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Original Article

Serum neuron specific enolase levels correlate with patient prognosis for advanced lung cancer

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Abstract: To analyze the clinical and prognostic value of neuron specific enolase (NSE) levels in serum of advanced lung cancer patients, we analyzed serum NSE level of 110 advanced lung cancer patients (case group), 100 benign lung disease patients (benign disease group), and 100 healthy persons (control group). Case group patients were divided by NSE level into ≥25 ng/mL (52 cases) and <25 ng/mL (58 cases) groups to analyze overall survival (OS) and progression-free survival (PFS). The results showed the serum NSE levels of case group patients were significantly higher than those of control or benign disease group patients (P<0.05). Serum NSE levels of small cell lung cancer patients were significantly higher than those of patients with other tumor pathologies (all P<0.05). Median OS significantly differed between patients with NSE levels ≥25 ng/mL (23.7 months) and <25 ng/mL (31.4 months) (P<0.05). Median PFS also significantly differed between patients with NSE levels ≥25 ng/mL (13.5 months) and <25 ng/mL (17.6 months) (χ^2=9.992; P<0.05). Tumor pathology (RR=4.136), patient performance status score (RR=2.903), and serum NSE level (RR=2.338) were factors influencing OS (P<0.05). Patient performance status score (RR=2.903), number of chemotherapy lines (RR=1.776), and serum NSE level (RR=2.075) were influencing factors in patients' PFS (P<0.05). In brief, serum NSE level significantly correlates with advanced lung cancer patient prognosis and may be useful as an auxiliary index to predict prognosis.

Keywords: Neuron specific enolase, advanced lung cancer, serum markers, prognosis

Introduction

The high morbidity and mortality of lung cancer have become a popular, yet concerning, central topic in the medical field. According to World Health Organization statistics, the total number of new lung cancer cases worldwide is more than 1,200,000 per year, and the number of deaths is more than 1,100,000 per year [1]. Data from 2009 showed that, in the year alone, World Health Organization statistics, the total number of new lung cancer cases lung cancer morbidities and mortalities numbered 220,000 and 160,000, respectively [2, 3].

Lung cancer mortalities account for one-third of all cancer-caused deaths worldwide [4, 5]. The five-year survival rate of lung cancer is only 15% in the United States and even lower in Chinese populations [6]. In highly modernized metropolises of China, such as Beijing and Shanghai, lung cancer in the permanent population has had the highest morbidity and mortality in the past decade among all malignant tumor types [7].

Lung cancer is mainly divided into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Although SCLC accounts for less than 15% of all lung cancer cases, it is highly malignant and prone to early metastasis and multi-drug resistance. NSCLC accounts for more than 85% of lung cancer cases, but its malignancy is low. For NSCLC cases classified as clinical stage I or II and treated with standard surgical treatment, five-year survival rates can reach 40% [6, 7]. However, NSCLC is difficult to diagnose early, so most patients have advanced disease at diagnosis and thus have lost the best opportunity for radical surgery [8].

It is generally believed that lung cancer is a systemic disease, so it is difficult to treat with one therapeutic approach. The current ideal thera-

peutic mode for lung cancer is comprehensive therapy that combines chemotherapy (based on surgical operation), radiotherapy, and other treatment approaches [9]. The prognosis of lung cancer patients is closely correlated with tumor pathology and gene expression. Driver mutations that are correlated with onset and outcome of NSCLC include *EGFR*, *KRAS*, *HER2*, *BRAF*, and *ALK* [10]. However, there are few studies on driver genes of highly malignant adenosquamous carcinoma or SCLC.

Relevant studies of lung cancer, especially those on suspected carcinogenic factors, mechanisms of action, malignant transformation theory, experimental treatment, and prognosis prediction, are performed on animals instead of patients and thus have poor extrapolation. In addition, lung cancer-influencing factors are complex; therefore it is inaccurate and incomplete to analyze prognostic factors of lung cancer merely from macroscopic aspects. Lung cancer patient prognosis is currently predicted mainly based on clinical staging and pathology. However, even for lung cancer patients with similar clinical characteristics and who receive the same therapeutic regimen, disease progression and survival time can differ greatly [11].

Identifying driver gene-related molecular markers, such as single nucleotide polymorphisms (SNPs), has been a central issue for lung cancer studies. Several studies show that SNPs in multiple biological pathways can influence expression, structure, and function of genes and thus can influence tumorigenesis and patient prognosis. Expression of p16, p21, p27, cyclin A, CDK2, cyclin E, and other cell-cycle regulatory proteins and chemotactic factors are genetic markers that are closely correlated with lung cancer prognosis [12]. However, these markers require costly detection conditions and so are not ubiquitous in clinical practice. Therefore, serum tumor markers are still the most promising prognostic indicators for lung cancer in clinical applications. This study focuses on the clinical value of serum neuron specific enolase (NSE) in the prognosis of advanced lung cancer patients.

Subjects and methods

General information

Subjects enrolled at Heilongjiang Provincial Hospital (Harbin, Heilongjiang Province, China) between June 2011 and June 2014 were divided into case, benign disease, and control groups. Case, benign disease, and control groups did not significantly differ in age or gender ($P>0.05$). This study was approved by the Hospital Ethics Committee and the informed consent was obtained from all subjects.

The case group was comprised of 110 patients with advanced (stage IIIb~IV) primary lung cancer. All lung cancer cases were diagnosed through pathology or cytology examination and further confirmed with CT, MRI, and other imaging modalities to exclude patients with secondary lung cancer complicated with cerebral stroke, craniocerebral injury, or other neuronal injury-related diseases. The case group consisted of 62 males and 48 females, aged 49-78 years with an average age of 58.6±9.3 years. There were 12 cases of SCLC, 71 cases of adenocarcinoma, 23 cases of squamous carcinoma, and 4 cases of large cell carcinoma and other pathology types. The group included 26 cases of stage IIIb cancer, 84 cases of stage IV cancer, 29 cases with bone metastasis, 17 cases with brain metastases, 6 cases with liver metastasis, 66 cases with peripheral lymph node metastasis, and 40 cases with malignant pleural effusion. Eastern Cooperative Oncology Group performance status (ECOG PS) scores divided the case group into 78 cases scoring 0-1 and 32 cases scoring ≥2. Of the case group patients, 66 only received first-line chemotherapy and 44 received both first-line and second-line chemotherapy. The group included 41 current smokers or patients with smoking history and 69 non-smokers.

The benign disease group included 100 patients with benign lung diseases treated in Heilongjiang Provincial Hospital (Harbin, Heilongjiang Province, China) during the same time period. This group included 59 males and 41 females, aged 51-75 years with an average age of 57.3±7.8 years. All enrolled benign disease subjects had neither lung cancer nor neuronal injuries.

The control group consisted of 100 healthy individuals who underwent routine physical examination in Heilongjiang Provincial Hospital (Harbin, Heilongjiang Province, China) during the same time period. This group included 58 males and 42 females, aged 48-76 years with an average age of 58.9±8.9 years. All enrolled control subjects had no pulmonary disease, tumor, or neuronal injuries.

Table 1. Comparison of serum NSE levels among groups

Group	*Category*	*Number*	NSE (ng/mL)
Control group		100	8.52±4.61[a]
Benign disease group		100	9.66±5.63[b]
Case group		110	33.15±16.28
	SCLC	12	53.62±18.91
	Adenocarcinoma	71	22.38±15.32[c]
	Squamous cell carcinoma	23	23.69±16.33[d]
	Other pathological types	4	20.93±17.14[e]

Note: [a]*P*<0.05, q=0.475, *vs* case group; [b]*P*<0.05, q=0.458, *vs* case group; [c]*P*<0.05, q=4.508, *vs* SCLC patients; [d]*P*<0.05, q=4.366, *vs* SCLC patients; [e]*P*<0.05, q=4.602, *vs* SCLC patients.

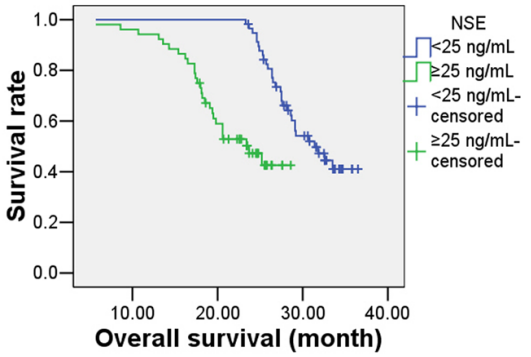

Figure 1. Kaplan-Meier survival curve of overall patient survival based on serum NSE level.

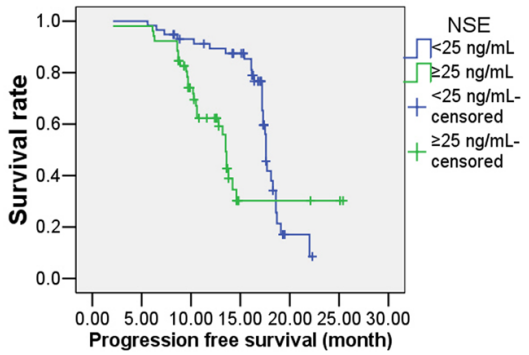

Figure 2. Kaplan-Meier survival curve of progression-free patient survival based on serum NSE level.

Observational index

Blood samples were collected from the median cubital vein of all subjects during a morning fast (on the following day after hospitalization for patients in case and benign disease groups; on examination day for patients in control group) and centrifuged at 4000 RPM for 15 min. An electrochemiluminescence analyzer (Roche) was used to analyze serum NSE levels using built-in instrument kits per manufacturer's instructions. Follow-up was performed for patients in the case group to determine overall survival (OS) and progression-free survival (PFS).

Statistical methods

SPSS 13.0 statistical package (SPSS Inc.) was used to establish a database for this study and to perform statistical analysis. Measurement data were expressed as mean ± standard deviation. Multi-group comparisons were performed with single factor analysis of variance, and pairwise comparisons were performed with SNK method (Q method). OS and PFS median were compared with Kaplan-Meier survival analyses, and log-rank test was used to detect statistical significance. Relevant factors influencing OS and PFS were analyzed through Cox multivariate regression analysis. The test level for all statistical tests was α=0.05.

Results

Serum NSE levels are increased in advanced lung cancer cases

Serum NSE levels significantly differed among all three groups (F=5.267; P<0.05). Serum NSE levels of case group patients were significantly higher than those of control (q=0.475, P<0.05) and benign disease group patients (q=0.458; P<0.05). No statistically significant difference was observed between benign disease and control groups (q=0.408; P>0.05). Among lung cancer patients with various pathologies, SCLC patients' NSE levels were significantly higher than patients with other tumor pathologies (q=4.508, 4.366, 4.602; P<0.05), while no sta-

Table 2. Cox multivariate analysis of variables' effect on overall survival (OS)

Variables		*HR*	*Wald*χ^2	P
Gender	Male	1.635	1.706	>0.05
Age	<60 years	1.728	1.026	>0.05
Smoking history	Never	0.442	0.488	>0.05
Pathology	Non-adenocarcinoma	4.136	8.755	<0.05
ECOG PS	≥2	2.903	6.731	<0.05
Chemotherapy lines	≥2	0.332	2.048	>0.05
Bone metastases	Yes	1.036	0.754	>0.05
Brain metastases	Yes	1.756	0.996	>0.05
Liver metastases	Yes	1.094	0.491	>0.05
Other lobes metastases	Yes	1.308	0.608	>0.05
Pleural effusion	Yes	0.769	0.602	>0.05
Serum NSE	≥25 ng/mL	2.338	4.895	<0.05

Table 3. Cox multivariate analysis of variables' effect on progression-free survival (PFS)

Variables		*HR*	*Wald*χ^2	P
Gender	Male	1.438	2.077	>0.05
Age	<60 years	1.168	0.207	>0.05
Smoking history	Never	0.876	0.575	>0.05
Pathology	Non-adenocarcinoma	1.773	1.902	>0.05
ECOG PS	≥2	2.055	5.128	<0.05
Chemotherapy lines	≥2	1.776	5.327	<0.05
Bone metastases	Yes	0.953	0.682	>0.05
Brain metastases	Yes	0.527	1.658	>0.05
Liver metastases	Yes	1.898	0.932	>0.05
Other lobes metastases	Yes	1.113	0.108	>0.05
Pleural effusion	Yes	0.736	0.648	>0.05
Serum NSE	≥25 ng/mL	2.075	4.379	<0.05

tistically significant difference was observed among patients with other tumor pathologies (q=0.411~0.736; P>0.05) (**Table 1**).

Serum NSE level correlates with patient survival

The follow-up time for patients in the case group was 6-39 months, up to December 31, 2014, with a median follow-up time of 19 months. The case group was divided into two groups based on serum NSE level: ≥25 ng/mL (52 cases) and <25 ng/mL group (58 cases). Median OS of patients in the NSE ≥25 ng/mL group was 23.7 months (95% CI: 18.984, 48.416) and that of patients in the NSE <25 ng/mL group was 31.4 months (95% CI: 27.329, 35.471). These differences were statistically significant (χ^2=23.474; P<0.05). **Figure 1** shows the Kaplan-Meier survival curve of OS between the two groups. Median PFS of patients in the NSE ≥25 ng/mL group was 13.5 months (95% CI: 13.019, 13.981) and that of patients in the NSE <25 ng/mL group was 17.6 months (95% CI: 17.367, 17.833). These differences were also statistically significant (χ^2= 9.992; P<0.05). **Figure 2** shows the Kaplan-Meier survival curve of PFS between the two groups.

Additional factors correlate with patient survival

Cox multivariate regression analyses assessed the effects of gender, age, smoking history, tumor pathology, ECOG PS score, number of chemotherapy lines, metastases, pleural effusion, and serum NSE level on patients' OS (**Table 2**) and PFS (**Table 3**). Results showed that tumor pathology (RR= 4.136), ECOG PS score (RR= 2.903), and serum NSE level (RR=2.338) influenced patients' OS (*P*<0.05) (**Table 2**). ECOG PS score (RR=2.903), number of chemotherapy lines (RR=1.776), and serum NSE level (RR=2.075) influenced patients' PFS (*P*<0.05) (**Table 3**).

Discussion

This study shows that serum NSE levels are significantly higher in advanced lung cancer patients than control or benign lung disease patients. Serum NSE levels also are significantly higher in SCLC cases than other tumor pathologies, including adenocarcinoma and squamous cell carcinoma These results show that serum NSE levels significantly correlate with prognosis of advanced lung cancer patients and suggest that NSE can be used as a secondary indicator in prognosis prediction, especially for SCLC.

NSE is a highly acidic protein that was first isolated from nervous tissue in 1965 by scholars who were investigating nervous system-specific proteins using DEAE-cellulose column chromatography and starch gel electrophoresis [13]. NSE is a key enzyme of the glycolytic pathway and is generally located in the cytoplasm of neurons and, characteristically, neuroendocrine cells. NSE leaks out of neurons during necrosis, so it is also an important marker of neuronal damage [14].

Patients with brain damage-related diseases show significantly increased NSE levels in serum or cerebrospinal fluid. NSE has been widely applied in diagnosis, disease evaluation, and prognosis assessment of cerebral infarction, cerebral hemorrhage, subarachnoid hemorrhage, epilepsy, brain trauma, cerebral anoxia, encephalitis, Creutzfeldt-Jakob disease, and other diseases related to central nervous system injury [15]. NSE levels are correlated with infarction volume, degree of neurological function defect, location of infarction, prognosis, and other indicators [16].

NSE is also a serum tumor marker that is correlated with malignant lung and neuroendocrine tumors. NSE in combination with cytokeratin 19 fragment, tumor specific growth factor, carcinoembryonic antigen, and other tumor markers is diagnostic for lung cancer [17, 18]. Further, when a single marker is used during detection, NSE has the highest positive correlation to SCLC. Therefore, many clinicians use NSE in differential diagnosis of SCLC and NSCLC, therapeutic effect monitoring, and prognosis estimation for SCLC.

This study also shows that tumor pathology, ECOG PS score, and serum NSE level influence patients' OS. ECOG PS score, number of chemotherapy lines, and serum NSE level influence patients' PFS. These results indicate that serum NSE level is correlated with patient survival and that serum NSE level is an independent risk factor that influences lung cancer prognosis. It is worth noting that-in addition to performance status, pathology, serum NSE level, and other clinical characteristics-number of chemotherapy lines is the only extrinsic factor affecting patient prognosis.

Median OS significantly differed between patients with NSE levels ≥25 ng/mL (23.7 months) and <25 ng/mL (31.4 months). Median PFS also significantly differed between patients with NSE levels ≥25 ng/mL (13.5 months) and <25 ng/mL (17.6 months). These results indicate that serum NSE level correlates with survival time of lung cancer patients, with higher serum NSE levels correlated to poorer prognosis. Recent studies have shown that the relevance between NSE level and patient prognosis for SCLC is significantly higher than other indicators, such as carcinoembryonic antigen and lactate dehydrogenase [19]. Serum NSE level may therefore be used as an auxiliary index to predict patient prognosis.

SCLC is lowly differentiated and prone to early hematogenous metastasis with poor prognosis. Only about 30%-40% of SCLC patients are in limited stage at first clinical treatment [20]. But even these patients' median survival is unlikely to exceed 20 months; only 20%-40% of SCLC patients in limited stage have a survival time exceeding two years. Comprehensive therapy is the standard treatment for such patients, and its efficacy can be affected by metabolism, speed of chemotherapy treatment (i.e., number of lines), DNA damage repair, and other factors [20].

Among lung cancer types, SCLC is more sensitive to initial chemotherapy but prone to multidrug resistance, thus leading to failure of chemotherapy, early recurrence and metastasis, and eventually death due to multiple organ failure. Multidrug resistance mechanisms of SCLC mainly refer to pharmacologic resistance, microenvironment resistance, apoptosis resistance, and biochemical drug resistance. Among these, apoptosis evasion is a common characteristic of tumor cells and also a driver for SCLC chemoresistance. In cell adhesion-mediated drug resistance, extracellular matrix proteins can antagonize apoptosis signals induced by cytotoxic drugs. Although chemotherapy is an important treatment for NSCLC, its effects are not ideal. The efficiency of combined chemotherapy has plateaued at 30%-40%, mainly due to tumor cell resistance to anticancer drugs. Multidrug resistance mechanisms of NSCLC may be related to resistance protein, glutathione S-transferase, topoisomerase II, protein kinase, microtubule gene mutation, telomerase, DNA damage repair functions, or apoptosis inhibition [21].

In addition, a development trend of individualized molecular targeted drugs, such as epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKIs), has added important comprehensive treatment choices for NSCLC patients. This appearance helps alleviate the clinical inefficacy of traditional surgery, radiotherapy, and chemotherapy. Molecular targeted drugs are selective for patients and have better tolerability in clinical applications; compared to conventional chemotherapy, such drugs are able to produce a higher effective rate and prolong patient survival [22]. Nevertheless, there are still many patients who develop primary and secondary resistance to molecular targeted drugs in clinical applications. For example, after an average period of 10-14 months, tumors treated with erlotinib, gefitinib, and other EGFR-TKIs tend to develop secondary resistance. Recent studies have found EGFR-TKI resistance is correlated with T790M mutation, MET amplification, phenotypic transformation, and other mechanisms [23].

These findings provide sound basis for molecular mechanism studies on next-generation targeted drugs that antagonize resistance [24]. In short, to produce additional clinical benefits for advanced lung cancer patients, clinicians should not only consider patients' clinical characteristics, but also develop personalized and sensitive chemotherapy regimens in a timely manner to effectively improve patient prognosis.

Disclosure of conflict of interest

None.

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