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Original Article

Serum expression of NSE, ProGrp and Cyfra21-1 in patients with small cell lung cancer and prognosis

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Abstract: Objective: To investigate the value of neuron-specific enolase (NSE), pro-gastrin-releasing peptide precursor (ProGrp) and cytokeratin-19 fragment (Cyfra21-1) in assisted diagnosis, treatment efficacy monitoring and prognosis evaluation of small cell lung cancer (SCLC) patients. Methods: The subjects were divided into an observation group (patients with SCLC, n = 86), a control group (patients with benign lung disease, n = 60) and normal group (healthy individuals, n = 80). The observation group was treated with EP chemotherapy regimen for two cycles (etoposide 80 mg/m^2 daily for 1-5 days and cisplatin 100 mg/m^2 for 3 or 4 days). Results: The serum expressions of NSE, ProGrp and Cyfra21-1 were detected before and after treatment. The serum expression of NSE, ProGrp and Cyfra21-1 in the observation group were significantly higher than those in the control group and normal group (all P<0.05). The expression levels of NSE, ProGrp and Cyfra21-1 in patients of extensive disease stage were significantly higher than those in patients of limited disease stage (all P<0.05). Spearman test showed that all indicators decreased with the improvement of clinical efficacy (all P<0.05). Conclusion: Univariate and multivariate Cox regression analysis found that NSE, ProGrp, Cyfra21-1, metastasis and stage were independent prognostic factors of SCLC patients. Cyfra21-1, ProGrp and NSE are highly expressed in SCLC and their expression levels are associated with clinical stage and prognosis. The serum expression of Cyfra21-1, ProGrp and NSE is negatively correlated with the therapeutic efficacy, suggesting their values are important in the monitoring of SCLC treatment.

Keywords: Neuron-specific enolase, pro-gastrin-releasing peptide, cytokeratin-19 fragment, small cell lung cancer, clinical features

Introduction

Cancer is one of the top ten malignant diseases, seriously threatening human health [1]. In 2015, the World Health Organization (WHO) released that as of 2012 there were approximately 14.1 million new cancer cases worldwide and more than 8 million deaths, among which lung cancer (LC) patients ranked first in both new cases (12.77%) and deaths (about 19.39%); with so high morbidity and mortality, LC has become one of the most urgent problems in the clinic [2]. According to the histological types of LC, it can be divided into two types: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC); in which NSCLC accounts for 85% and SCLC accounts for 15% [3]. SCLC is a special tumor type that originates from bronchial neuroendocrine cells. SCLC has several characteristics such as high malignancy, rapid disease progression, easy metastasis and poor prognosis in the early stage of the disease [4]. The treatment options for SCLC mainly include radiotherapy, chemotherapy and targeted therapy; at present, EP (etoposide+cisplatin) chemotherapy regimen is mainly used in clinical treatment [5].

The cytokeratin 19 fragment (Cyfra21-1) is the smallest fragment of the cytokeratin (CK) 19 protein and it is an acidic peptide present in the epithelial stroma cells of lung cancer patients; it can be released into the blood due to cytolysis and protein degradation caused by cell death, and it has a high sensitivity and specificity in the diagnosis of clinical lung squamous cell carcinoma [6]. Neuron-specific enolase (NSE) is a type of glycolysis enzyme mainly existing in nervous tissue and the neuroendocrine system [7]. A study showed that NSE

expression exhibited a significant elevation during tumorigenesis and the progression of SCLC, reflecting tumor load in SCLC patients, which was an important indicator for clinical diagnosis of SCLC [8]. In recent years, pro-gastrin-releasing peptide (ProGrp) has been frequently used as a biomarker for early diagnosis and screening of SCLC, and it mainly functions as a hormone in human gastric nerve fibers, cranial nerve fibers and fetal lung neuroendocrine tissues [9]. A study showed that ProGrp was expressed in various SCLC tumor tissues and cell lines [10]. Schneider et al. reported that ProGrp exhibited a higher diagnostic value than NSE in terms of SCLC progression and recurrence [11]. There is a lot of literature on the diagnosis of SCLC using the expressions of NSE, ProGrp and Cyfra21-1, but there is little literature on the value of NSE, ProGrp and Cyfra21-1 in the clinical treatment efficacy monitoring and prognosis evaluation [11, 12].

Therefore, we aimed to investigate the value of NSE, ProGrp, and Cyfra21-1 in the assisted diagnosis, treatment efficacy monitoring, and prognostic evaluation of SCLC patients by detecting their serum expression levels, which provides a reference for the application of NSE, ProGrp, and Cyfra21-1 in clinical diagnosis, treatment and prognosis of SCLC.

Materials and methods

General information

Eighty-six patients with SCLC admitted in to the Department of Respiratory of Heping Hospital Affiliated to Changzhi Medical College from January 2016 to December 2017, were selected in this study as the observation group, including 57 males and 29 females. All patients were diagnosed with SCLC by pathological examination. Clinical staging criteria were performed according to US Veterans Hospital standards [13]. Sixty patients with benign lung disease were selected as the control group, including 35 males and 25 females. The disease types included bronchiectasis, pneumonia, tuberculosis, emphysema and chronic obstructive pneumonia. In addition, 80 healthy individuals in Heping Hospital Affiliated to Changzhi Medical College were selected as the normal group, including 49 males and 31 females. There were no significant differences in gender and age among three groups (all $P>0.05$). The SCLC patients in this study were all newly diagnosed and hadn't received any cancer treatments. This study was approved by the Ethics Committee of Heping Hospital Affiliated to Changzhi Medical College, and all subjects signed an informed consent.

Agents

Etoposide (Hainan Qilu Pharmaceutical Co., Ltd., China, 5 mL); Cisplatin (Qilu Pharmaceutical Co., Ltd., China, 20 mg); Electrochemiluminescence Cyfra21-1, NSE Kit (CP011040, CP011070, Shanghai Toujing Life Technology Co., Ltd., China); ELISA kit ProGrp (DY7847-05, R&D Systems Company, America).

Treatment for patients with SCLC

The patients with SCLC were treated with EP (etoposide+cisplatin) chemotherapy regimen for two cycles. EP chemotherapy regimen was as follows: etoposide 80 mg/m^2 daily for 1-5 days; cisplatin 100 mg/m^2 for 3-4 days.

Specimen collection and testing

Fasting venous blood (5 mL) from the three groups and venous blood (5 mL) from the observation group the next morning after chemotherapy were collected. The samples were placed into gel separation tubes. The serum was collected by centrifugation at 4,000 rpm for 5 min and subpackaged. The samples were stored at -70°C for testing. NSE and Cyfra21-1 were tested using the Roche Cobas8000 fully automated electrochemiluminescence immunoassay. ProGrp was detected by ELISA. The protocol was as follows. Sample or standards (100 mL) were added to the reagent diluent, followed by sealing. The samples were incubated for 2 h at room temperature. After washing the plates, 100 μL of antibody solution was added to each well, followed by sealing and incubation for 2 h at room temperature. The plates were washed again and 100 μL of streptavidin-HRP working dilution was added to each well. Then the plates were sealed, incubated for 20 min at room temperature in the dark and washed. Substrate solution (100 mL) was added into each well and then incubated at 37°C for 20 min in dark, and 50 μL of stop solution was then

Table 1. Assignment

Factors	Number assignment
Gender	Male = 1; Female = 0
Stages	LD = 1; ED = 0
Metastasis	Metastasis = 1; no metastasis = 0
Tumor diameter	>3 cm = 1; ≤3 cm = 0
Smoking status	Smoker = 1; non-smoker = 0
NSE	≥17 ng/mL = 1; <17 ng/mL = 0
ProGrp	≥65 pg/mL = 1; <65 pg/mL = 0
Cyfra21-1	≥3.3 ng/mL = 1; <3.3 ng/mL = 0
Age	Analyzing according to continuous data

Note: LD, limited disease; ED, extensive disease; NSE, neuron-specific enolase; ProGrp, pro-gastrin-releasing peptide precursor; Cyfra21-1, cytokeratin-19 fragment.

added. The absorbance at 450 nm was detected using an ELXS00 microplate reader within 15 min. The blood samples were centrifuged, and the serum in the upper layer was detected by Roche Cobas8000 fully automated electrochemiluminescence immunoassay. The system parameters were set as follows. The sampling needle, washing station and measuring chamber were all perfused three times. The range of measured light background BGW was 400-1,600, CV% <5. Positive threshold values of each index: Cyfra21-1 ≥3.3 ng/mL, NSE ≥17 ng/mL, ProGrp ≥65 pg/mL.

Evaluation of clinical efficacy

Two weeks after treatment, the clinical efficacy was evaluated according to the American Cancer Institute's customized RECIST criteria [14]. The conditions of SCLC patients were divided into four stages: complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD).

Observation indexes

Main observation indexes: The serum expression of NSE, ProGrp and Cyfra21-1 in the normal group, control group and observation group were detected. The relationship between the expression changes of NSE, ProGrp and Cyfra21-1 and the clinical efficacy in the observation group was observed. Cox regression analysis was used to determine the independent prognostic factors of SCLC patients. The K-M survival curve was drawn according to the indicators with differences in single factor. The follow-up frequency was once every 3 months in the first year, followed by once a year. Secondary observation indexes: The relationship between the expression of NSE, ProGrp, Cyfra21-1 and patients' clinical features in the observation group, including gender, age, stage, metastasis, tumor size, and smoking status was observed.

Statistical analysis

Experimental data were statistically analyzed using SPSS 20.0 software (Guangzhou Bo-mai, China), and plotted using GraphPad Prism 7 (Shanghai Beka, China). The enumeration data were expressed as percentage (%). Measurement data were expressed as mean ± standard deviation (mean ± SD). Comparison between two groups was performed using independent sample t test. Comparison of clinical features before and after treatment was performed by paired t test, indicated by t value. Comparisons of more than two groups were done by one-way ANOVA, indicated by F value. Comparison between two groups was analyzed using LSD-t test. The correlation between the expression levels of NSE, ProGrp and Cyfra21-1 and clinical efficacy after chemotherapy were analyzed by Spearman correlation analysis. Survival analysis was performed using Kaplan-Meier and tested by the Log-rang test. Cox regression analysis was used to analyze the independent prognostic factors for SCLC patients. The value assignment for included variables was shown in **Table 1**. P<0.05 was considered statistically significant.

Results

Serum expression of NSE, ProGrp and Cyfra21-1 in three groups of patients

The serum expression of NSE, ProGrp and Cyfra21-1 in the observation group were significantly higher than those in the control group and normal group (all P<0.05). There was no significant difference in serum expression of NSE, ProGrp and Cyfra21-1 between the normal group and control group (all P>0.05). See **Figure 1**.

The correlation between clinical data of patients with negative/positive expression of NSE, ProGrp and Cyfra21-1

The clinical data of patients in the observation group were as follows: 57 males and 29

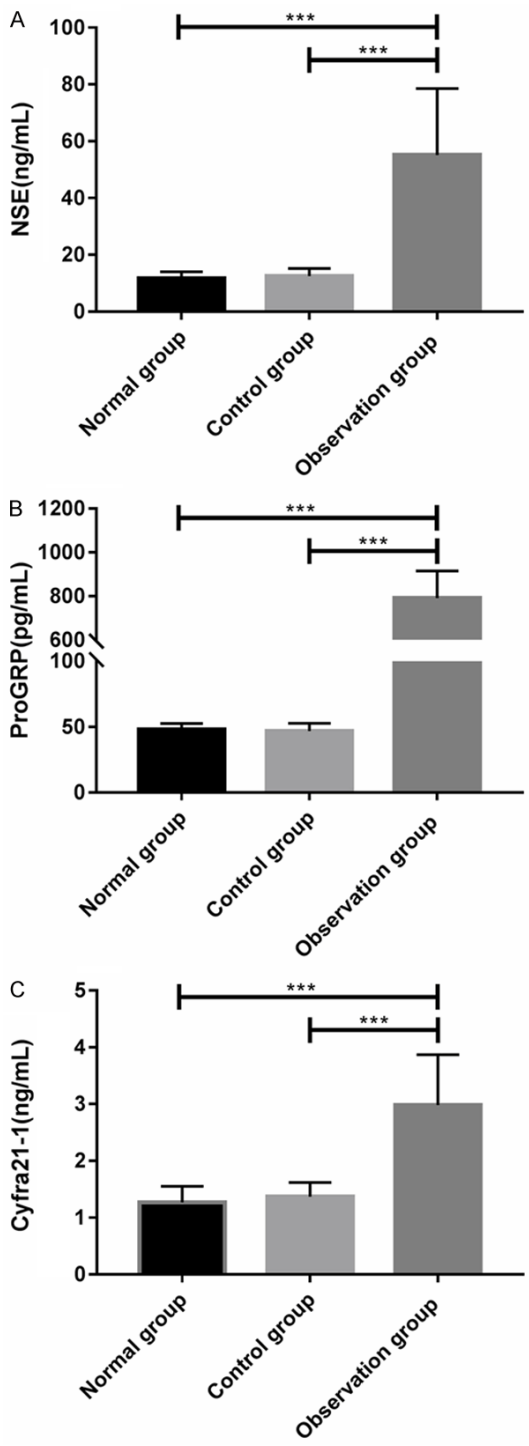

Figure 1. The serum expressions of NSE, ProGrp and Cyfra21-1 in all three groups. A. The serum expression of NSE in the subjects was detected, indicating that NSE expression of the normal group and control group was significantly decreased compared with that of the observation group. B. The serum expression of ProGrp in the subjects was detected, indicating that NSE expression of the normal group and control group was significantly decreased compared with that of the observation group. C. The serum expression of Cyfra21-1 in the subjects was detected, indicating that NSE expression of the normal group and control group was significantly decreased compared with that of the observation group. *Showed that there was a significant difference between two groups, ***P<0.001. NSE - neuron-specific enolase, ProGrp - pro-gastrin-releasing peptide precursor, Cyfra21-1 - cytokeratin-19 fragment.

females; the average age (57.91±11.18) years old; 52 patients of limited disease (LD) stage and 34 patients of extensive disease (ED) stage; there were 47 cases with tumor diameter >30 mm and 39 cases with tumor size ≤30 mm; 48 cases of smokers and 38 cases of non-smokers. By analyzing the relationship between NSE, ProGrp and Cyfra21-1 and clinical data, we found that there was no significant difference between the serum expression of NSE, ProGrp and Cyfra21-1 and other clinical indicators of patients (all P>0.05). See **Table 2**.

The correlation between serum expression of NSE, ProGrp and Cyfra21-1 and clinical efficacy evaluation after treatment

After being treated with EP chemotherapy regimen, there were 3 patients classified as CR, 56 as PR, 18 as SD and 9 as PD in the observation group. Compared with before treatment, the serum levels of NSE, ProGrp and Cyfra21-1 in the CR, PR and SD groups were significantly decreased after chemotherapy (all P<0.05). In patients with PD, the expression levels of NSE and Cyfra21-1 were significantly increased after chemotherapy while ProGrp was decreased (all P<0.05). See **Tables 3-5**. The relationship between different clinical efficacy and the three indicators was analyzed by Spearman test, suggesting that each indicator was decreased with the improvement of clinical efficacy (all P<0.05). See **Table 6**.

Cox regression analysis and patients' survival condition

The clinical data of patients were collected for univariate Cox regression analysis in the observation group. We found that stage (P = 0.000), metastasis (P = 0.003), NSE (P = 0.0021), ProGrp (P = 0.000) and Cyfra21-1 (P = 0.000) were significant prognostic factors in patients with SCLC. Sequentially, the indicators with significant differences were analyzed by

Table 2. The correlation between clinical data of patients with negative/positive expression of NSE, ProGrp and Cyfra21-1 (n, %)

Factors	NSE (ng/mL)		ProGrp (pg/mL)		Cyfra21-1 (ng/mL)	
	Negative	Positive	Negative	Positive	Negative	Positive
Gender						
Male (n = 57)	6 (10.53)	51 (89.47)	0 (0.00)	57 (100.00)	28 (49.12)	29 (50.88)
Female (n = 29)	1 (3.45)	28 (96.55)	0 (0.00)	29 (100.00)	14 (48.28)	15 (51.72)
Age						
≥60 (n = 39)	3 (7.69)	36 (92.31)	0 (0.00)	39 (100.00)	22 (56.41)	17 (43.59)
<60 (n = 47)	4 (8.51)	43 (91.49)	0 (0.00)	47 (100.00)	20 (42.55)	27 (57.45)
Stage						
ED (n = 34)	3 (8.82)	31 (91.18)	0 (0.00)	34 (100.00)	14 (41.18)	20 (58.82)
LD (n = 52)	4 (7.69)	48 (92.31)	0 (0.00)	52 (100.00)	28 (53.85)	24 (46.15)
Metastasis						
Metastasis (n = 12)	0 (0.00)	12 (100.00)	0 (0.00)	12 (100.00)	4 (33.33)	8 (66.67)
No Metastasis (n = 74)	7 (9.46)	67 (90.54)	0 (0.00)	74 (100.00)	38 (51.35)	36 (48.65)
Tumor diameter						
>30 mm (n = 47)	2 (4.26)	45 (95.74)	0 (0.00)	47 (100.00)	25 (53.19)	22 (46.81)
≤30 mm (n = 39)	5 (12.82)	34 (87.18)	0 (0.00)	39 (100.00)	17 (43.59)	22 (56.41)
Smoking status						
Smoker (n = 48)	6 (12.50)	42 (87.50)	0 (0.00)	48 (100.00)	27 (56.25)	21 (43.75)
Non-smoker (n = 38)	1 (2.63)	37 (97.37)	0 (0.00)	38 (100.00)	15 (39.47)	23 (60.53)

Note: LD, limited disease; ED, extensive disease; NSE, neuron-specific enolase; ProGrp, pro-gastrin-releasing peptide precursor; Cyfra21-1, cytokeratin-19 fragment.

Table 3. The differences in the serum expression of NSE before and after treatment in the observation group (ng/mL)

Clinical efficacy	NSE		T	P
	Before treatment	After treatment		
CR+PR (n = 59)	55.00±23.62	15.91±3.55	12.594	0.000
SD (n = 18)	48.74±24.40	19.52±5.58	4.884	0.000
PD (n = 9)	68.86±14.61	143.24±18.45$^{***,###}$	-8.339	0.000
F	0.844	240.975		
P	0.434	0.000		

Note: NSE, neuron-specific enolase; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease. Significant difference in CR+PR (***P<0.001); significant difference in SD ($^{###}$P<0.001).

Table 4. The differences in the serum expression of ProGrp before and after treatment in the observation group (pg/mL)

Clinical efficacy	ProGrp		T	P
	Before treatment	After treatment		
CR+PR (n = 59)	785.28±111.46	105.19±33.21	43.496	0.000
SD (n = 18)	785.62±133.87	428.10±89.99***	10.364	0.000
PD (n = 9)	842.08±176.67	580.13±152.81$^{***,##}$	5.739	0.000
F	2.284	1,344.738		
P	0.108	0.000		

Note: ProGrp, pro-gastrin-releasing peptide precursor; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease. Significant difference in CR+PR, ***P<0.001; significant difference in SD, $^{##}$P<0.001.

multivariate Cox regression analysis, which suggested that NSE (HR = 0.984, 95% CI: 0.973-0.995), ProGrp (HR = 1.006, 95% CI: 1.003-1.009), Cyfra21-1 (HR = 1.503, 95% CI: 0.938-2.410), metastasis (HR = 2.410, 95% CI: 1.091-5.320), stage (HR = 1.792, 95% CI: 0.932-3.444) were independent prognostic factors for patients with SCLC. See **Tables 7** and **8**. The survival curves were drawn according to univariate Cox regression analysis. The results showed that there was a significant difference in the overall survival rate between patients in ED stage and patients in LD stage (P = 0.001); there was a significant difference in the overall survival rate between patients with metastasis and patients without metastasis (P = 0.001); there was a significant differ-

Table 5. The differences in the serum expression of Cyfra21-1 before and after treatment in the observation group (ng/mL)

Clinical efficacy	Cyfra21-1		T	P
	Before treatment	After treatment		
CR+PR (n = 59)	3.03±0.63	2.51±0.62	23.069	0.000
SD (n = 18)	4.45±0.15***	3.78±0.26***	24.349	0.000
PD (n = 9)	4.41±0.27***,##	4.95±0.09***,#	-8.434	0.000
F	74.795	83.941		
p	0.000	0.000		

Note: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; Cyfra21-1, cytokeratin-19 fragment. Significant difference in CR+PR, ***P<0.001; significant difference in SD, #P<0.05; ##P<0.01.

ence in the overall survival rate between patients in NSE normal group and patients in NSE elevated group (P = 0.001); there was a significant difference in the overall survival rate between patients in ProGrp normal group and patients in ProGrp elevated group (P = 0.001); there was a significant difference in the overall survival rate between patients in Cyfra21-1 normal group and patients in Cyfra21-1 elevated group (P = 0.001). See **Figure 2**.

Discussion

LC has become one of the most common malignancies worldwide. US cancer statistics report showed that there were more than 22.3 million new cases in 2017, and the number of LC-related new deaths had reached 15.6 million, showing an increasing trend year by year [15, 16]. SCLS, as a subtype of LC, is difficult to treat due to early metastasis, high invasiveness and its insensitivity to radiotherapy and chemotherapy [17].

In recent years, great advances have been achieved in the chemotherapy regims for SCLC. Post et al. reported that chemotherapy significantly improved the overall survival of SCLC patients [18]. In the past decade, EP and EC (etoposide+carboplatin) regimen have become the most important treatment for SCLC [19]. Recently, there was a study that reported irinotecan+cisplatin exhibited better therapeutic efficacy than the EP regimen; currently EP and EC still dominate, mainly due to lower costs [20].

The evaluation of SCLC is mainly based on the RECIST standard established by the American Cancer Institute, which is diagnosed mostly by imaging techniques, and only a small part of SCLC can be diagnosed with tumor biomarkers. At present, there are many tumor biomarkers for SCLC in the clinic, but very few specific tumor biomarkers [21]. Several studies have reported that NSEP, ProGrp and Cyfra21-1 can be used as biomarkers for SCLC [22, 23]. Therefore, this study was conducted to observe the expression of NSE, ProGrp and Cyfra21-1 before and after EP chemotherapy in patients with SCLC, so as to provide a reference for clinical prognosis.

As a high-acid protein-glycolytic enolase found in nerve tissue, differential expression of NSE is of great clinical significance for the observation of clinical treatment effect of SCLC and the assessment of patients' disease progression and recurrence [24]. ProGrp, as a SCLC-specific biomarker, it can be used as an important factor for early diagnosis of SCLC; it is also used for histological identification of LC, which is a benefit for making quick decisions on the specific clinical characters of patients [25]. Cyfra21 is a soluble fragment of cytokeratin 19 protein, located in human epithelial cells. Existing study has shown that Cyfra21-1 can be used as a new type of lung cancer biomarker, and high expression of Cyfra21-1 is significantly associated with tumor severity [26]. In this study, the serum expressions of NSE, ProGrp and Cyfra21 in the normal group, control group and observation group were monitored. The results showed that there was no significant difference in the serum expression of NSE, ProGrp and Cyfra21 between the control group and normal group; while all three indicators were significantly increased in the observation group. The relationship between the expression of each indicator and clinical data of patients in the observation group was also analyzed. The results suggested that the expression of NSE, ProGrp, and Cyfra21-1 was not associated with the clinical data of patients in the observation group. Subsequently, we performed an EP chemotherapy regimen for two weeks on patients with SCLC. The results indicated that the serum expression of NSE, ProGrp, and

Table 6. The correlation between clinical efficacy and indicators

Indicators	CR+PR (n = 59)	SD (n = 18)	PD (n = 9)	R	P
NSE (ng/mL)	15.91±3.55	19.52±5.58	143.24±18.45	0.529	0.001
ProGrp (pg/mL)	105.19±33.21	428.10±89.99	580.13±152.81	0.799	0.001
Cyfra21-1 (ng/mL)	2.51±0.62	3.78±0.26	4.95±0.09	0.816	0.001

Note: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NSE, neuron-specific enolase; ProGrp, pro-gastrin-releasing peptide precursor; Cyfra21-1, cytokeratin-19 fragment.

Table 7. Univariate Cox regression analysis

Factors	β	SD	χ^2	P	HR (95% CI)
Gender	0.288	0.280	1.061	0.303	1.334 (0.771-2.308)
Age	0.007	0.012	0.295	0.587	1.007 (0.983-1.031)
Stage	-1.067	0.301	12.523	0.000	0.344 (0.191-0.621)
Metastasis	-1.124	0.384	8.550	0.003	0.325 (0.153-0.690)
Tumor diameter	0.141	0.262	0.289	0.591	1.151 (0.689-1.923)
Nse	0.008	0.004	5.299	0.021	1.008 (1.001-1.015)
ProGrp	0.004	0.001	34.473	0.000	1.004 (1.003-1.005)
Cyfra21-1	0.810	0.175	21.440	0.000	2.248 (1.595-3.167)
Smoking status	-0.505	0.262	3.705	0.054	0.604 (0.361-1.009)

Note: SD, standard deviation; HR, hazard ratio; NSE, neuron-specific enolase; ProGrp, pro-gastrin-releasing peptide precursor; Cyfra21-1, cytokeratin-19 fragment.

Table 8. Multivariate Cox regression analysis

Factors	β	SD	χ^2	P	Adjusted HR (95% CI)
NSE	-0.016	0.006	7.788	0.005	0.984 (0.973-0.995)
ProGrp	0.006	0.002	13.493	0.000	1.006 (1.003-1.009)
Cyfra21-1	0.407	0.241	2.863	0.001	1.503 (0.938-2.410)
Metastasis	0.879	0.404	4.737	0.030	2.410 (1.091-5.320)
Stage	0.583	0.333	4.058	0.042	1.792 (0.932-3.444)

Note: SD, standard deviation; HR, hazard ratio; NSE, neuron-specific enolase; ProGrp, pro-gastrin-releasing peptide precursor; Cyfra21-1, cytokeratin-19 fragment.

Cyfra21-1 of patients in CR, PR and SD stage was significantly decreased after chemotherapy, and for patients in PD stage, the expression of NSE and Cyfra21-1 was significantly increased after chemotherapy, while ProGrp was decreased. These data demonstrated that the serum expression of NSE, ProGrp and Cyfra21-1 in patients can objectively reflect the chemotherapy efficacy. In addition, the expression and changing trends of patients in PD stage also demonstrated that NSE and Cyfra21-1 were significantly associated with the chemotherapy efficacy. Previous studies have reported that NSE, ProGrp, and Cyfra21-1 can be used as prognostic factors for patients with SCLC after chemotherapy, which is consistent with our results [27, 28]. In addition, we also performed multivariate Cox regression analysis on the overall survival of patients with SCLC. The results indicated that NSE, ProGrp, Cyfra21-1, tumor metastasis, and tumor stage were independent prognostic factors for SCLC patients, which was consistent with previous study [29]. However, another study also finds that ProGrp is not associated with the prognosis of SCLC, and we speculate that it may be due to the use of different thresholds [30]. Previous studies explored the correlation of every two indicators with the prognosis of SCLC patients. In this study, we simultaneously analyzed the expression of NSE, ProGrp and Cyfra21-1 after chemotherapy in patients with SCLC, confirming the prognostic values of these three indicators, which has filled in the gaps in the field.

However, there are still some limitations in this study. The sample size in the present study was relatively small and prone to bias. Second, we did not study the action mechanism of chemotherapy on the serum expression of NSE, ProGrp and Cyfra21-1. Therefore, we expect to increase the sample size in future research, and perform more in-depth studies on the mechanisms for improvements of various indicators after chemotherapy to support the results of this study.

Cyfra21-1, ProGrp, and NSE are highly expressed in the serum of patients with SCLC, and the expression levels are significantly associated with the clinical stage and prognosis.

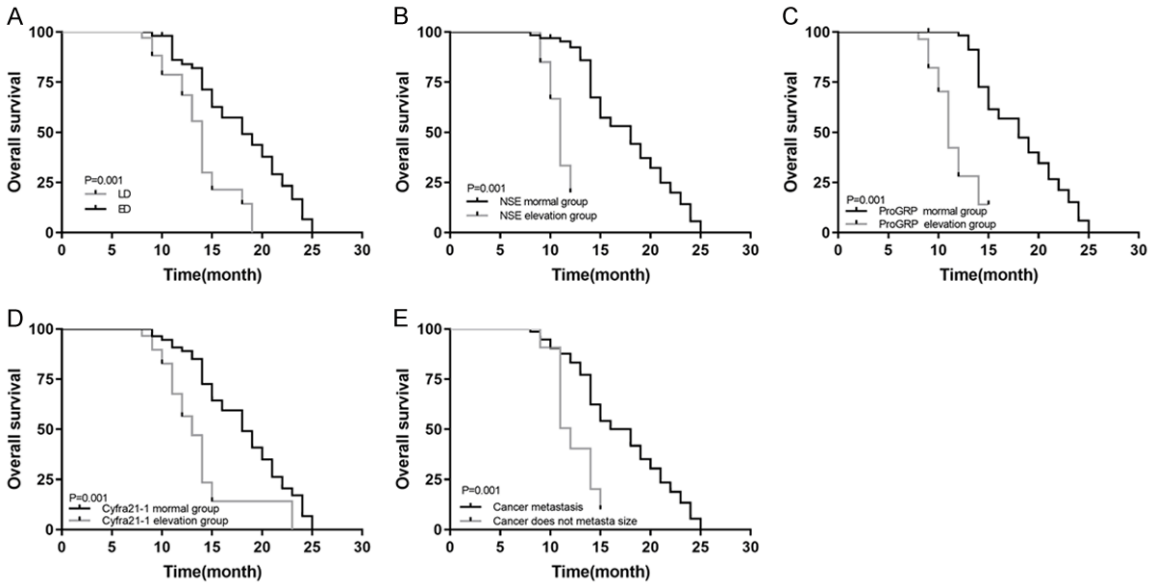

Figure 2. Survival curve. A. There was a significant difference in the overall survival rate between patients in ED stage and patients in LD stage (P=0.001). B. There was a significant difference in the overall survival rate between patients in normal NSE group and patients in NSE elevated group (P=0.001). C. There was a significant difference in the overall survival rate between patients in ProGrp normal group and patients in ProGrp elevated (P=0.001). D. There was a significant difference in the overall survival rate between patients in Cyfra21-1 normal group and patients in Cyfra21-1 elevated group (P=0.001). E. There was a significant difference in the overall survival rate between patients with metastasis and patients without metastasis (P=0.001). ED - extensive disease, LD - limited disease, NSE - neuron-specific enolase, ProGrp - pro-gastrin-releasing peptide precursor, Cyfra21-1 - cytokeratin-19 fragment.

Moreover, Cyfra21-1, ProGrp, and NSE are associated with the clinical chemotherapy efficacy of patients, which suggests value for their monitoring.

Disclosure of conflict of interest

None.

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