

The Clinical Value of Neuron-Specific Enolase as a Tumor Marker in Bronchoalveolar Lavage

M. Concepción Prados, M.D., Ph.D.,* Rodolfo Alvarez-Sala, M.D., Ph.D.,*
Rafael Blasco, M.D., Ph.D.,† Tomás Chivato, M.D.,‡ José L. García Satué, M.D.,*
Francisco J. García Ríó, M.D.,* F. Javier Gómez de Terreros,§
and José Villamor, M.D., Ph.D.*

Background. Neuron-specific enolase (NSE) is used in the staging and monitoring of responses to therapy and the detection of recurrences in lung cancer. The diagnostic value of NSE has been under discussion. This may be because NSE usually has been studied in the sera of patients with bronchogenic carcinoma and not in the bronchoalveolar lavage (BAL).

Methods. The NSE levels in the BAL of three groups—control subjects, patients with chronic bronchitis, and patients with tumors—were analyzed. The fluid obtained was centrifuged. The NSE was analyzed in the supernatant of the BAL (NSE, Pharmacia, Columbia, MD). Its concentrations were calculated in relation to milligrams of total protein.

Results. A significant difference was noted in the level of NSE in the BAL of the tumor group compared with those of the other two groups. No differences were observed between the other two groups or between healthy smokers and nonsmokers. No correlation was found with the histologic type of pulmonary carcinoma and NSE levels in BAL. The NSE levels were higher in the lavages of patients with primary pulmonary carcinomas than in those with metastases.

Conclusions. Neuron-specific enolase could be of aid in the early diagnosis of solitary pulmonary nodules and lung cancer. More studies would be required to identify a correlation between NSE levels in BAL and those in serum, or between NSE levels in BAL and tumor size and location and disease stage of lung cancer. *Cancer* 1994; 74: 1552-5.

Key words: neuron-specific enolase, lung cancer, bronchoalveolar lavage.

Neuron-specific enolase (NSE) is a biochemical marker proposed as a tumor marker for several types of carcinomas, such as lung cancer. It is a useful procedure in staging and monitoring the response to therapy and the detection of early recurrences and extrathoracic involvement in these patients.¹⁻³ Some authors³⁻⁶ have demonstrated the pretreatment prognostic value of NSE in the sera of patients with small cell lung cancer (SCLC). Although other authors^{6,7} have established a possible diagnostic value of NSE in the sera of patients with SCLC, the role played by NSE in the diagnosis of lung cancer is relatively unknown. This may be because the analyses of NSE have focused on the serum, but not on the bronchoalveolar lavage (BAL), of patients with pulmonary carcinomas. BAL allows us to obtain the bronchial and alveolar components of the lung.⁸ As different authors^{9,10} have reported, we think that the measurement of NSE in the BAL fluid obtained from the corresponding lobar bronchus might be a more suitable procedure as a diagnostic marker.⁹ Our aim was to analyze the diagnostic value of NSE as a tumor marker in the BAL of patients with different types of lung cancer.

Patients and Methods

We studied 126 subjects classified in three groups.

Control Group

The control group had 41 individuals, 27 men and 14 women, with a mean age of 39.5 ± 2.9 years (range, 18-73 years). Of these, 12 were smokers and 29 were nonsmokers. Bronchoscopy was performed for one of

From the Departments of *Pneumology, †Nuclear Medicine, ‡Clinical Immunology and Allergy, and §Pneumology Services, La Paz and Aire Hospitals, Autónoma and Complutense Universities, Madrid, Spain.

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Address for reprints: M. Concepción Prados, M.D., Ph.D., c/o Julio Dánvila 1, 3ºB, 28033 Madrid, Spain.

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the following reasons: possible hemoptysis that later revealed nonpulmonary bleeding (10 patients), dysphonia (5 patients), and vocal chord paralysis (4 patients) which were not attributable to a pulmonary disease, and because of unclear radiologic images, such as enlargement of pulmonary arteries, unclear pulmonary hilia, granulomas, or diaphragmatic elevations (22 patients). Subjects were monitored for a 1.5 years after bronchoscopy to verify the absence of pulmonary disease.

Chronic Bronchitis Group

This group contained 12 patients, 11 male patients and 1 female patient, with a mean age of 64.5 ± 9.0 years (range, 14–80 years). Eight of them were smokers and four were nonsmokers. The diagnosis was established according to the clinical history.

Tumor Group

The tumor group had 73 patients, 64 men and 9 women, with a mean age of 63.7 ± 4.9 years (range, 18–80 years). Fifty-nine of the patients were smokers and 14 were not. The histologic groups are as follows: 39 patients had squamous cell carcinoma, 12 had adenocarcinoma, 12 had SCLC, 1 had a highly malignant neuroendocrine tumor, and 9 had metastatic carcinoma.

BAL was performed according to the European BAL Task Group norms.¹¹ The tip of the fiber-optic bronchoscope was wedged into the affected bronchiole or the closest one to the lesion in the patients in the tumor group and into the lingula or middle lobe in the control subjects or patients with chronic bronchitis to perform the BAL. Subsequently, 150 ml of 0.9% sterile saline serum was instilled in three aliquots of 50 ml. The fluid of each one was recovered by gentle suction. The total aspirated volume was quantified and immediately transferred to the laboratory. BAL fluid was centrifuged at 500 g for 15 minutes to separate the cellular components from the supernatant. NSE measurements were performed using a commercial kit and following the manufacturer's instructions (NSE, Pharmacia, Columbia, MD). Total proteins (TP) of the BAL were analyzed using the Lowry method. The results are expressed in nanograms per milligrams of TP.

The values were expressed as the mean and standard deviation and the range. Student *t* test was used to directly compare the BAL tumor marker concentrations in the control group and patients with chronic bronchitis and those with lung cancer. Multivariate analysis and the Kruskal–Wallis test were used to study the

Table 1. Comparison of the NSE Levels in the Bronchoalveolar Lavage Fluid of Healthy Smokers vs. Nonsmokers, the Control Group, the Tumor Group, and the Chronic Bronchitis Group

NSE level (ng/mg TP)	Group
2.2 ± 2.0	Smokers
1.0 ± 1.0	Nonsmokers
1.4 ± 1.4	CG
23.5 ± 19.3	TG
1.0 ± 0.5	CBG

NSE: neuron-specific enolase; TP: total protein; CG: control group; TG: tumor group; CBG: chronic bronchitis group.

P was not significant for S vs. nonsmokers and for CG vs. CBG. *P* < 0.001 for CG vs. TG and for CBG vs. TG.

differences between the tumor markers and the histologic types of neoplasia.

Results

The mean value of NSE in the BAL of the control group was 1.1 ± 1.4 ng/mg TP (range, 0.1–6 ng/mg TP). There were no differences when the NSE levels in BAL of smokers and nonsmokers were compared (Table 1). Two of the subjects in this group had concentrations higher than the control group's mean value of NSE, corresponding to a vocal chord paralysis (5.3 ng/mg TP) and a diaphragmatic elevation (6.0 ng/mg TP). The mean NSE concentrations in the BAL fluid of the chronic bronchitis group were 1.0 ± 0.5 ng/mg TP (range, 0–1.8 ng/mg TP). In this group, two patients had mean levels of NSE higher than the mean value (1.8 and 1.4 ng/mg TP, respectively). The mean NSE concentrations in the BAL fluid of the tumor group were 23.5 ± 29.3 ng/mg TP (range, 0.2–158.5 ng/mg TP) (Table 2). When the concentrations obtained in BAL between the control and the chronic bronchitis groups were compared, no differences were found (Table 2).

However, a significant difference was obtained between the mean values of NSE in the BAL of the control

Table 2. Comparison of the Bronchoalveolar Lavage Fluid NSE Levels in Patients with Primary Pulmonary Carcinomas vs. the Metastases Group

Group	NSE level (ng/mg TP)
Primary carcinoma	24.4 ± 31.8
Metastases	11.1 ± 15.6
Small cell lung cancer	28.4 ± 33.1
Non-small cell lung cancer	25.6 ± 30.7

NSE: neuron-specific enolase; TP: total protein.

P < 0.05 for primary carcinoma vs. metastases.

P was not significant for small cell lung cancer vs. non-small cell lung cancer.

group and the tumor group, and between the levels of NSE in the lavage of the patients with chronic bronchitis and those with tumor when they were compared ($P < 0.001$) (Table 2). The mean NSE concentrations in the BAL fluid depending on the histologic type were as follows: squamous carcinoma, 28.8 ± 32.5 ng/mg TP (range, 2.9–158.5 ng/mg TP); adenocarcinoma, 17.0 ± 22.7 ng/mg TP (range, 1.5–72.5 ng/mg TP); SCLC, 28.4 ± 33.7 (range, 1.45–74.5 ng/mg TP), and the malignant neuroendocrine tumor, 12.8 ng/mg TP. There was no correlation between NSE levels in the lavage and the histologic types of tumors. Three of the cancers proved difficult to access for the bronchoscope (two squamous carcinomas and one SCLC). All of them had high concentrations (158.5, 82.5, and 72.3 ng/mg TP, respectively). This could be of great value because it would allow the origin of a solitary pulmonary nodule or pulmonary mass to be distinguished. Among the patients with lung cancer, 13 (17.9%) had levels lower than the mean: 4 of 39 had squamous carcinomas, 3 of 12 had adenocarcinomas, 2 of 12 had SCLC, 3 of 9 had metastases, and 1 had malignant neuroendocrine cancer.

If all lung cancers of the tumor group are classified into primary bronchopulmonary carcinomas and pulmonary metastases, NSE levels in the BAL fluid of the former are greater than in those of the latter (Table 2). If all primary lung cancers are classified into two groups (small cell carcinomas and nonsmall cell carcinomas), no significant difference can be found (Table 2). This might be explained by the small number of patients with diagnoses of small cell bronchial carcinomas.

Discussion

Increased sera levels of NSE have been found in patients with extensive disease (65–69%), especially in those with SCLC.^{1,12,13} Several authors^{4,12,14–16} have shown a good correlation between serum NSE variations and the evolution of the disease. However, its diagnostic value has not been proven. Plasma might not be an ideal biologic medium for measuring the level of NSE in the early stages. BAL fluid might be a better procedure in this stage.¹⁷ Thus, we have analyzed the NSE of BAL fluid in patients with lung cancers, and we have compared these results with those obtained from the control group and in the patients with chronic bronchitis. We noticed that the NSE levels in the BAL of the tumor group were significantly higher than those found in the two other groups, although some subjects of the control group and patients in the chronic bronchitis group had elevated levels, but the levels were not as high as those of the patients in the tumor group. In this sense, NSE studied in the BAL of patients with bron-

chial carcinomas could assist in the early diagnosis of this disease. Three of the patients had tumors that were difficult for bronchoscope to access. All three of these patients had high levels of NSE in the lavage. De Diego et al.¹⁸ considered that NSE in the BAL might have a diagnostic value when used to study peripheral lung cancers. This means that increased concentrations of NSE in the BAL fluid of patients with pulmonary solitary nodules or masses could indicate malignancy.

According to other reports,^{3,12,19} enolase is localized in non-neuron cells and non-neuroendocrine tissues. Fujita et al.¹² seem to show that NSE concentrations in all histologic types of lung cancer are enhanced several times compared with those in normal lung tissue. This may be because of the activity of glycolytic enzymes in tumor cells accompanied by an increase in anaerobic glycolysis. This may explain why NSE and other neural markers are elevated in lung cancer, regardless of the histologic type.^{10,12} Scagliotti et al.¹⁰ considered that several neural markers, such as gastrin releasing peptide, would be of great assistance in the diagnosis of lung cancer if they were studied in the BAL of these patients, rather than in their sera. Among all nonsmall cell lung cancers, large cell carcinomas are those that have neuroendocrine properties.²⁰ We have found no relation between the NSE levels in the BAL and the different types of lung cancer; we also found no relation between NSE in the lavage of SCLC and nonsmall cell lung cancers. Nevertheless, higher concentrations of NSE were obtained in the BAL of patients with primary pulmonary carcinomas than in those with metastatic disease. This could mean that metastases could have less neuroendocrine properties.¹² Despite that, we think that more studies would be necessary to prove whether a relationship might exist between NSE in the BAL and some histologic types of bronchial carcinomas. In this way, greater accuracy would be obtained.

We conclude that NSE analyzed in the BAL fluid could be of assistance in the early diagnosis of lung cancers. However, we think additional research is required into a correlation with the disease stage, tumor size, tumor location, and NSE levels in the sera of such patients.

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