Prognostic value of neuron specific enolase in patients with advanced and metastatic non-neuroendocrine non-small cell lung cancer

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Abstract

Background: Increased serum NSE levels were found in a substantial proportion (30%-69%) of patients with NSCLC, but little is known about the clinical properties of neuron specific enolase (NSE). **Objective:** We aimed to assess the level of serum NSE to predict the prognosis and treatment response in advanced or metastatic non-neuroendocrine non-small cell lung cancer. **Methods:** We retrospectively analyzed 363 patients with advanced and metastatic NSCLC (without neuroendocrine immunohistochemistry stain and brain metastasis) between January 2011 and October 2016. The serum NSE level was measured before initiation of treatment. **Results:** According to the X-tile program, the optimum cut-off value for NSE level was 26.1 ng/ml. Patients with elevated NSE level (≥ 26.1 ng/ml) showed significantly shorter progression-free survival (PFS) (median PFS, 4.31 months vs 9.93 months; $P < 0.001$) and significantly shorter overall survival (OS) (median OS, 8.13 months vs 17.11 months; $P < 0.001$). In subgroup analysis, patients with elevated NSE level (≥ 26.1 ng/ml) showed significantly shorter PFS than those with low NSE level (< 26.1 ng/ml) in EGFR mutations group and EGFR-wide type group (6.58 months vs 16.02 months, $P = 0.0016$; 3.42 months vs 7.46 months, $P < 0.001$, respectively). In terms of OS, the patients with elevated NSE was also significantly shorter than those with low NSE in EGFR mutations group and EGFR-wide type group (9.51 months vs 27.73 months, $P < 0.001$; 7.76 months vs 13.55 months, $P < 0.001$, respectively). Univariate and multivariate analysis demonstrated that elevated NSE level was independent prognostic factors for short PFS (hazard ratio [HR] = 2.40 (1.71-3.38), $P < 0.001$; HR = 1.81 (1.28-2.56), $P = 0.001$, respectively) and OS (HR, 2.40 (1.71-3.37), $P < 0.001$; HR, 1.76 (1.24-2.50), $P = 0.002$, respectively). **Conclusion:** The survival of NSCLC with elevated serum NSE level was shorter than that of NSCLC with low NSE level. Serum NSE level is a predictor of treatment and independent prognostic factor.

Background

Worldwide, lung cancer is the leading cancer in terms of incidence and mortality, with 2.1 million new lung cancer cases and 1.8 million deaths predicted in 2018^[1]. Traditionally, lung cancer has been divided into two main histological types: small cell lung cancer (SCLC) accounts for 15%-25% of all new lung cancer cases, and non-small-cell lung cancer (NSCLC) accounts for the remaining 75%-85%^[2], the latter is mainly subdivided into adenocarcinoma, squamous cell carcinoma (SCC), and other subtypes, including sarcomatoid carcinoma and neuroendocrine large cell carcinoma, are uncommon variants^[3]. Despite advances in early detection and standard therapies, approximately 57% of patients are diagnosed at an advanced stage and have a poor prognosis, with an overall 5-year survival rate of 10% to 15%^[4].

Serum biomarkers provide valuable information about the diagnosis and prognosis of a wide variety of malignant tumors, and the best known and most widely studied tumor markers of lung cancer are carcinoembryonic antigen (CEA), SCC, cytokeratin-19 fragments (Cyfra21-1) and neuron-specific enolase (NSE)^[5, 6]. Serum NSE has been established as a clinically useful marker for staging, monitoring treatment, and predicting relapse of SCLC^[7].

Increased serum NSE levels were found in a substantial proportion (30%-69%) of patients with NSCLC^[8, 9]. However, few studies have assessed the clinical value of NSE elevation in NSCLC, especially squamous cell carcinoma and adenocarcinoma. Therefore, the purpose of this study was initially to explore the role and value of NSE in predicting prognosis in advanced and metastatic non-neuroendocrine non-small-cell lung cancer patients.

Methods

Patient enrolment

We reviewed all non-neuroendocrine NSCLC patients who underwent treatments at Jinan Central Hospital Affiliated to Shandong University between January 2011 and October 2016. We drafted the inclusion and exclusion criteria.

Eligible patients were enrolled according to the following criteria: 1) patients with a histological diagnosis of squamous cell carcinoma or adenocarcinoma; 2) patients with complete clinical and histological information as well as follow-up data; 3) patients aged > 18 years old; 4) epidermal growth factor receptor (EGFR) mutations identified in adenocarcinoma; 5) patients with no previous treatment for cancer; 6) patients with complete tumor marker data; 7) patients with clinical stage IIIB, IIIC, IVA and IVB disease defined by the tumor node metastasis staging guidelines (TNM, Eighth Edition of the American Joint Committee on Cancer).

The exclusion criteria were as follows: 1) the patient had other malignancies; 2) the patient had systemic infection, autoimmune disease or inflammation; 3) the patient had an Eastern Cooperative Oncology Group (ECOG) score ≥ 2 ; 4) the patient had brain metastases; and 5) the patient had any positive immunohistochemical staining for CgA, Syn, or NSE.

Finally, 363 patients remained and were analysed in the study. The study was approved by the Ethical Committee of Jinan Central Hospital Affiliated to Shandong University. Due to the retrospective nature of the study, informed consent was obtained verbally from the subjects' family members or guardians. The ethics committee approved this procedure. This study was conducted in accordance with the Declaration of Helsinki.

Treatment and Follow-Up

Eighty-seven patients with EGFR mutations used gefitinib 250 mg/d orally or erlotinib 150 mg/d orally as first-line therapy, and the other patients used platinum-based doublet therapy. Patients were treated with chemotherapy, which was composed of 4-6 cycles. Comprehensive evaluation was performed after every two cycles of chemotherapy. After chemotherapy cycles were completed, the follow-up periods were set as every 3-4 months for the 1st and 2nd years, every 6 months for the 3rd year, and annually thereafter.

The post-treatment surveillance programme consisted of physical examination, cytological assessment, chest computed tomography (CT), brain magnetic resonance imaging (MRI), bone emission CT scans, and additional tests as needed to confirm patient response and to assess disease progression. All responses were defined according to the revised Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1)^[10]. The objective response rate (ORR) referred to the percentage of patients who had complete response (CR) or partial response (PR). The disease control rate (DCR) referred to the percentage of patients who had CR, PR, or stable disease (SD). Progression-free survival (PFS) was measured from the date of initiation of treatment to either the date of disease progression or death. Overall survival (OS) was defined as the interval from the date of initiation of treatment to the date of death from any cause. PFS was the primary ending. Data collection was terminated in October 2019.

Data collection

The main clinical characteristics, such as age, gender, smoking history and pathological differentiation, clinical stage, ECOG score, and NSE level, were extracted from medical records. The eighth edition of the American Joint Committee on Cancer (AJCC-8th) TNM classification was used to define TNM stage. Serum concentrations of NSE were measured within 2 weeks before treatment by electrochemiluminescence immunoassay on a Roche Analytics E170 Immunology Analyzer (Roche Diagnostics, China). Before April 2013, EGFR mutations were identified by direct sequencing. After April 2013, EGFR mutations were detected by the peptide nucleic acid-locked nucleic acid polymerase chain reaction (PCR) clamp method. Because squamous cell carcinoma has a low EGFR mutation rate, only adenocarcinoma requires EGFR detection.

Statistical analysis

The X-tile program was used to calculate the optimal cut-off value for NSE. The Kolmogorov-Smirnov test was used to reveal a normal distribution. The Chi-squared test was used to compare the clinical characteristics grouped by NSE. Multivariate logistic regression was applied to explore the correlation between NSE level and the clinicopathological characteristics. OS and PFS were calculated and depicted using the Kaplan-Meier method and compared using the log-rank test. The Kaplan-Meier method was used to calculate brain metastasis. A Cox proportional hazards model was applied to explore the risk factors for PFS and OS, with proportional hazard ratio (HR), and 95% confidence interval (CI). $P < 0.05$ was regarded as statistically significant. All statistical analyses were conducted with SPSS Statistics 25 (IBM Corporation, NY, USA).

Results

Baseline characteristics

From January 2011 to October 2016, 363 patients with advanced and metastatic non-neuroendocrine non-small-cell lung cancer were enrolled based on the inclusion and exclusion criteria. The baseline characteristics are summarized in Table 1. Among these 363 patients, 239 (65.8%) patients were male

and 124 (34.2%) were female, and age ranged from 27 to 95 (mean 66.1 ± 11.4). Based on the TNM staging system, 75 patients presented with stage IIIB, followed by 9 with IIIC, 146 with stage IVA, and 133 with stage IVB. Regarding the pathological differentiation level of tumors, 0.6%, 4.9%, and 94.5% of patients were histologically diagnosed with well, moderately, and poorly differentiated disease, respectively. Eighty-seven patients had EGFR mutations, and 276 patients were EGFR-wild type or unknown. The average value of NSE level was 20.1 ng/ml (range 0.1-285.2 ng/ml).

The optimal cut-off value for NSE level

According to the X-tile program, the optimum cut-off value for NSE level was 26.1 ng/ml (Figure 1A-C). Based on the optimal cut-off value, patients were subsequently divided into two groups (NSE < 26.1 ng/ml and NSE $\geq$ 26.1 ng/ml) for further analyses.

Correlation between NSE level and other clinicopathological characteristics

Potential correlations of NSE levels with other clinicopathological variables were explored (Table 2). The chi-squared test revealed that the NSE level was correlated with the ECOG score ($P = 0.003$), histological source ($P = 0.008$), histological type ($P = 0.014$), and clinical stage ($P < 0.001$).

Multivariate logistic regression analysis of NSE level and clinical factors showed that NSE level was associated with clinical stage ($P = 0.006$). No significant difference in the other clinicopathological variables with NSE level was found (Table 3).

Effectiveness of first-line treatment

According to RECIST 1.1 guidelines, among the 363 patients, the outcomes were CR: 0.0% (0/363); PR: 16.0% (58/363); SD: 25.6% (93/363); PD: 58.4% (212/363); ORR: 16.0% (58/363); and DCR: 41.6% (151/363) (Table 4A).

In addition, in the low NSE group (NSE < 26.1 ng/ml), the outcomes were CR: 0.0% (0/298); PR: 18.8% (56/298); SD: 27.2% (81/298); PD: 54.0% (161/298); ORR: 18.8% (56/298); and DCR: 46.0% (137/298). In the high NSE group (NSE $\geq$ 26.1 ng/ml), the outcomes were CR: 0.0% (0/65); PR: 3.1% (2/65); SD: 18.5% (12/65); PD: 78.5% (51/65); ORR: 3.1% (2/65); and DCR: 21.6% (14/65) (Table 4B).

The outcome of PFS and OS

Among 363 patients, at the end of follow-up, 199 (54.8%) patients had been confirmed dead.

The median PFS was 7.96 months (95% confidence interval [CI] = 6.42-9.50). The 1- and 3-year PFS rates were 40.4% and 9.0%, respectively (Figure 2A). The median OS was 14.41 months (95% CI = 11.83-16.98). The 1- year and 3-year OS rates were 56.4% and 16.6%, respectively (Figure 2B).

The median PFS was 9.93 months (95% CI = 7.41-12.46) in the low NSE group (< 26.1 ng/ml). The mPFS was 4.31 months (95% CI = 2.20-6.42) in the high NSE group (NSE $\geq$ 26.1 ng/ml) ($P < 0.001$, log-rank

test).

The 1-, and 3-year PFS rates of the low NSE group (NSE < 26.1 ng/ml) were 44.9% and 11.7%, respectively. The 1-year and 3-year PFS rates of the high NSE group (NSE ≥ 26.1 ng/ml) were 14.5% and 0.0%, respectively (Figure 3A). A significant difference in PFS was observed between the two groups ($P < 0.001$, log-rank test).

The median OS was 17.11 months (95% CI = 13.71-20.50) in the low NSE group (<26.1 ng/ml). The mOS was 8.13 months (95% CI = 6.23-9.97) in the high NSE group (NSE ≥ 26.1 ng/ml) ($P < 0.001$, log-rank test).

The 1-year and 3-year OS rates of the low NSE group (NSE < 26.1 ng/ml) were 63.6% and 18.6%, respectively. The 1-year and 3-year OS rates of the high NSE group (NSE ≥ 26.1 ng/ml) were 22.6% and 0.0%, respectively (Figure 3B). A significant difference in OS was observed between the two groups ($P < 0.001$, log-rank test).

The total number of EGFR mutations in adenocarcinoma patients was 87. In this subgroup, patients with NSE < 26.1 ng/ml, mPFS was 16.02 months (95% CI = 10.47-21.57), and the 1-year and 3-year PFS rates were 66.7% and 18.7%, respectively. For patients with NSE ≥ 26.1 ng/ml, the mPFS was 6.58 months (95% CI = 2.36-10.80), and the 1-year and 3-year PFS rates were 13.2% and 0%, respectively. A significant difference in PFS was observed between the two groups ($P = 0.0016$, log-rank test) (Figure 4A).

The median overall survival of patients with EGFR mutations and low serum NSE levels (< 26.1 ng/ml) was 27.73 months (95% CI = 11.52-15.59). The 1- and 3-year OS rates were 77.7% and 21.3%, respectively. The median overall survival of patients with EGFR wild-type and high serum NSE levels (≥ 26.1 ng/ml) was 9.51 months (95% CI= 3.53-15.48). The 1- and 3-year OS rates were 21.9% and 0%, respectively. A significant difference in OS was observed between the two groups ($P < 0.001$, log-rank test) (Figure 4B).

There were 171 cases of EGFR-wild type adenocarcinomas. In this subgroup, patients with NSE < 26.1 ng/ml, mPFS was 7.46 months (95% CI = 6.14-8.80), and 1-year and 3-year PFS rates were 29.8% and 0%, respectively. For patients with NSE ≥ 26.1 ng/ml, the mPFS was 3.42 months (95% CI = 2.11-4.73), and the 1-year, 3-year PFS rates were 11.1% and 0%, respectively. A significant difference in PFS was observed between the two groups ($P < 0.001$, log-rank test) (Figure 4C).

The median overall survival of patients with EGFR-wild type and low serum NSE levels (< 26.1 ng/ml) was 13.55 months (95% CI= 11.52-15.59). The 1- and 3-year OS rates were 56.0% and 13.8%, respectively. The median overall survival of patients with EGFR mutations and high serum NSE levels (≥ 26.1 ng/ml)) was 7.76 months (95% CI= 5.79-9.74). The 1- and 3-year OS rates were 18.9% and 5.1%, respectively. A significant difference in OS was observed between the two groups ($P < 0.001$, log-rank test) (Figure 4D).

In the patients with serum NSE < 26.1 ng/ml, the cumulative risk of brain metastasis at 3 months, 6 months and 9 months was 11.4%, 26.7% and 50.3%, respectively.

In the patients with serum NSE ≥ 26.1 ng/ml, the cumulative risk of brain metastasis at 3 months, 6 months and 9 months was 55.4%, 79.2% and 97%, respectively. There was a significant difference between the two groups ($P < 0.001$, log-rank test) (Figure 5).

Univariate and multivariate analysis of PFS

As shown in Figure 6A, the results from univariate analysis indicated that gender (HR = 0.72, $P = 0.028$), smoking history (HR = 1.44, $P = 0.013$), clinical stage (HR = 2.58, $P < 0.001$), pathological differentiation (HR = 5.23, $P < 0.001$), NSE level (HR = 2.40, $P < 0.001$), and EGFR mutation status (HR = 0.58, $P = 0.001$) were significant prognostic factors for PFS. Multivariate analysis indicated that clinical stage (HR = 1.93, $P = 0.001$), pathological differentiation (HR = 3.24, $P = 0.007$), NSE level (HR = 1.81, $P = 0.001$), and EGFR mutation status (HR = 0.54, $P < 0.001$) were independent prognostic parameters for PFS (Figure 6B).

Univariate and multivariate analysis of OS

Univariate analysis in all patients showed that gender (HR = 0.67, $P = 0.008$), smoking history (HR = 1.46, $P = 0.010$), clinical stage (HR = 2.34, $P < 0.001$), pathological differentiation (HR = 4.59, $P < 0.001$), NSE level (HR = 2.40, $P < 0.001$), and EGFR mutation status (HR = 0.57, $P = 0.001$), were associated with OS (Figure 7A). To identify possible independent prognostic factors, multivariate OS analysis next was conducted in which ECOG score (HR = 1.33, $P = 0.016$), clinical stage (HR = 1.94, $P = 0.001$), pathological differentiation (HR = 2.85, $P = 0.015$), NSE level (HR = 1.76, $P = 0.002$), and EGFR mutation status (HR = 1.58, $P = 0.002$) were identified as independent factors predicting OS (Figure 7B).

Discussion

NSE, also known as enolase- γ , is neuro- and neuroendocrine-specific isoenzyme of enolase. It was originally described by Moore and McGregor in 1965 as an enzyme enriched in neurons in general and in peripheral neuroendocrine cells.^[11] NSE is considered to be a useful tumor marker for tumors of neural and neuroendocrine origin, such as neuroblastoma, neuroendocrine tumors and SCLC.^[12, 13]

In this study, we found that the pretreatment serum NSE level in lung cancer patients is an independent prognostic and predictive factor of advanced or metastatic NSCLC patients. In addition to predicting patient prognosis, it can also help clinicians predict chemotherapy or epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) response and susceptibility to brain metastasis.

PFS and OS were shorter in patients with elevated NSE levels (≥ 26.1 ng/ml), regardless of EGFR mutation status.

We hypothesized that the reason high serum NSE predicts poor prognosis might be: 1) A Mixed SCLC-NSCLC component is present in a certain proportion of lung cancers.^[14] The prognosis of SCLC or neuroendocrine cancer is worse than that of NSCLC, and the 5-year relative survival for SCLC (6%) is

lower than that for NSCLC (23%) for all stages.^[15] 2) Histologic transformation from NSCLC to SCLC occurs, after EGFR-TKIs therapy, which is a known mechanism of resistance to first-generation EGFR-TKIs that dramatically impacts patient prognosis.^[16, 17] 3) NSE is considered a key enzyme in glycolysis, and it plays an important role in aerobic glycolysis.^[18] The faster aerobic glycolysis is, the faster the tumor cell growth. 4) In this study, logistic regression analysis showed a correlation between NSE and staging. Compared to patients in other stages, more stage IV patients had elevated serum NSE.

Some studies came to the same conclusions: Ferrigno D et al^[19] enrolled 448 NSCLC patients (stage I to IV), assessed NSE before treatment, and found that patients with NSE >11 ng/ml had a worse prognosis than patients with lower NSE levels. Further, there are several studies that investigated this phenomenon in a limited number of patients. Van Zandwijk^[20] assessed the prognostic value of NSE and lactic dehydrogenase (LDH) in 42 locally advanced or metastatic NSCLC patients treated with chemotherapy. This study showed that high levels of both markers (NSE and LDH) were associated with shorter survival. Another study with 84 NSCLC patients found that a high serum NSE concentration conferred lower survival and greater probability of relapse after radical surgery than a low serum NSE concentration^[21].

These studies were conducted in an earlier era, so in our study, we analysed subsets of EGFR mutations. We found that a high NSE level also predicted short survival in both EGFR-mutated and non-mutated groups.

Suh KJ et al^[22] determined that patients with elevated NSE levels (> 16.3 ng/ml) had a median PFS of 10.5 months, which was shorter than that for patients with low NSE levels (< 16.3 ng/ml) who had a median PFS of 15.4 months after EGFR-TKI treatment. Fiala et al^[23] also reported that in 163 patients with advanced NSCLC treated with EGFR TKIs, high pretreatment serum NSE levels (> 12.5 ng/mL) were associated with short PFS.

We also found that this subset of patients with high NSE was innately resistant to drugs, either EGFR-TKIs or chemotherapy. In EGFR-mutated NSCLCs, half of the patients progressed at just 6.58 months, and in EGFR-wild type NSCLCs, half of the patients progressed at just 3.42 months. Innate resistance to EGFR-TKIs could be explained by a mixed disease component and by heterogeneity. A study reported that NSE mRNA expression was inversely correlated with sensitivity to gefitinib in advanced NSCLC patients.^[24]

However, for EGFR wild-type NSCLCs, patients with high NSE concentrations are also resistant to systematic chemotherapy, which cannot be explained by the presence of small cell components, which are sensitive to platinum. Patients with positive immunohistochemical staining for NSE were more sensitive to chemotherapy.^[25] Two other studies showed the opposite results—patients with neuroendocrine differentiation had a higher response rate to cytotoxic drugs.^[26, 27] However, the above three studies were all contained immunohistochemical analysis of neuroendocrine features, while our exclusion criteria excluded patients with positive immunohistochemical staining for CgA, Syn, and NSE.

More than 147 potential mechanisms of platinum resistance have been proposed.^[28] High-throughput next-generation sequencing (NGS) technologies have been developed to cover a wider spectrum of predictive genomic abnormalities. To determine why chemotherapy resistance occurs in these patients with high NSE levels, gene sequencing is needed.

In our study, high NSE levels were more frequently associated with brain metastases. The possible reason for this phenomenon is that NSCLCs with neuroendocrine differentiation are more aggressive.^[29,30] A study also observed the same situation—a high level of serum NSE might be associated with brain metastases in patients with lung cancers.^[31] Their conclusions are consistent with our findings.

Regarding clinical staging, pathological differentiation, ECOG score and EGFR mutation status, NSE level was an independent prognostic factor for NSCLC by univariate and multivariate Cox regression analysis.

The innovations of our study include the following: 1) we excluded patients with brain metastases during enrolment, so that we could observe when brain metastases occurred; 2) the indicators of endocrine differentiation in immunohistochemistry (CgA, Syn, NSE) were excluded; 3) to keep treatment strategies consistent, only advanced and metastatic NSCLC were included; and 4) different from other studies, we used a cut-off value (26.1 ng/ml) calculated by the X-tile software instead of using the NSE concentration range set by the device (0-16 ng/ml).

There are some limitations in our study. First, for nearly 80% patients whose tissues were obtained by percutaneous transthoracic biopsy or bronchoscopy, histologic heterogeneity was hard to assess because of the limited amount of tissue. Biopsy of both primary lesions and metastatic lesions should be performed to avoid missing lung neuroendocrine components. Second, NSE level was assessed only during the pretreatment period, and was not followed at the time of EGFR-TKIs and systemic chemotherapy failure. Third, re-biopsy and NGS were not performed at the time of disease progression to detect transformation to small cell carcinoma or neuroendocrine phenotype or agent resistance. In addition, the retrospective design of our study may have led to selection bias.

This study had two key findings: one is that NSCLC patients with high NSE levels have a poor prognosis, and the other is that multisite biopsy should be performed when possible to facilitate a precise pathological diagnosis. Prospective studies with larger sample sizes should be designed to confirm the value of NSE in NSCLC in the future.

Conclusion

The survival of NSCLC patients with high serum NSE levels was shorter than that of NSCLC patients with low NSE levels. Patients with high serum NSE were innately resistant to first-line antitumor therapies, such as chemotherapy and epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs). These patients were more prone to brain metastases. The serum NSE level is a predictor of treatment and an independent prognostic factor.

Abbreviations

SCLC: small cell lung cancer; NSCLC: non-small-cell lung cancer; SCC: squamous cell carcinoma; CEA: carcinoembryonic antigen; Cyfra21-1:cytokeratin-19 fragments; NSE: neuron-specific enolase; EGFR: epidermal growth factor receptor; ECOG: Eastern Cooperative Oncology Group; CT: computed tomography; MRI: magnetic resonance imaging; RECIST: Response Evaluation Criteria in Solid Tumors; ORR: objective response rate; CR: complete response; PR: partial response; DCR: disease control rate; SD:stable disease; PFS: progression-free survival; OS: overall survival; AJCC: American Joint Committee on Cancer; CI: confidence interval; LDH: lactic dehydrogenase; NGS: next-generation sequencing; EGFR-TKIs: epidermal growth factor receptor tyrosine kinase inhibitors.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethical Committee of Jinan Central Hospital Affiliated to Shandong University. Due to the retrospective nature of the study, informed consent was obtained verbally from subjects' family members or guardian. The ethics committee of Jinan Central Hospital Affiliated to Shandong University approved this procedure. This study was conducted in accordance with the Declaration of Helsinki.

Consent for publication

Consents for publication obtained from that persons or legal guardians verbally.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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There is no funding for this work.

Authors' contributions

P.Y. and CY.L. designed the study.

YM.H and XY.W analyzed the data and wrote the manuscript.

YM.H. and TY.Z. collected the clinical specimen and analyzed the clinical data.

L.J. contributed to the data interpretation and analysis.

P.Y. and L.J. have drafted the work.

All authors read and approved the final manuscript.

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Figures

Figure 1

X-tile analyses. The optimum cut-off points for NSE level was 26.1 ng/ml according to the X-tile program.

Figure 2

Kaplan-Meier survival curves of PFS (A) in all patients. Kaplan-Meier survival curves of OS (B) in all patients.

Figure 3

Kaplan-Meier survival curves of PFS (A) according to NSE level (< 26.1 ng/ml or ≥ 26.1 ng/ml) in all patients. Kaplan-Meier survival curves of OS (B) according to NSE level(< 26.1 ng/ml or ≥ 26.1 ng/ml) in all patients.

Figure 4

Kaplan-Meier survival curves according to NSE level (< 26.1 ng/ml or ≥ 26.1 ng/ml). A: PFS probability in EGFR-mutations patients. B: OS probability in EGFR-mutations patients. C: PFS probability in EGFR-wild type patients. D: OS probability in EGFR-wild type patients.

Figure 5

Cumulative incidence of subsequent brain metastasis in patients after treatment according to NSE level (< 26.1 ng/ml or ≥ 26.1 ng/ml) in all patients.

Figure 6

Result of univariate analysis(A) and multivariate analysis(B) for the association of clinical factors with PFS.

Figure 7

Result of univariate analysis(A) and multivariate analysis(B) for the association of clinical factors with OS.