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ORIGINAL ARTICLE

Neuron specific enolase tissue expression as a prognostic factor in advanced non small cell lung cancer

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Summary

Purpose: *To determine the frequency of neuron specific enolase (NSE) tissue expression and its possible influence on survival of patients treated for advanced non small cell lung cancer (NSCLC).*

Patients and methods: *We studied 158 patients with histological diagnosis of NSCLC (stage III/IV). Combined chemoradiotherapy was used in stage III disease (without pleural effusion), whereas chemotherapy only was used in stage III (with pleural effusion) as well as in stage IV disease. Immunohistochemical analysis of NSE expression was determined using antibodies to NSE (DAKO, Den). One- and 2-year overall survival were assessed.*

Results: *45 (28.5%) patients had positive NSE expression. The most frequent NSE expression was seen in 6/9 (66.7%) patients with large cell carcinoma and in 23/50 (46%) with adenocarcinoma. One- and 2-year survival rates were 70% and 30% in the group of patients with positive NSE expression compared to 26% and 3% in the group with negative NSE expression (p=0.000). The median survival time was 16.4 vs. 11.4 months in the groups of patients with and without NSE expression, respectively (p < 0.001). One- and 2-year survival rate was higher in NSCLC patients with more than 50% of NSE positive cancer cells (p=0.0004 and 0.000, respectively).*

Conclusion: *A total of 28.5% of advanced NSCLC patients had positive NSE expression. Median 1- and 2-year survival time was significantly longer in patients with positive NSE expression.*

Key words: lung cancer, neuroendocrine, neuron specific enolase, non small cell, survival

Introduction

Lung cancers with neuroendocrine differentiation belong to an heterogeneous group of carcinomas with different clinical behavior. These tumors are characterized by the expression of panendocrine markers, neuroamines, neuropeptides and by ultrastructural evidence of dense-core secretory granules identified using immunohistochemical methods or by electron microscopy [1]. It has been found that a broad spectrum of immunohistochemical markers can highlight neuroendocrine differentiation in lung tumors, although NSE remains the most suitable marker.

The finding of neuroendocrine differentiation in some NSCLC has led to the hypothesis that these tumors may form a subgroup with a prognosis and response to treatment somewhere between NSCLC and small cell lung cancer (SCLC). Almost 10-30% of NSCLCs express neuroendocrine properties confirmed by immunohistochemistry. Therefore, these tumors are referred to collectively as NSCLC with neuroendocrine differentiation (NSCLC-ND) [2]. Recent studies, in which neuroendocrine expression was described as prognostic factor, have provided inconsistent and sometimes conflicting results. Some series have shown NSCLC-ND to be associated with longer survival [3,4], whereas others have not [5,6]. Carnaghi et al. confirmed these controversial data by analyzing 13 large clinical trials. In 2 of them, the authors described shorter survival, in 8 studies expression did not correlate with

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survival, but in the last 3 significantly longer survival was registered [7].

The aim of this trial was to determine the frequency and influence of NSE expression on survival in patients treated for advanced NSCLC.

Patients and methods

A total of 158 patients with advanced NSCLC treated in the Military Medical Academy during the period 2001-2005, were analyzed. Fresh material obtained via endo/transbronchial biopsy was studied. The diagnosis was histologically confirmed using standard haematoxylin-eosin method. NSE expression was determined by immunohistochemical analysis using monoclonal mouse antihuman NSE antibody (1:50; Dako Ltd, Den). Staining intensity was assessed as follows: 0=none, 1+=weak, 2+=moderate, 3+=strong, and the percentage of NSE-positive tumor cells was graded as follows: 0=none, 1+=<10%, 2+=10-50%, 3+=>50% [8]. For NSE antibody the score for intensity was multiplied by that for distribution to give an intensity-distribution (ID) score. An ID score of >2 was used as the criterion for evidence of NSE expression.

Patients were staged using the Mountain Staging System (TNM) [9]. Staging was performed prior to the most recent update of the therapy protocol. Therapy was determined according to disease stage.

Stage IIIA and IIIB (without pleural effusion) patients were treated with chemoradiotherapy, while stage IIIB (with pleural effusion) and IV patients were treated with chemotherapy alone. Chemotherapy was carbo/cisplatin-based and was administered up to 6 cycles or until disease progression. Assessment of response to therapy was carried out every 2nd cycle. When disease progression was apparent in stage IIIA and IIIB (without pleural effusion) treatment continued with radiotherapy alone (split course, total tumor dose 55-60 Gy).

Statistics

The survival of the treated patients was assessed in 1 and 2 years. Survival was calculated by the Kaplan-Meier method and differences between groups were analyzed by the log-rank test. Differences in patient characteristics between groups were evaluated by the chi-square test.

Results

117 male and 41 female patients were evaluated. The patient mean age was 62.35±11.57 years (range 37-74). The majority of patients (42.41%) belonged to 60-69 years age group. Of 158 eligible patients, 45 (28.5%) had positive NSE expression.

The distribution of NSE expression in relation to NSCLC histological subtypes is shown in Table 1.

The most frequent expression of NSE was obtained in the group of patients with large cell carcinoma and adenocarcinoma. Of 9 patients with large cell lung cancer, 6 (66.7%) had positive NSE expression and of 50 patients with adenocarcinoma 23 (46.0%) showed positive NSE expression. Of 14 patients with adenosquamous carcinoma 4 (28.6%) displayed positive NSE expression, while squamous cell subtype showed positive NSE expression in only 12 of 85 (14.0%) patients. There was significant correlation between NSE expression and NSCLC histological subtypes (p=0.000).

One- and 2-year survival rates were 70% and 30% in the group of patients with positive NSE expression compared to 26% and 3% in the group without NSE expression (p=0.000; Figures 1,2). The median survival time was 16.4 vs. 11.4 months in the group of patients with and without positive NSE expression, respectively.

Table 1. NSE expression distribution in relation to NSCLC histological subtypes

Histological subtypes	*Positive n (%)*	*Negative n (%)*
Squamous cell Ca	12 (14.0)	73 (86.0)
Adenosquamous Ca	4 (28.6)	10 (71.4)
Adeno Ca	23 (46.0)	27 (54.0)
Large cell Ca	6 (66.7)	3 (33.3)

p=0.000

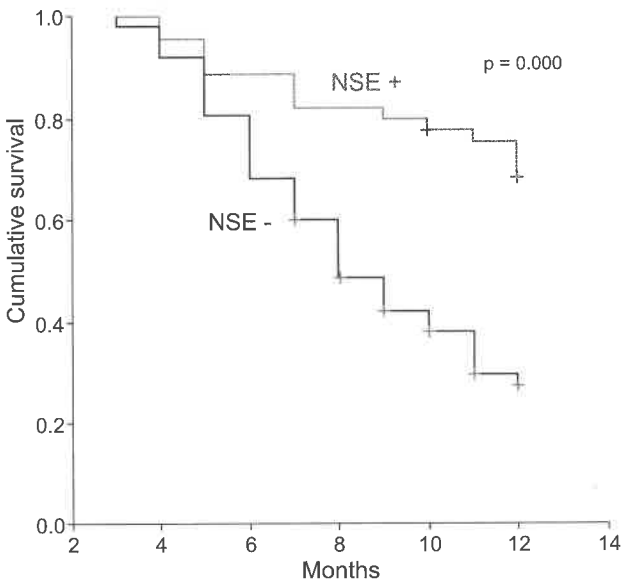


Figure 1. One-year survival curve of NSCLC patients with or without NSE expression.

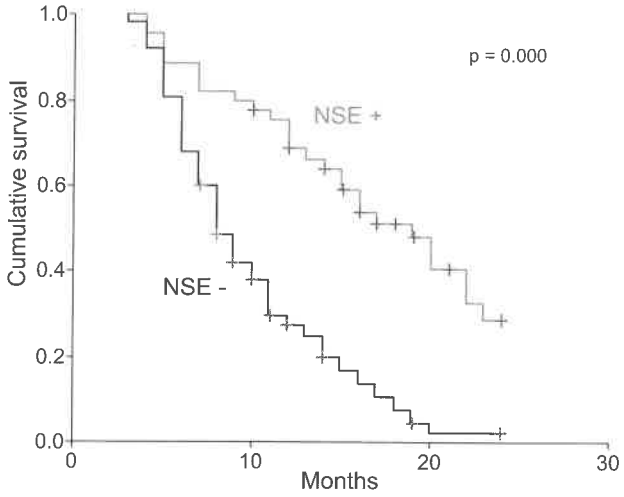


Figure 2. Two-year survival curve of NSCLC patients with or without NSE expression.

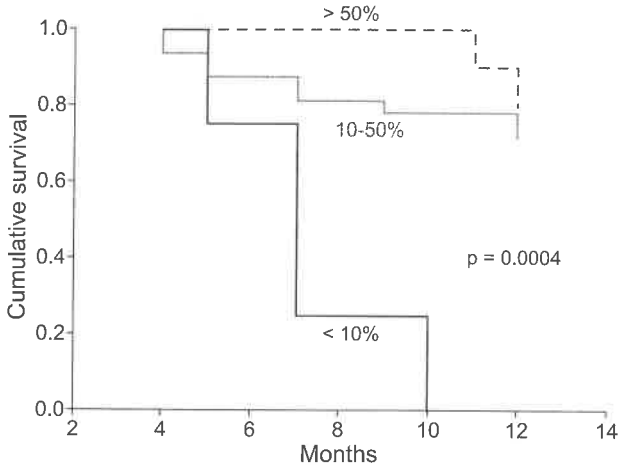


Figure 3. One-year survival curve of NSCLC patients with neuroendocrine expression in relation to percentage of NSE-positive cancer cells.

Significant difference was detected in 1-year survival of NSCLC patients with neuroendocrine expression in relation to the percentage of NSE-positive cancer cells. One-year survival rate in patients with more than 50% NSE-positive cancer cells was 80%, whereas survival in patients with less than 10% NSE-positive cancer cells was less than 10 months (p=0.0004; Figure 3). Two-year survival rate in patients with more than 50% NSE-positive cancer cells was 60%, whereas in patients with 10-50% NSE-positive cancer cells it was 38% (p=0.000; Figure 4).

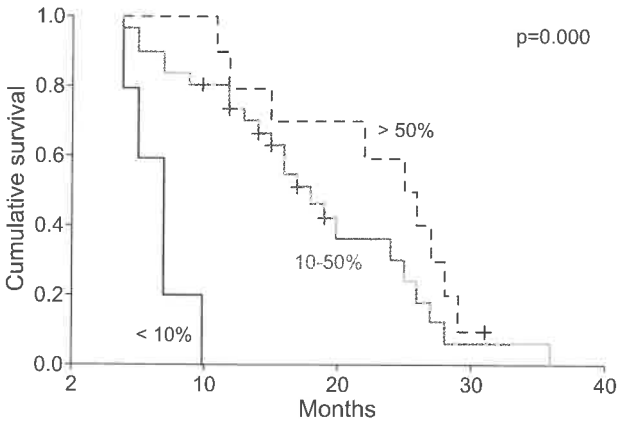


Figure 4. Two-year survival curve of NSCLC patients with neuroendocrine expression in relation to percentage of NSE-positive cancer cells.

Discussion

Neuroendocrine features of NSCLC have been reported in many studies with considerable variation in results ranging from 30 to 79% of the cases [5,8,10]. Slodkowska et al. [5] and a group of Chinese investigators [10] described higher frequency of NSE expression (79.3% and 59.3%, respectively) in patients with NSCLC. Graziano et al. [6] described results similar to ours.

Differences in tissue processing, techniques or markers used for highlighting neuroendocrine differentiation, definitions of positive results and study population selection may account for these contradictory results reported in the clinical meaning of neuroendocrine differentiation in patients with NSCLC [3,6,11]. Investigating the NSCLC neuroendocrine differentiation, a group of Norwegian researchers suggested that the frequency of neuroendocrine expression fluctuated from 5% using standard immunohistochemical methods to 25% using tyramide signal amplification [12].

Relative ambiguity exists in the relevant literature over the definition of NSE positivity [3,11,13,14]. Neuroendocrine differentiation was detected in 12% of the cases when the criterion for NSE positivity was 10% or more of the stained tumor cells [8]. Sundaresan et al. described neuroendocrine expression in 30% of all patients with NSCLC (n=107) when the criterion for NSE positivity was 5% or more of NSE positive cancer cells [15].

In our study, there was a significant correlation between NSE expression and histological subtypes of NSCLC (p=0.000). NSE expression was detected most often in large cell carcinomas followed by adenocarcinomas. Peng et al. have published similar results. Large cell lung cancer with neuroendocrine expression is clinicopathologically similar to SCLC because of high biological neuroendocrine differentiation level [16].

In our study squamous cell lung cancer displayed

the lowest NSE expression compared to other NSCLC subtypes, a fact confirmed also by other investigators [17]. Carnaghi et al. and Baldi et al. described high frequency of NSE expression in lung adenocarcinoma [7,18]. However, Schleusener et al. indicated that there were no significant differences in NSE expression between various NSCLC histological subtypes [4].

In our study a significant correlation was detected between NSE expression and survival of patients with advanced stage III and IV NSCLC. Our results indicated that 1- and 2-year survival were significantly higher in patients with positive NSE expression compared to those with negative NSE expression. Hiroshima et al. [11] presented very similar results. However, Gajra et al. [3] indicated that neuroendocrine differentiation was not significant prognostic factor for response to chemotherapy and survival.

Skov et al. analyzed the percentage of NSE-positive cancer cells to see if this approach could be used as a prognostic factor in NSCLC patients. Patients with more than 10% of NSE-positive cancer cells had significantly longer survival time compared to those with less percentage of positive cells [19]. In our study, 1- and 2-year survival rate in the group of NSCLC patients with more than 50% NSE-positive cancer cells was significantly longer compared to the group with less than 10% of NSE-positive cancer cells.

Conclusion

Positive NSE tissue expression existed in 28.5% of patients with advanced NSCLC. NSE expression was detected most often in large cell carcinomas followed by adenocarcinomas. One- and 2-year survival time was significantly higher in patients with positive NSE expression. Moreover, longer survival was often associated with the presence of more than 50% of NSE-positive cancer cells.

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