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 access 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.

Introduction

Worldwide, lung cancer is the leading cause of cancer 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) represents approximately the remaining 75%-85%^[2], with the latter mainly being 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, about 57% of patients are diagnosed at an advanced stage and have a poor prognosis, with an overall 5-year survival rate of 10–15%^[4].

Serum biomarkers provide valuable information about the diagnosis and prognosis of a wide variety of malignant tumors, 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 was 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 have assessed the clinical value of elevation in NSCLC, especially squamous cell carcinoma and adenocarcinoma. Therefore, the purpose of this study here was initially to explore the prognostic role of NSE for predicting prognosis value in advanced and metastatic non-neuroendocrine non-small cell lung cancer patients.

Materials And Methods

Patients enrollment

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

The exclusion criteria was: 1) patient had other malignancies; 2) patient had systemic infection, autoimmune disease or inflammation; 3) Eastern Cooperative Oncology Group (ECOG) score $\geq$ 2; 4) brain metastases; 5) immunohistochemical staining CgA, Syn, NSE, any was positive.

At last, 363 patients remained and were analyzed 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 subjects' family members or guardian. The ethics committee approved this procedure. This study was conducted in accordance with the Declaration of Helsinki.

Treatment and Follow-Up

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

The post-treatment surveillance program consisted of physical examination, cytological assessment, chest computed tomography (CT), brain magnetic resonance imaging (MRI) and bone emission CT scans, and additionally as needed to confirm patients 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]. Objective response rate (ORR) referred to the percentage of patients who had complete response(CR), or partial response(PR). 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, NSE level, were extracted in 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 Roche Analytics E170 Immunology Analyzer (Roche Diagnostics, China). Before April 2013, EGFR mutations were identified by direct sequencing. Since 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 mutations rate, only adenocarcinoma requires EGFR detection.

Statistical analysis

The X-tile program was performed to calculate the optimal cut-off value for NSE. 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. Use the Kaplan-Meier method to calculate brain metastasis. Cox proportional hazards model was applied to explore the risk factors for PFS and OS, with proportional hazard ratio (HR), calculating 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 were summarized in Table 1. Among these 363 patients, 239 (65.8%) patients were male

and 124 (34.2%) were female, aging 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 tumor, 0.6%, 4.9%, and 94.5% of patients were histologically diagnosed with well, moderately, and poor differentiated disease, respectively. 87 patients were with EGFR mutations and 276 patients were EGFR-wide type or unknown. The average value of NSE level was 20.1 ng/ml (range 0.1-285.2 ng/ml).

Table 1
Baseline characteristics of the enrolled patients

Characteristics	Number of Patients
Overall (%)	363 (100)
Age(years)	
< 67	176 (48.5)
≥ 67	187 (51.5)
Mean ± S.D	66.1 ± 11.4
Range	27–95
Gender, n (%)	
Male	239 (65.8)
Female	124 (34.2)
Smoking history, n (%)	
Former/current	219 (60.3)
Never	144 (39.7)
ECOG score	
0	224 (61.7)
1	139 (38.3)
Histological types, n (%)	
Adenocarcinoma	258 (71.1)
Squamous cell carcinoma	105 (28.9)
Clinical stage, n (%)	
IIIB	75 (20.7)
IIIC	9 (2.5)
IVA	146 (40.2)
IVB	133 (36.6)
Pathological differentiation, n (%)	
Well	2 (0.6)

Abbreviations: NSE = Neuron specific enolase. ECOG = Eastern Clinical Oncology Group. EGFR = Epidermal growth factor receptor.

Characteristics	Number of Patients
Moderate	18 (4.9)
Poor	343 (94.5)
NSE level (ng/ml)	
Mean $\pm$ S.D	20.1 $\pm$ 23.7
Range	0.1–285.2
EGFR status	
mutations	87 (24.0)
Wild	171 (47.1)
Unknown	105 (28.9)
Abbreviations: NSE = Neuron specific enolase. ECOG = Eastern Clinical Oncology Group. EGFR = Epidermal growth factor receptor.	

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 (Fig. 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 correlation of NSE level with other clinicopathological variables were explored (Table 2). Chi-squared test revealed that NSE level was correlated with ECOG score ($P = 0.003$), histological source ($P = 0.008$), histological types ($P = 0.014$), as well as clinical stage ($P < 0.001$).

Table 2

correlation of serum NSE level (NSE < 26.1/NSE ≥ 26.1 ng/ml) with clinicopathological characteristics

Characteristics	Number of all patients (%)		χ^2	P
	NSE < 26.1 ng/ml	NSE ≥ 26.1 ng/ml		
Overall	298 (100)	65 (100)		
Age (years)				
< 67	147 (49.3)	29 (44.6)	0.48	0.491
≥ 67	151 (50.7)	36 (55.4)		
Gender				
Male	196 (65.8)	43 (66.2)	0.00	0.953
Female	102 (34.2)	22 (33.8)		
Smoking history				
Former/current	176 (59.1)	43 (66.2)	1.12	0.290
Never	122 (40.9)	22 (33.8)		
ECOG score				
0	199 (66.8)	25 (38.5)	5.42	0.003
1	99 (33.2)	40 (61.5)		
Histological source				
Needle biopsy	162 (54.4)	41 (63.1)	11.73	0.008
Bronchoscopy	60 (20.1)	15 (23.1)		
Resection	53 (17.8)	1 (1.5)		
Cytology	23 (7.7)	8 (12.3)		
Histological types				
Squamous cell carcinoma	91 (30.5)	14 (21.5)	7.96	0.014
Adenocarcinoma	207 (69.5)	51 (78.5)		
Clinical stage				

Note: P < 0.05 was considered to indicate significant difference. The P value was calculated by Chi-squared test.

Abbreviations: NSE = Neuron specific enolase. ECOG = Eastern Clinical Oncology Group. EGFR = Epidermal growth factor receptor. Ki-67 = Antigen KI 67.

Characteristics	Number of all patients (%)		χ^2	P
	NSE < 26.1 ng/ml	NSE $\geq$ 26.1 ng/ml		
IIIB + IIIC	82 (27.5)	2 (3.1)	17.92	< 0.001
IVA + IVB	216 (72.5)	63 (96.9)		
Pathological differentiation				
Well	2 (0.7)	0 (0.0)	4.77	0.098
Moderate	18 (6.0)	0 (0.0)		
Poor	278 (93.3)	65 (100)		
EGFR status				
mutations	75 (86.2)	12 (13.8)	5.29	0.071
Wide	132 (77.2)	39 (22.8)		
Unknown	91 (86.7)	14 (13.3)		
Ki-67				
< 0.3	68 (22.8)	9 (13.8)	2.57	0.109
$\geq$ 0.3	230 (77.2)	56 (86.2)		
Note: P < 0.05 was considered to indicate significant difference. The P value was calculated by Chi-squared test.				
Abbreviations: NSE = Neuron specific enolase. ECOG = Eastern Clinical Oncology Group. EGFR = Epidermal growth factor receptor. Ki-67 = Antigen KI 67.				

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 of the other clinicopathological variables with NSE level was found (Table 3).

Table 3
Multivariate logistic regression analysis for NSE level

Variable	OR (95% CI)	P
ECOG score		
0	0.56(0.03,1.75)	0.325
1		
Histological source		
Needle biopsy		0.275
Bronchoscopy	1.15(0.58,2.30)	0.693
Resection	0.15(0.02,1.12)	0.064
Cytology	1.17(0.48,2.82)	0.733
Histological types		
Adenocarcinoma	0.77(0.39,1.53)	0.453
Squamous cell carcinoma		
Clinical stage		
IIIB + IIIC	7.68(1.79,33.03)	0.006
IVA + IVB		
Note: P < 0.05 was considered to indicate significant difference.		
Abbreviations: OR = odds ratio. CI = Confidence interval.		

Table 4
A The responses in all patients

Variable	N (%)
Overall	363(100)
Complete response	0 (0)
Partial response	58 (16.0)
Stable disease	93 (25.6)
Progressive disease	212 (58.4)
ORR	58(16.0)
DCR	151(41.6)

Effectiveness of first-line treatment

According to the RECIST 1.1, among 363 patients, 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); DCR: 41.6% (151/363) (Table 4A).

As well as, in the group (NSE < 26.1 ng/ml), 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); DCR: 46.0% (137/298). And in the group (NSE ≥ 26.1 ng/ml), CR: 0.0% (0/100); PR: 3.1% (2/100); SD: 18.5% (12/100); PD: 78.5% (51/100); ORR: 3.1% (2/100); DCR: 21.6% (14/100) (Table 4B).

The outcome of PFS and OS

Among 363 patients, at the end of follow-up, 199 (54.8%) patients were confirmed died.

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

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

The 1-, and 3-year PFS rates of the group (NSE < 26.1 ng/ml) were 44.9%, 11.7%, respectively. The 1-year and 3-year PFS rate of the group (NSE ≥ 26.1 ng/ml) was 14.5%, 0.0%, respectively (Fig. 3A). 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 low NSE group (< 26.1 ng/ml). The mOS was 8.13 months (95% CI = 6.23–9.97) in elevated NSE group (NSE ≥ 26.1 ng/ml) ($P < 0.001$, log-rank test).

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

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

The median overall survival of patients with EGFR mutations with low level serum NSE (< 26.1 ng/ml) was 27.73 months (95%CI = 11.52–15.59). The OS rates in 1-, and 3-year were 77.7%, 21.3%, respectively. The median overall survival of patients with EGFR wide-type with high level serum NSE (≥ 26.1 ng/ml)

was 9.51 months (95%CI = 3.53–15.48). The OS rates in 1-, and 3-year were 21.9%,0%, respectively. Significant difference in OS was observed between the two groups ($P < 0.001$, log-rank test)(Fig. 4B).

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

The median overall survival of patients with EGFR-wide type in low level serum NSE (< 26.1 ng/ml) was 13.55 months (95%CI = 11.52–15.59). The OS rates in 1-, and 3-year were 56.0%,13.8%, respectively. The median overall survival of patients with EGFR mutations in high level serum NSE (≥ 26.1 ng/ml) was 7.76 months (95%CI = 5.79–9.74). The OS rates in 1-, and 3-year were 18.9%, 5.1%, respectively. Significant difference in OS was observed between the two groups ($P < 0.001$, log-rank test)(Fig. 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 statistical difference between the two groups ($P < 0.001$, log-rank test) (Fig. 5).

Univariate and multivariate analysis of PFS

As shown in Fig. 6A, 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 mutations 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 mutations status (HR = 0.54, $P < 0.001$) were independent prognostic parameters for PFS (Fig. 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 mutations status (HR = 0.57, $P = 0.001$), were associated with OS (Fig. 7A). To find possible independent prognostic factors, multivariate OS analysis next were 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 mutations status (HR = 1.58, $P = 0.002$) were identified as independent factors predicting OS (Fig. 7B).

Discussion

NSE, also known as enolase- γ , is a 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 the serum NSE level in pretreatment lung cancer is an independent prognostic and predictive factor of advanced or metastatic NSCLC patients. In addition to predicting patients' prognosis, it can also help the clinician predict chemotherapy or epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) respond and susceptibility to brain metastasis.

PFS and OS were shorter in patients with elevated NSE group (≥ 26.1 ng/ml), whether in EGFR mutations subgroup or EGFR wide type group.

We hypothesized that the reason for the high elevated serum NSE predicts poor prognosis might be: 1) Mixed SCLC-NSCLC component in certain proportion of lung cancers.^[14] The prognosis of SCLC or neuroendocrine cancer is worse than that of NSCLC 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, after EGFR-TKIs therapy, which is a known mechanism of resistance to first-generation EGFR-TKI that dramatically impacts patients' prognosis.^[16, 17] 3) NSE is considered a key enzyme in glycolysis and it plays an important role in aerobic glycolysis,^[18] the faster the aerobic glycolysis, the faster the tumor cell growth; 4) In this study, logistic regression showed correlation between NSE and staging. we found that higher proportion of stage IV in patients with elevated serum NSE.

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

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

Suh KJ, *et al*^[22] assessed elevated NSE levels (> 16.3 ng/ml) had median PFS of 10.5 months shorter than low NSE levels (< 16.3 ng/ml) had 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 shorter PFS.

We also found that this subset of patients with high NSE is innate resistant to drugs, either EGFR-TKIs or chemotherapy. In EGFR-mutations NSCLCs, half of the patients progressed at just 6.58 months, and in

EGFR-wide type NSCLCs, half of the patients progressed at just 3.42 months. Innate resistance to EGFR-TKIs could be explained by mixture component and heterogeneity, and a study have shown reported that NSE mRNA expression was inversely correlated with sensitivity to Gefitinib in advanced NSCLC patients^[24].

But, for EGFR wild-type NSCLCs, 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] Another two studies showed the opposite results—neuroendocrine differentiation had a higher response rate to cytotoxic drugs.^[26, 27] However, the above three studies were all immunohistochemical with neuroendocrine features, while our exclusion criteria excluded patients with positive immunohistochemical staining CgA, Syn, NSE.

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

In our study, high NSE levels are 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—high level of serum NSE might be associated with brain metastases in patients with lung cancers.^[31] Their conclusions are consistent with our findings.

As the clinical staging, the pathological differentiation, ECOG score and EGFR mutations 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 when enrollment, so that we could observe when brain metastases occurred; 2) The indicators of endocrine differentiation in immunohistochemistry (CgA, Syn, NSE) were excluded; 3) For the consistency of treatment, only advanced and advanced NSCLC were included; 4) Different from other studies, we used the cut-off value (26.1 ng/ml) calculated by the x-tile software instead of using the range of NSE concentration set by the device (0-16 ng/ml).

There are some limitations in our study. First, nearly 80% patients tissues obtained by percutaneous transthoracic biopsy or bronchoscopy, histologic heterogeneity is hardly to observed because of less tissue. Both primary lesions and the metastatic lesions biopsy should be performed avoid missing lung neuroendocrine component. Second, NSE level was assessed pre-treatment period only, and was not followed at the time of EGFR-TKIs and systemic chemotherapy failure. Third, re-biopsy and NGS didn't be performed at the time of disease progression to detect transformation to small cell carcinoma or neuroendocrine phenotype or agent resistance. In addition, due to the retrospective design of our study, which may have led to selection bias.

This study gave us two hints, one is that NSCLC with elevated NSE level has a poor prognosis, and the other is to strive for multi-site biopsy to make a precise pathological diagnosis. Prospective studies with larger samples should be designed to confirm the value of NSE in NSCLC in the future.

Conclusion

The survival of NSCLC with elevated serum NSE level was shorter than that of NSCLC with low NSE level. Patients with elevated serum NSE were innate resistance to first-line anti-tumor therapies such as chemotherapy and epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs). And those were more prone to brain metastases. Serum NSE level is a predictor of treatment and 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.