunoassay in the sera of the patients with neuroendocrine tumours, including SCLC.[6-8]

Therefore, this study was designed to re-evaluate the role of serum NSE in the diagnosis and differential diagnosis of the patients with lung carcinoma; to re-evaluate whether serum NSE levels are in the correlation with the extent of tumour dissemination or stages of the disease, and to re-evaluate the role of NSE as a marker for monitoring a therapeutic response in the patients with lung carcinoma.

Patients and methods

Patients

In this study 123 persons divided in three groups were included: first group - 71 newly diagnosed untreated patients with a different type of lung carcinoma; second group - 24 patients with non malignant lung diseases and third group - 28 healthy adults.

According to the histological types of lung carcinoma, the patients from the first group were further divided in two groups: 33 patients with SCLC and 38 patients with no-small-cell lung cancer (NSCLC).

Methods

Clinical assessment

The routine pre-treatment staging procedures consisted of physical examination; biochemistry; chest X ray; lung functional tests; fiberoptic bronchoscopy (with bronchial biopsy and cytological examination of brushings and washings); ultrasound procedures; radionuclide scan of bone. Biopsy or fine needle aspiration specimens of enlarged lymph-nodes, subcutaneus nodules and pleural effusions were taken when clinically indicated. According to these findings, the patients with SCLC were staged as having limited disease (tumour confined to one hemithorax, including the ipsilateral lymph nodes) or extensive disease (outside these limits). The patients with NSCLC were divided in five stages of TNM classification. Only 16 patients were followed up and a response to chemotherapy and/or radiotherapy was evaluated. The response was judged to be: complete (CR) when both clinical and pathological evidence of tumour totally disappeared; partial (PR) when there was a reduction of 50% or more in the sum of all measurable and evaluable tumour masses. Lesser degrees of tumour reduction were judged as no response.

The diagnosis of all patients was confirmed at the Institute for Lung Diseases and Tuberculosis; the patients were treated at the Institute for Radiology and Oncology, Medical Faculty in Skopje.

Immunoassay

Blood specimens were collected from each of the 71 patients with lung carcinoma at diagnosis, as well as from the patients with non malignant lung diseases and healthy adults. Serial samples were obtained from 16 of 71 patients with lung carcinoma, usually at 6-week intervals, after each course of

chemotherapy or after the end of radiotherapy. The serum was separated immediately after the collection and was stored at - 20 °C before the assay. NSE levels were determined by a double-antibody solid phase radioimmunoassay technique (Pharmacia NSE-RIA test) at the Institute for Immunology, Medical Faculty, Skopje.

Student's t test, Newman- Keuls test and x^2 test were used to determine the statistical significance between the mean values and between raised frequencies separately.

Results

Twenty eight healthy adults had NSE serum level ranging from 2.58 to 17.41 ng/ml (mean level 8.01 +/- 4.40 ng /ml). The upper limit of the normal interval for serum NSE 16.6 ng/ml is defined as the mean value for healthy controls plus 1.96 standard deviations. Only two patients from this group (7.14%) had a raised serum level above the normal value.

In the group of patients with non-malignant lung diseases NSE serum level was ranged from 2.61ng/ml to 41.87ng/ml, with the mean level of 11.79 +/- 9.53 ng/ml. Among them, five were serum NSE positive (20.83%). On the basis of these findings 88.13% was determined as a specificity of NSE test.

The mean level of NSE in the group of 71 patients with LC was 127.96 +/- 442.53 ng/ml. Thirty-four of them were found to have raised serum NSE concentrations that determined the sensitivity of NSE in LC of 47.82%. A statistical analysis between the positive NSE findings in the three groups showed a significant difference ($x^2 = 18.19$ $p< 0.001$). With Newman-Keuls test we obtained a statistical significant difference between the mean values in the three groups ($F=1.84$, $p<0.05$).

When the upper normal limit for serum NSE was taken to be 16.6 ng/ml, 73% of patients with SCLC were found to have raised NSE concentrations compared with 38% of patients with NSCLC ($x^2 = 12.78$, $p<0.001$).

Eighteen of 33 patients with SCLC had a limited stage disease and 56 had an extensive disease. NSE was raised in 10 of 18 (55.55%) limited-stage patients and in all 15 (100%) patients with an extensive-stage disease ($x^2 = 8.8$. $p<0.005$). The mean pre-treatment NSE in the limited-stage disease was 46.94 +/- 56.92 ng /ml, versus 290.48 +/- 325.24 ng/ml for the extensive-stage disease (Student's t test = 2.69, $p<0.001$; Table 1).

There was not a significant statistical difference between the number of patients with NSCLC in different TNM stages who had the raised serum NSE level above 16.6 ng/ml. The results are shown in the Table 2 ($x^2 = 7.27$, p non significant).

Serum NSE was measured again in 16 pa-

Table 1. Mean value and sensitivity of NSE in the patients with SCLC

Group	Mean value	Frequency of raised value >16,6ng/ml	Sensitivity
SCLC limited diseases	46.94 +/- 56.92	10/18	55.5 %
SCLC extensive diseases	290.48 +/- 325.24	15/15	100 %

Table 2. Sensitivity of NSE in patients with NSCLC in different TNM stages

Group	Number	Freqensy of raised value> 16,6ng/ml	Sensitivity (%)
Stage I	13	4 / 13	30.7
Stage II	12	1 / 8	12.5
Stage IIIa	10	2 / 6	33.3
Stage IIIb	9	2 / 5	40.0
Stage IV	11	5 / 6	83.3

tients after the end of each course of chemotherapy or at the end of radiotherapy when staging procedures were repeated. Eleven of these 16 patients were with SCLC, and 5 with NSCLC. In Figure 1a, there are the changes of serum NSE level in 13 patients who responded to therapy (responders); 9 were with SCLC and 2 with NSCLC. Eight patients had the elevated serum NSE level above 16.6 ng/ml and had a predominantly extensive disease.

According to the clinical signs, when a complete or a partial response was obtained, the serum NSE level in 6 patients decreases to a normal range; in one patient the serum NSE level decreased, but between 16 and 20 ng / ml, and in one patient the level remained stable. In other five patients who had the serum NSE level in a normal range at diagnosis, at the moment when CR or PR was clinically achieved, the serum NSE level decreased in 2 patients, versus slightly elevation in three patients, even in the normal range. However, when they had a relapse, the serum NSE rose again: in 11 patients above a normal range and in 2 patients to a normal value.

Figure 1b shows the changes in the serum level of NSE in three patients who did not response to chemotherapy. In one patient the pre-treatment level was in a normal range; in the other two patients NSE level was above 16.6 ng/ml. In all three patients there is a clear elevation in NSE during the chemotherapy that predicted the clinical recognition of relapse.

Discussion and conclusions

A raised serum NSE was observed in 47.8% of the patients with LC; the sensibility of NSE was 72.72% in patients with SCLC *versus* 38.89% in NSCLC. Our results correspond to the findings of other authors: Carney et al.[6] - NSE sensibility of 69% in SCLC with the cut of level of 12 ng/ml, Cooper et al.[8] - NSE sensibility of 77% in SCLC with the cut of level of 13 ng/ml, Esscher et al.[1] - NSE sensibility of 85% in SCLC and 25% in NSCLC with the cut of level of 12 ng/ml, Lorenz et al.[9] - NSE sensibility of 98% in SCLC and 4% in NSCLC with the cut of level of 15 ng/ml.

NSE is a marker specific for the neuroendocrine system and for tumours that arise from it, the so-called ADUP neoplasms. The characteristics of these tumours are defined

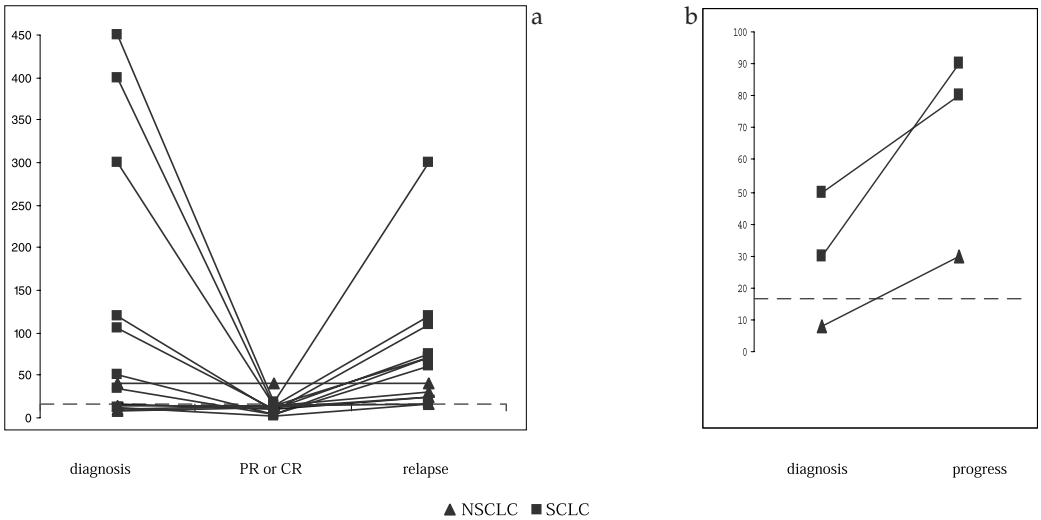

Figure 1. Serum NSE level changes (ng/ml) in patients with SCLC and NSCLC during treatment.

in vivo and *in vitro* studies: many neurosecretory granules, the ability for the production of different hormones and polypeptides, the high level of L-Dopa decarboxylasa and NSE.[10] When the characteristic features of SCLC were first described, this tumour was considered to be an anaplastic malignancy with which a variety of paraneoplastic syndromes were associated.[11] In the 1960, however, the presence of neurosecretory granules within the cells was described, which led to the inclusion of SCLC within the APUD system. It was thus presumed that the anaplastic cells were derived, in the normal bronchial mucosa, from the Kulchitsky cells which posses APUD properties. It is interesting why serum NSE is elevated in some of patients with NSCLC. The mechanisms by which NSCLC cells are capable of producing APUD-derived enzymes and hormones are not known. According to the findings of Gazard, there is a possibility of *in vitro* "conversion" of small-cell lung cacinoma to large cell cancer morphology. Although "transformed" cells had lost most or all of their "amine precusor uptake and decarboxylation characteristics" certain neuroendocrine features such as NSE were retained. Thus, it is possible that the large cell lung cancers were originally small cell tumours that "changed" histology, but "retained" NSE activity or were mixed tumours with the small cell component. The appearance of heterogeneous cell populations in the carcinomas is the reason why different parts of the tumour tissue show a different immunohistochemical expression for the same marker and why the tumour metastasises do not release some tumour marker which is released by the primary tumour.[12]

The results regarding the extension of the disease in patients with SCLC are comparable with the results reported by other authors (mean value of limited disease *versus* mean value of extensive disease): Fischbah and Berthold[13] - 8.4 +/- 0.8 / 47.7 +/- 8.8 ng/ml; Carney *et al.*[6] - 13.8 / 59.0 ng/ml; Johnson *et al.*[7] - 33.4 +/- 4.7 / 94.5 +/- 13.8 ng/ml; Cooper *et al.*[8] - 14 / 42 ng/ml; Splinter *et al.*[14] - 25 / 51 ng/ml. According to Carney *et al.*[6], Johnson *et al*[7] and others, the serum level of NSE, is more in correlation with the tumour burden and the number of metastatic site than with the individual ability of tumour to produce NSE.

Our follow-up studies in 16 patients showed the correlation between the tumour burden, the clinical response and serum NSE concentrations: the elevated, pre-treatment NSE levels decline to normal or nearly normal when CR or PR was achieved, while 1 responding patient maintained the essentially stable NSE level. When the relapse or progression in the disease occurred, the NSE level rose again; what is more important, raising was obtained before the clinical recognition of the relapse; a rising NSE level may predict relapse weeks to months in advance of other clinical evidence and signal the need to change a therapy sooner. A progressive rise in NSE levels during the treatment indicates the tumour resistance or relapse and also need to change the therapy. With serial NSE measurements we can follow up the disease course of the patients with SCLC, and give a different therapy at a time when it is likely to be most beneficial, i.e., when the tumour burden is low.[7,8,15] NSE is a useful tumour marker for the diagnosis and the differential diagnosis in patients with SCLC. This means that NSE measurements in patients with SCLC are at least a useful addition to standard investigational methods. Serial NSE measurements are useful for monitoring the course of the disease and therapeutical response; they provide information relevant to patient management which could not be obtained by the physical examination or routine staging procedures.

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