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Neuron-Specific Enolase Is an Independent Prognostic Factor in Resected Lung Adenocarcinoma Patients with Anaplastic Lymphoma Kinase Gene Rearrangements

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

E 1,2,3 **Shouying Li***
D 1,2,3 **Lianjing Cao***
C 1,2,3 **Xinyue Wang**
B 1,2,3 **Fan Wang**
F 1,2,3 **Liuchun Wang**
A 1,2,3 **Richeng Jiang**

1 Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer, Tianjin, P.R. China
2 Key Laboratory of Cancer Prevention and Therapy, Tianjin's Clinical Research Center for Cancer, Tianjin, P.R. China
3 Department of Thoracic Oncology, Tianjin Lung Cancer Center, Tianjin Cancer Institute and Hospital, Tianjin Medical University, Tianjin, P.R. China

* These authors contributed equally to this work and share first authorship

Corresponding Author: Richeng Jiang, e-mail: jiangricheng@tjmuch.com

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Background: An extensive body of research reveals the clinical value of serum tumor markers in lung cancer patients, including carcinoembryonic antigen (CEA), squamous cell carcinoma antigen (SCCA), cytokeratin-19 fragments (Cyfra21-1), and neuron-specific enolase (NSE), but little is known about the clinical properties of these serum tumor markers in anaplastic lymphoma kinase (ALK)-positive lung cancer patients.

Matreial/Methods: We retrospectively analyzed 54 patients harboring ALK rearrangements and 520 patients without ALK rearrangements, and all these patients were treated exclusively by surgery between 2011 and 2016.

Results: NSE level (P=0.007 for OS) was identified as an independent prognostic factor among patients with resected ALK-positive adenocarcinoma of the lung.

Conclusions: A high level of NSE is associated with worse outcome among resected lung adenocarcinoma patients harboring ALK rearrangements.

MeSH Keywords: **Adenocarcinoma • Carcinoembryonic Antigen • Phosphopyruvate Hydratase**

Abbreviations: **ALK** – anaplastic lymphoma kinase; **EML4-ALK** – echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase; **CEA** – carcinoembryonic antigen; **Cyfra21-1** – cytokeratin-19 fragments; **SCCA** – squamous cell carcinoma antigen; **NSE** – neuron-specific enolase; **AFP** – α-fetoprotein; **EGFR** – epidermal growth factor receptor; **VEGF** – vascular endothelial growth factor; **PFS** – progression-free survival; **OS** – overall survival; **NLR** – neutrophil-lymphocyte ratio; **PLR** – platelet- lymphocyte ratio; **NSCLC** – non-small-cell lung cancer; **SCLC** – small cell lung cancer

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Background

The prognostic factors of lung cancer might be important in daily clinical practice due to its high prevalence and mortality rates. In recent years, the efficacy of cancer treatment has achieved a qualitative leap since many oncogenic drivers have been discovered, such as anaplastic lymphoma kinase (ALK) genes, epidermal growth factor receptor (EGFR), and vascular endothelial growth factor (VEGF). These developments have improved molecular diagnosis and accurate therapy for lung cancer patients.

The ALK gene was first described as NPM-ALK fusion protein, mainly in anaplastic large-cell lymphomas [1]. It accounts for about 6.7% of NSCLCs [2] and 11% of lung adenocarcinoma [3]. Although a low proportion of lung cancer patients have ALK rearrangements, the total number of the cases is considerable and these patients would benefit from molecular-targeted treatment. Thus, it is important to explore predictive factors among lung cancer patients that harbor ALK rearrangements in clinical practice.

Serum tumor markers, including carcinoembryonic antigen (CEA) [4], squamous cell carcinoma antigen (SCCA) [5], cytokeratin-19 fragments (Cyfra21-1) [6], and neuron-specific enolase (NSE) [7], have been found to be useful biomarkers in lung cancer, but no consistent view has been reached yet. Indeed, the clinical significance of these serum tumor markers in ALK-positive lung adenocarcinoma have not been thoroughly investigated.

In this study, we carried out a retrospective clinical research of a total 574 operable lung adenocarcinoma patients in which 54 patients had ALK rearrangements. We covered clinical factors between serum tumor markers and the clinical features in patients of stage I, II, and III who underwent partial or complete surgical operation. We explored the clinical features in ALK-positive lung adenocarcinoma and assessed whether these serum tumor markers are independent indicators for prognosis in these patients.

Material and Methods

Patient selection

We retrospectively analyzed a database of consecutive lung adenocarcinoma patients treated by surgery between March 2011 and August 2016 at Tianjin Medical University Cancer Institute and Hospital. Approval by the Research Ethics Committee of Tianjin Medical University Cancer Institute was gained and its purpose was to explore the clinical significance of serum tumor markers at the beginning of diagnosis as prognostic factors in the subset of lung adenocarcinoma patients with ALK rearrangements. Examinations prior to the operation included physical examination, level of serum tumor biomarkers, blood chemistry analysis, CT scan, fiberoptic bronchoscopy, brain MRI, and bone scanning. All patients underwent partial or complete surgical resection exclusively. The selection criteria were as follows: (a) the pathological diagnosis was lung adenocarcinoma; (b) ALK-rearrangement positive (by the Ventana ALK [D5F3] IHC Kit, Roche, Switzerland); (c) clinical stages I, II, IIIA, or IIIB disease defined by the tumor node metastasis (TNM) criteria of NSCLC (AJCC,7th edition) and the TNM stage assessment was according to CT scans, brain MRI, and bone emission CT scans; (d) patients were untreated at the time routine blood analyses were performed; and (e) preoperative evaluation showed that surgery was appropriate for patients. Adjuvant treatment included chemotherapy, radiotherapy, or targeted therapy. Follow-up data were gathered directly from the clinical record or telephone follow-up. CT scans were checked every 3 or 4 months to monitor recurrence and then annually after 3 years. Disease-free survival (DFS) was from the date of surgery to recurrence, or the date of final follow-up. Overall survival (OS) was measured to the date to death caused by disease or to the date of final follow-up.

Tumor-associated antigens

The concentrations of serum tumor biomarkers were detected by immunoenzymometric assay (Roche Diagnostics, China) within 1 week before the operation. The cut-off values of serum tumor markers were as follows: CEA 5.0 ng/ml, SCCA 1.5 ng/ml, Cyfra21-1 3.3 ng/ml, and NSE 15.2 ng/ml, using the reference values provided by the manufacturer.

Statistical analysis

SPSS Statistics 24 (IBM Corporation, NY, USA) was used for all statistical analyses. Univariate analyses of the comparisons of baseline clinical characteristics between cohorts were carried out using the chi-squared test. Survival was conducted using Cox regression analysis. To examine differences in survival between different cohorts, the log-rank test was used. Cox proportional hazard method was applied to calculate hazard ratio (HR) and the 95% confidence intervals (CI), and the multivariate Cox method was performed using the backward selection method. In all analyses, a p-value less than 0.05 was regarded as significant.

Results

Patient characteristics

Our study cohort included 574 lung adenocarcinoma patients who underwent partial or complete surgical resection between 2011 and 2016. As shown in Table 1, 267 (46.5%) of the patients were male and 307 (53.5%) were female. Histological

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Table 1. All patient characteristics (n=574).

Characteristics	n (%)	
Age		
≤60	336	(58.5)
>60	238	(41.5)
Sex		
Male	267	(46.5)
Female	307	(53.5)
Smoking history		
Former/current smoker	275	(47.9)
Never smoker	291	(50.7)
Pathological stage		
I	288	(50.2)
II	92	(16)
IIIA	178	(31)
IIIB	16	(2.8)
Tumor size		
≤3 cm	348	(60.6)
>3 cm	226	(39.4)
Regional LN metastasis		
No	342	(59.6)
Yes	232	(40.4)

Characteristics	n (%)	
Surgical resection		
Pneumonectomy	36	(6.3)
Lobectomy	517	(90.1)
Wedge resection	17	(3.0)
Adjuvant treatment		
No	298	(51.9)
Yes	276	(48.1)
CEA		
≤5.0 ng/ml	364	(63.4)
>5.0 ng/ml	208	(36.2)
SCCA		
≤1.5 ng/ml	525	(91.5)
>1.5 ng/ml	47	(8.2)
Cyfra21–1		
≤3.3 ng/ml	382	(66.6)
>3.3 ng/ml	190	(33.1)
NSE		
≤15.2 ng/ml	451	(78.6)
>15.2 ng/ml	120	(20.9)

LN – lymph node; CEA – carcinoembryonic antigen; Cyfra21-1 – cytokeratin-19 fragments; SCCA – squamous cell carcinoma antigen; NSE – neuron-specific enolase.

diagnoses were all adenocarcinoma. The clinical stages were as follows: 288 stage I, 92 stage II, 178 stage IIIA, and 16 stage IIIB. There were 517 patients who underwent lobectomy, 36 underwent pneumonectomy, and 17 underwent wedge resection.

Of all 574 patients, 276 (48.1%) received adjuvant treatment including platinum-based adjuvant chemotherapy, postoperative radiotherapy, and anaplastic lymphoma kinase inhibitor, and the other 298 (51.9%) received no postoperative treatment. The average DFS in this cohort was 25.20 months and the average OS was 30.59 months. By the final follow-up date, 148 patients had died.

Among the ALK-positive patients, 25 (46.3%) were male and 29 (53.7%) were female (Supplementary Table 1). The clinical stages were as follows: 27 were stage I, 11 were stage II, 13 were stage IIIA, and 3 were stage IIIB. Fifty-one patients received lobectomy, 2 received pneumonectomy, and 1 received wedge resection. The average DFS in this cohort was 18.47 months and the average OS was 25.43 months. By the last follow-up date, 7 patients had died.

Among the ALK-negative patients, 242 (46.5%) were male while 278 (53.5%) and were female (Supplementary Table 1). The clinical stages were as follows: 261 stage I, 81 stage II, 165 stage IIIA, and 13 stage IIIB. There were 466 patients who underwent lobectomy, 34 had pneumonectomy, and 16 had wedge resection. The average DFS in this cohort was 25.90 months and the average OS was 31.12 months. By the last follow-up date, 141 patients had died.

Correlation between ALK expression and the clinicopathological characteristics

We further analyzed the correlation between ALK expression and clinical characteristics (Supplementary Table 2). Univariate

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Table 2. Univariate and multivariate analyses of DFS and OS in all patients.

Variables	DFS					OS				
	Univariate analysis			Multivariate analysis		Univariate analysis			Multivariate analysis	
	n	Median DFS (mo)	P*	HR (95%CI)	P*	n	Median OS (mo)	P*	HR (95%CI)	P*
Age (years)										
≤60	336	24.93	0.893			336	30.57	0.918		
>60	238	25.59				238	30.61			
Sex										
Male	267	24.87	0.600			267	29.87	0.179		
Female	307	25.49				307	31.21			
Smoking history										
Former/current smoker	275	24.73	0.494			275	30.03	0.267		
Never smoker	291	25.47				291	30.84			
Pathological stage										
I	288	27.33	**≤0.001**	1.479 (1.298, 1.684)	**≤0.001**	288	31.21	**≤0.001**	1.425 (1.208, 1.682)	**≤0.001**
II	92	25.54				92	31.08			
IIIA	178	22.11				178	29.54			
IIIB	16	19.25				16	27.50			
Tumor size										
≤3 cm	348	25.67	**0.003**		0.601	348	30.15	0.239		
>3 cm	226	24.48				226	31.26			
Regional LN metastasis										
No	342	27.12	**≤0.001**		0.274	342	30.94	**≤0.001**		0.193
Yes	232	22.37				232	30.06			
Surgical resection										
Pneumonectomy	36	22.38	**0.031**	1.354 (1.060, 1.731)	**0.015**	36	39.62	**0.019**	1.477 (1.093, 1.996)	**0.011**
Lobectomy	517	25.29				517	30.47			
Wedge resection	17	27.82				17	34.50			
Adjuvant treatment										
No	298	24.94	0.394			298	29.17	0.066		
Yes	276	25.49				276	32.14			
CEA										
≤5.0 ng/ml	364	26.26	**≤0.001**	1.308 (1.013, 1.688)	**0.039**	364	30.68	**0.005**		0.203
>5.0 ng/ml	208	23.36				208	30.44			

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Table 2 continued. Univariate and multivariate analyses of DFS and OS in all patients.

Variables	DFS					OS				
	Univariate analysis			Multivariate analysis		Univariate analysis			Multivariate analysis	
	n	Median DFS (mo)	P*	HR (95%CI)	P*	n	Median OS (mo)	P*	HR (95%CI)	P*
SCCA										
≤1.5 ng/ml	525	25.55	0.096			525	31.04	0.164		
>1.5 ng/ml	47	21.31				47	25.59			
Cyfra21–1										
≤3.3 ng/ml	382	26.28	**≤0.001**	1.482 (1.156, 1.900)	**0.002**	382	31.32	**≤0.001**	1.818 (1.312, 2.521)	**≤0.001**
>3.3 ng/ml	190	23.04				190	29.14			
NSE										
≤15.2 ng/ml	451	25.33	0.145			451	30.64	0.059		
>15.2 ng/ml	120	24.67				120	30.42			

LN – lymph node; CEA – carcinoembryonic antigen; Cyfra21-1 – cytokeratin-19 fragments; SCCA – squamous cell carcinoma antigen; NSE – neuron-specific enolase; DFS – disease-free survival; OS – overall survival; HR – hazard ratio; CI – confidence interval.

analysis revealed ALK expression was associated with clinicopathologic characteristics, including age (P=0.006), smoking history (P=0.008), CEA level (P≤0.001), and SCCA level (P=0.018). Multivariate logistic regression analysis of ALK expression and clinical factors showed that ALK expression was associated with age (P=0.006) and smoking history (P=0.008) (Supplementary Table 3).

Univariate and multivariate analysis of all patients

Univariate analysis in all patients showed that clinical stage, surgical resection, metastasis in regional lymph node, tumor size, CEA level, and Cyfra21-1 level were associated with DFS, while OS was associated with clinical stage, surgical resection, metastasis in regional lymph node, CEA level, and Cyfra21-1 level (Table 2, Supplementary Figures 1A, 1B, 2A, 2B). To find possible independent prognostic factors, we next conducted multivariate DFS and OS analysis in which clinical stage (P≤0.001 for DFS, P≤0.001 for OS), surgical resection (P=0.015 for DFS, P=0.011 for OS), and Cyfra21-1 (P=0.002 for DFS, P≤0.001 for OS) were identified as independent factors predicting DFS and OS (Table 2).

Univariate and multivariate analysis of ALK-positive and -negative patients

For ALK-positive patients, by univariate analysis, we found that DFS was related to smoking history, clinical stage, metastasis in regional lymph node, CEA level, and Cyfra21-1 level, while clinical stage, CEA, NSE, and Cyfra21-1 were correlated with OS (Table 3, Supplementary Figure 1C, 1D, 2C, 2D). In multivariate Cox regression analysis, clinical stage (P=0.013) and Cyfra21-1 level (P=0.030) were predictive factors for DFS., while clinical stage (P=0.004) and NSE level (P=0.007) were independent prognostic factors for OS.

For ALK-negative patients, by univariate analysis, we found that DFS was related to clinical stage, surgical resection, maximum size of tumor, metastasis in regional lymph node, CEA, and Cyfra21-1, while clinical stage, surgical resection, adjuvant treatment, metastasis in regional lymph node, CEA level, and Cyfra21-1 level were correlated with OS (Supplementary Table 4, Supplementary Figures 1E, 1F, 2E, 2F). In multivariate Cox regression analysis, clinical stage (P≤0.001), surgical resection (P=0.013), CEA (P=0.022), and Cyfra21-1 level (P=0.008) were independent factor predicting DFS. Metastasis in regional lymph node (P≤0.001), surgical resection (P=0.013), and Cyfra21-1 level (P≤0.001) were independent prognostic factors predicting OS.

NSE analysis

For all 574 patients, the average NSE level was 14.35 ng/ml. Patients were stratified into lower and higher NSE groups, one with NSE level ≤15.2 ng/ml (n=451) and another with NSE level >15.2 ng/ml (n=120) (Table 2). The average DFS in high-NSE patients was 24.67 months as compared with 25.33 months for low-NSE patients. The average OS for high-NSE patients

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Table 3. Univariate and multivariate analyses of DFS and OS in ALK positive patients.

Variables	DFS					OS				
	Univariate analysis			Multivariate analysis		Univariate analysis			Multivariate analysis	
	n	Median DFS (mo)	P*	HR (95%CI)	P*	n	Median OS (mo)	P*	HR (95%CI)	P*
Age (years)										
≤60	41	18.25	0125			41	23.24	0.370		
>60	13	19.16				13	32.34			
Sex										
Male	25	16.98	0.519			25	21.37	0.298		
Female	29	19.75				29	28.92			
Smoking history										
Former/current smoker	17	14.34	**0.019**		0.194	17	20.42	0.281		
Never smoker	37	20.36				37	27.73			
Pathological stage										
I	27	20.16	**0.004**	17.37 (1.122, 2.687)	**0.013**	27	26.12	**0.004**	4.043 (1.551, 10.535)	**0.004**
II	11	21.77				11	31.52			
IIIA	13	13.59				13	20.16			
IIIB	3	12.27				3	19.72			
Tumor size										
≤3 cm	36	19.42	0.098			36	25.92	0.362		
>3 cm	18	16.57				18	24.45			
Regional LN metastasis										
No	30	19.97	**0.030**		0.997	30	25.51	0.114		
Yes	24	16.59				24	25.33			
Surgical resection										
Pneumonectomy	2	6.73	0.521			2	13.50	0.303		
Lobectomy	51	18.92				51	26.03			
Wedge resection	1	18.83				1	18.83			
Adjuvant treatment										
No	15	21.30	0.082			15	28.47	0.198		
Yes	39	17.38				39	24.26			
CEA										
≤5.0 ng/ml	50	19.26	**0.023**		0.153	50	25.80	**0.002**		0.767
>5.0 ng/ml	4	8.62				4	20.81			

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Table 3 continued. Univariate and multivariate analyses of DFS and OS in ALK positive patients.

Variables	DFS					OS				
	Univariate analysis			Multivariate analysis		Univariate analysis			Multivariate analysis	
	n	Median DFS (mo)	P*	HR (95%CI)	P*	n	Median OS (mo)	P*	HR (95%CI)	P*
SCCA										
≤1.5 ng/ml	45	19.17	0.449			45	26.47	0.818		
>1.5 ng/ml	9	14.94				9	20.24			
Cyfra21–1										
≤3.3 ng/ml	36	20.66	**0.009**	2.659 (1.099, 6.433)	**0.030**	36	26.16	**0.048**		0.231
>3.3 ng/ml	18	14.09				18	23.96			
NSE										
≤15.2 ng/ml	44	19.51	0.249			44	26.83	**0.005**	12.552 (1.977, 79.692)	**0.007**
>15.2 ng/ml	10	13.89				10	19.26			

LN – lymph node; CEA – carcinoembryonic antigen; Cyfra21-1 – cytokeratin-19 fragments; SCCA – squamous cell carcinoma antigen; NSE – neuron-specific enolase; DFS – disease-free survival; OS – overall survival; HR – hazard ratio; CI – confidence interval.

was 30.64 months as compared with 30.42 months for low-NSE patients. The survival analysis revealed that high serum NSE level was not relevant to OS (P=0.059) (Figure 1A).

For ALK-positive patients, the average NSE level was 13.86 ng/ml. Patients were also stratified into lower (n=44) and higher (n=10) NSE groups (Table 3). The average DFS in high-NSE patients was 13.89 months, as compared with 19.51 months for low-NSE patients. The average OS for high-NSE patients was 19.26 months as compared with 26.83 months for low-NSE patients. The survival analysis revealed that high serum NSE level was significantly relevant to decreased OS (P=0.005) (Figure 1B). Multivariate Cox regression analysis showed that NSE was an independent prognostic factor for OS (P=0.007).

For ALK-negative patients, the survival analysis revealed that high serum NSE level was not associated with OS (P=0.189) (Figure 1C).

Discussion

Anaplastic lymphoma kinase is a receptor protein-tyrosine kinase of the insulin receptor protein-tyrosine kinase superfamily. EML4-ALK fusion was recently reported as a neoteric targeted therapy molecular in lung cancer and has become increasingly important in the diagnosis of lung cancer. Several studies have reported that more ALK-positive cases could be seen in light- or never-smoking patients [8], type of adenocarcinoma [9], and females and young patients [10]. ALK rearrangement has also been reported to be more common in males (P=0.039) [11], but other studies reported no sex difference [8,12]. In our study, consistent with the above-mentioned studies, ALK expression was strongly correlated with age and smoking history and was more common in never-smoking and young patients. However, no sex difference was found in our study cohort. Due to the low positive rate or other reasons, the prognostic factors in ALK-positive lung adenocarcinoma patients are unclear and no prognostic models have been developed. Therefore, it is necessary to find effective prognostic factors among these patients.

A recent study including 173 ALK-positive NSCLC patients reported that neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) were closely related to survival, and PLR was a significant prognostic factor in ALK-positive NSCLC [13]. In the study by Suzuki et al. [14], $CD56^{+}$ was reported to be associated with poor prognosis (P=0.002) in both ALK-negative and ALK-positive subgroups. Further research is needed to better predict the prognosis of ALK-positive patients. Serum tumor makers are routinely used in examinations after admission to help guide diagnosis, classification, and treatment of these patients. To the best of our knowledge, the present study is the first to report that the level of NSE in blood is an independent prognostic factor in ALK-positive lung adenocarcinoma patients.

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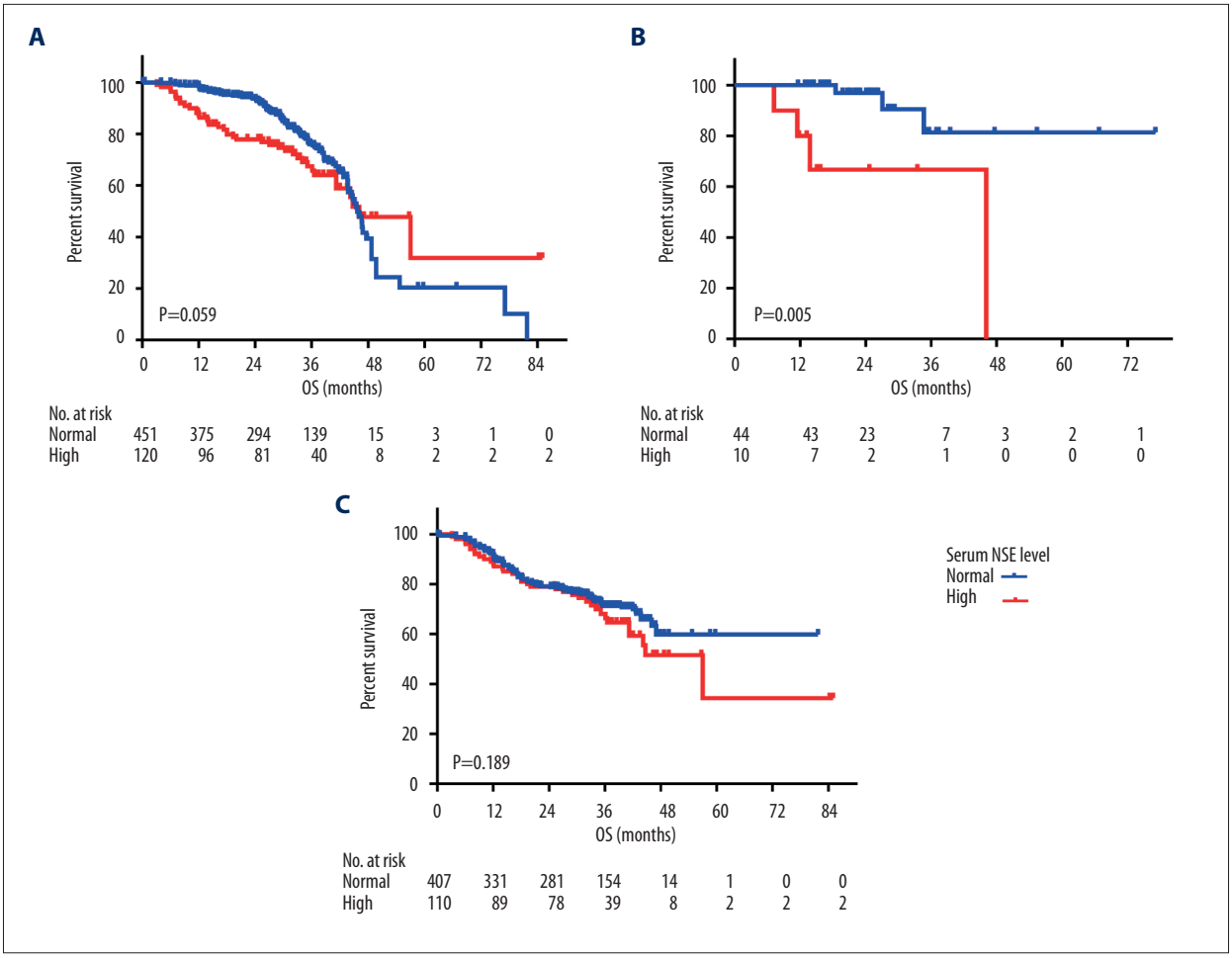

Figure 1. Kaplan-Meier survival curves of OS (**A**) according to NSE level in all patients. Kaplan-Meier survival curves of OS (**B**) according to NSE level in ALK-positive patients. Kaplan-Meier survival curves of OS (**C**) according to NSE level in ALK-negative patients.

CEA is a kind of glycoprotein involved in programmed cell death and cell adhesion, and is normally secreted during embryonic development, but the secretion stops before birth. Its specialized sialofucosylated glycoforms serve as functional ligands that may be vital to cancer cell metastasis [15]. CEA was reported to be an unspecific serum tumor marker with abnormal expression in various solid tumors. No consensus has been reached on the prognostic value of pretreatment CEA levels in lung cancer. Tomita et al. [16] reported that CEA was a useful serum tumor marker in NSCLC and that CEA was an important prognostic factor in NSCLC patients ($p<0.0001$), but other studies did not find this [5] To date, there have been no related reports on the relationship between CEA and ALK status. In our study, increased level of CEA was correlated with decreased DFS and OS in ALK-positive patients. CEA is involved in adhesion between cells and extracellular matrix, and its overexpression can inhibit cell differentiation and damage the structure of tissues. This could also partially be attributed to glycosylation of glycoproteins, which promote metastasis and invasion. The elevated level of CEA is one of the manifestations of activation of glycosylated enzymes, and other relevant molecules include AFP (α-fetoprotein) and NSE (neuron-specific enolase). Glycosylase activation leads to glycosylation of glycoproteins, which can affect ALK phosphorylation and downstream signaling [17–19], and thus have an effect on the prognosis of ALK-positive patients. In the present study, our cases were all adenocarcinoma patients, and CEA has already been shown to be predict poor prognosis in patients with resected lung adenocarcinomas [20].

CYFRA 21-1 is a fragment of cytokeratin (CK) 19. CK is the principal structural element of the cytoskeleton (keratin filaments) of epithelial cells, including bronchial epithelial cells. It elevated in many cell types of lung cancer, but mainly in squamous cell carcinoma [21]. It is reported that preoperative serum CYFRA 21-1 was a poor independent prognostic factor for adenocarcinoma of lung after surgery [22], which is consistent with our study. However, CYFRA 21-1 was reported to be unrelated to cancer recurrence or survival by Matsuoka et al. and Blankenburg et al., respectively [23,24]. In our study,

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CYFRA 21-1 was found to be associated with decreased DFS and OS in ALK-positive patients. High serum CYFRA 21-1 level might reflect poor differentiation because multi-directional differentiation indicates tumor aggressiveness and is also considered as a poor prognostic factor in early-stage lung cancer.

NSE is a neuron-specific isoenzyme of enolase, also known as enolase-γ. The clinical significance of NSE in small cell lung cancer (SCLC) had been well accepted, since NSE is reported to be frequently elevated in patients diagnosed with SCLC [25]. However, the value of NSE in NSCLC remains unclear. In the study of Pujol et al. [7], NSE was reported to be a prognostic factor for survival. Similar results were found by other studies, including Jacot et al. [26] and Ferrigno et al. [27]. However, in the study of Reinmuth et al. [28], preoperative serum NSE level was not found to be related to prognosis. To the best of our knowledge, no research has focussed on determining the predictive role of NSE in ALK-positive lung adenocarcinoma patients. As described in our study, NSE is an independent prognostic factor for survival in ALK-positive lung adenocarcinoma patients. Since mixed element of SCLC-NSCLC may arise in a small fraction of all lung cancer cases [29] and some NSCLC patients do have an elevated NSE level, we hypothesized that the association between poor prognosis and elevated pretreatment serum NSE level in NSCLC patients might be due a co-existing neuroendocrine or small cell component in the solid tumor or tumor glucose metabolism [30]. Recently, Choi et al. [31] reported that higher glucose metabolism was found in EML4-ALK-rearranged NSCLC than in EML4-ALK-negative NSCLC patients, revealing that EML4-ALK may play a role in regulating glucose metabolism. Ma et al. [32] reported that EML4-ALK activates aerobic glycolysis, while fast ATP and a carbon source for biosynthesis require a high glycolysis rate, and thus make cancer cells replicate faster. NSE is considered a key enzyme in glycolysis and it plays an important role in aerobic glycolysis; this could partially explain how NSE affects the prognosis of ALK-positive patients. However, the adverse prognostic significance of elevated NSE was also presumed to be more malignant in lung cancers with neuroendocrine differentiation.

Adjuvant chemotherapy is not generally recommended for early-stage patients in daily clinical work. As described in our study, an elevated serum NSE level is associated with poor prognosis in ALK-positive patients. Therefore, we suggest postoperative adjuvant therapy for resected ALK-positive patients with elevated serum NSE levels.

Our study has some limitations. First, it was a single-center, retrospective investigation, so it is possible that selection bias may have affected our findings. Second, the study included an insufficient number of ALK-positive to provide strong conclusions. Nevertheless, it is the first study to show that elevated NSE level is a potential biomarker of poor prognosis for ALK-positive patients with resected lung adenocarcinoma. Further research is needed on the clinical value of NSE in prognosis.

Conclusions

High serum NSE level is not relevant to decreased OS in ALK-negative patients, but NSE is an independent prognostic factor in ALK-positive patients.

Conflicts of interest

None.

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Supplementary Files

Supplementary Table 1. Patient characteristics in ALK positive(n=54) and ALK negative (n=520).

Characteristics	ALK positive n (%)		ALK negative n (%)	
Age				
≤60	41	(75.9)	295	(56.7)
>60	13	(24.1)	225	(43.3)
Sex				
Male	25	(46.3)	242	(46.5)
Female	29	(53.7)	278	(53.5)
Smoking history				
Former/current smoker	17	(31.5)	258	(49.5)
Never smoker	37	(68.5)	254	(48.8)
Pathological stage				
I	27	(50.0)	261	(50.2)
II	11	(20.4)	81	(15.6)
IIIA	13	(24.1)	165	(31.7)
IIIB	3	(5.6)	13	(2.5)
Tumor size				
≤3 cm	36	(66.7)	312	(60)
>3 cm	18	(33.3)	208	(40)
Regional LN metastasis				
No	30	(55.6)	312	(60)
Yes	24	(44.4)	208	(40)
Surgical resection				
Pneumonectomy	2	(3.7)	34	(6.5)
Lobectomy	51	(94.4)	466	(89.6)
Wedge resection	1	(1.9)	16	(3.1)
Adjuvant treatment				
No	15	(27.8)	283	(54.4)
Yes	39	(72.2)	237	(45.6)
CEA				
≤5.0 ng/ml	50	(92.6)	314	(60.4)
>5.0 ng/ml	4	(7.4)	204	(39.2)
SCCA				
≤1.5 ng/ml	45	(83.3)	480	(92.3)
>1.5 ng/ml	9	(16.7)	38	(7.3)
Cyfra21–1				
≤3.3 ng/ml	36	(66.7)	346	(66.5)
>3.3 ng/ml	18	(33.3)	172	(33.1)
NSE				
≤15.2 ng/ml	44	(81.5)	407	(78.3)
>15.2 ng/ml	10	(18.5)	110	(21.2)

LN – lymph node; CEA – carcinoembryonic antigen; Cyfra21-1 – cytokeratin-19 fragments; SCCA – squamous cell carcinoma antigen; NSE – neuron-specific enolase.

Supplementary Table 2. Correlation between ALK expression and the clinicopathological characteristics.

Characteristics	n (%)		ALK positive		ALK negative		P*
Age							**0.006**
≤60	336	(58.5)	41	(7.1)	295	(51.4)	
>60	238	(41.5)	13	(2.3)	225	(39.2)	
Sex							
Male	267	(46.5)	25	(4.4)	242	(42.2)	0.973
Female	307	(53.5)	29	(5.1)	278	(48.4)	
Smoking history							
Former/current smoker	275	(47.9)	17	(3.0)	258	(45.6)	**0.008**
Never smoker	291	(50.7)	37	(6.5)	254	(44.9)	
Pathological stage							
I	288	(50.2)	27	(4.7)	261	(45.5)	0.353

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Characteristics	n (%)		ALK positive		ALK negative		P*
II	92	(16)	11	(1.9)	81	(14.1)	
IIIA	178	(31)	13	(2.3)	165	(28.7)	
IIIB	16	(2.8)	3	(0.5)	13	(2.3)	
Tumor size							
≤3 cm	348	(60.6)	36	(6.3)	312	(54.4)	0.340
>3 cm	226	(39.4)	18	(3.1)	208	(36.2)	
Regional LN metastasis							
No	342	(59.6)	30	(5.2)	312	(54.4)	0.526
Yes	232	(40.4)	24	(4.2)	208	(36.2)	
Surgical resection							
Pneumonectomy	36	(6.3)	2	(0.4)	34	(6.0)	0.609
Lobectomy	517	(90.1)	51	(8.9)	466	(81.8)	
Wedge resection	17	(3.0)	1	(0.2)	16	(2.8)	
Adjuvant treatment							
No	298	(51.9)	15	(2.6)	283	(49.3)	**≤0.001**
Yes	276	(48.1)	39	(6.8)	237	(41.3)	
CEA							
≤5.0 ng/ml	364	(63.4)	50	(8.7)	314	(54.9)	**≤0.001**
>5.0 ng/ml	208	(36.2)	4	(0.7)	204	(35.7)	
SCCA							
≤1.5 ng/ml	525	(91.5)	45	(7.9)	480	(83.9)	**0.018**
>1.5 ng/ml	47	(8.2)	9	(1.6)	38	(6.6)	
Cyfra21–1							
≤3.3 ng/ml	382	(66.6)	36	(6.3)	346	(60.5)	0.985
>3.3 ng/ml	190	(33.1)	18	(3.1)	172	(30.1)	
NSE							
≤15.2 ng/ml	451	(78.6)	44	(7.7)	407	(71.3)	0.636
>15.2 ng/ml	120	(20.9)	10	(1.8)	110	(19.3)	

LN – lymph node; CEA – carcinoembryonic antigen; Cyfra21-1 – cytokeratin-19 fragments; SCCA – squamous cell carcinoma antigen; NSE – neuron-specific enolase.

Supplementary Table 3. Multivariate logistic regression analysis for ALK expression.

Characteristics	HR (95%CI)	P*
Age	0.375 (0.190, 0.740)	**0.006**
Smoking history	0.435 (0.232, 0.813)	**0.008**

HR – hazard ratio; CI – confidence interval.

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Supplementary Table 4. Univariate and multivariate analyses of DFS and OS in ALK negative patients.

Variable	DFS					OS				
	Univariate analysis			Multivariate analysis		Univariate analysis			Multivariate analysis	
	n	Median DFS (mo)	P*	HR (95%CI)	P*	n	Median OS (mo)	P*	HR (95%CI)	P*
Age (years)										
≤60	295	25.85	0.646			295	31.59	0.694		
>60	225	25.96				225	30.51			
Sex										
Male	242	25.69	0.675			242	30.75	0.269		
Female	278	26.09				278	31.45			
Smoking history										
Former/current smoker	258	25.41	0.757			258	30.66	0.451		
Never smoker	254	26.21				254	31.30			
Pathological stage										
I	261	28.19	**0.026**	1.450 (1.265, 1.662)	**≤0.001**	261	31.75	**0.005**		0.370
II	81	25.69				81	30.99			
IIIA	165	22.78				165	30.34			
IIIB	13	20.86				13	29.29			
Tumor size										
≤3 cm	312	26.39	**0.006**		0.583	312	30.64	0.375		
>3 cm	208	25.17				208	31.84			
Regional LN metastasis										
No	312	27.81	**≤0.001**		0.292	312	31.46	**≤0.001**	2.674 (1.862, 3.842)	**≤0.001**
Yes	208	23.04				208	30.61			
Surgical resection										
Pneumonectomy	34	23.30	**0.029**	1.376 (1.070, 1.770)	**0.013**	34	30.57	**0.030**	1.482 (1.087, 2.019)	**0.013**
Lobectomy	466	25.99				466	30.96			
Wedge resection	16	28.39				16	35.49			
Adjuvant treatment										
No	283	25.13	0.761			283	29.21	**0.045**	0.440 (0.304, 0.637)	**≤0.001**
Yes	237	26.82				237	33.41			
CEA										

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Variable	DFS					OS				
	Univariate analysis			Multivariate analysis		Univariate analysis			Multivariate analysis	
	n	Median DFS (mo)	P*	HR (95%CI)	P*	n	Median OS (mo)	P*	HR (95%CI)	P*
≤5.0 ng/ml	314	27.37	≤**0.001**	1.367 (1.046, 1.787)	**0.022**	314	31.46	**0.034**		0.479
>5.0 ng/ml	204	23.65				204	30.63			
SCCA										
≤1.5 ng/ml	480	26.15	0.153			480	31.47	0.126		
>1.5 ng/ml	38	22.82				38	26.85			
Cyfra21–1										
≤3.3 ng/ml	346	26.87	**0.001**	1.421 (1.095, 1.843)	**0.008**	346	31.85	≤**0.001**	1.839 (1.313, 2.575)	≤**0.001**
>3.3 ng/ml	172	23.98				172	29.68			
NSE										
≤15.2 ng/ml	407	25.96	0.205			407	31.05	0.189		
>15.2 ng/ml	110	25.65				110	31.44			

LN – lymph node; CEA – carcinoembryonic antigen;Cyfra21-1 – cytokeratin-19 fragments; SCCA – squamous cell carcinoma antigen; NSE – neuron-specific enolase; DFS – disease-free survival; OS – overall survival; HR – hazard ratio; CI – confidence interval.

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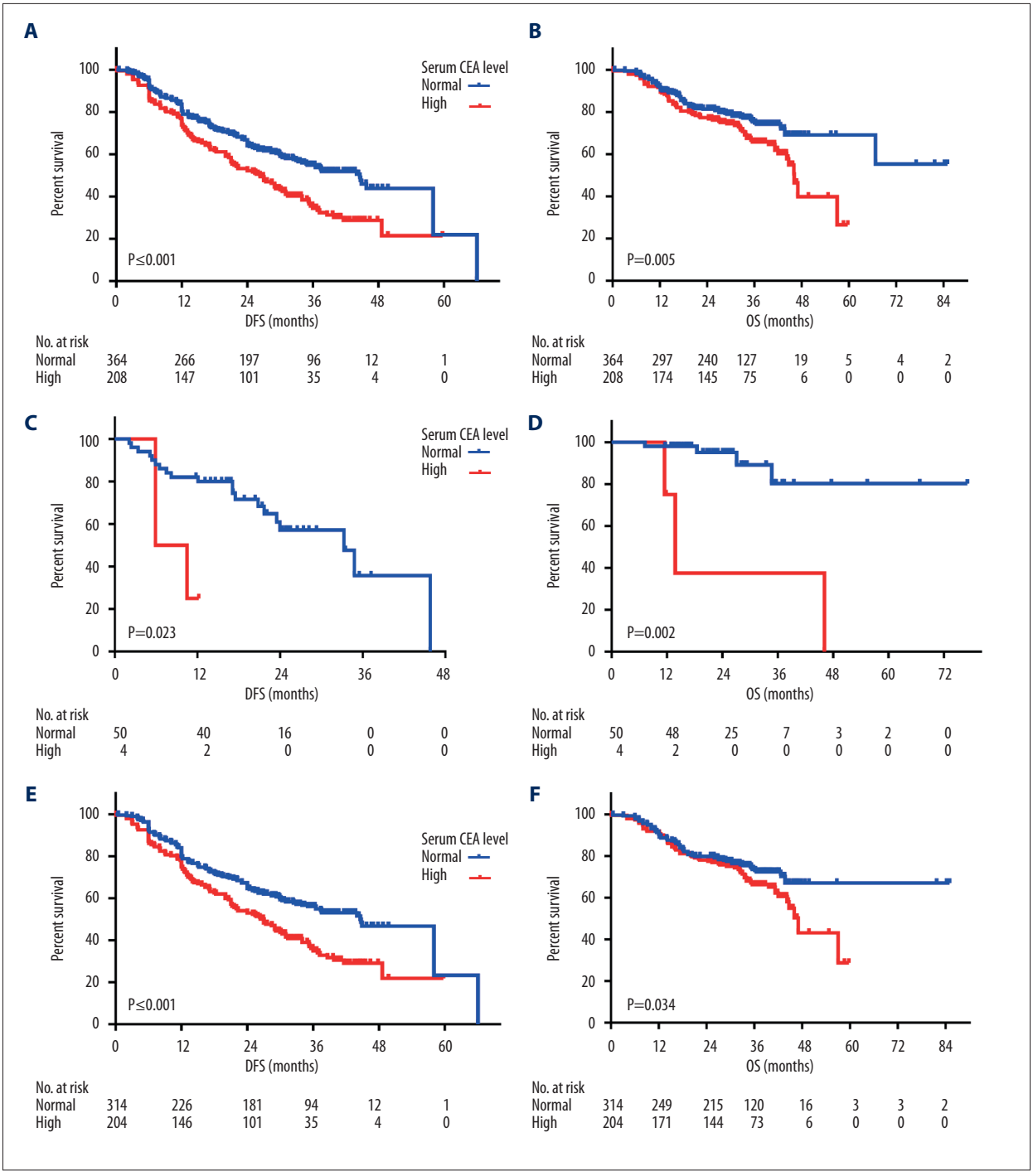

Supplementary Figure 1. Kaplan-Meier survival curves of DFS (**A**) and OS (**B**) according to CEA level in all patients. Kaplan-Meier survival curves of DFS (**C**) and OS (**D**) according to CEA level in ALK-positive patients. Kaplan-Meier survival curves of DFS (**E**) and OS (**F**) according to CEA level in ALK-negative patients.

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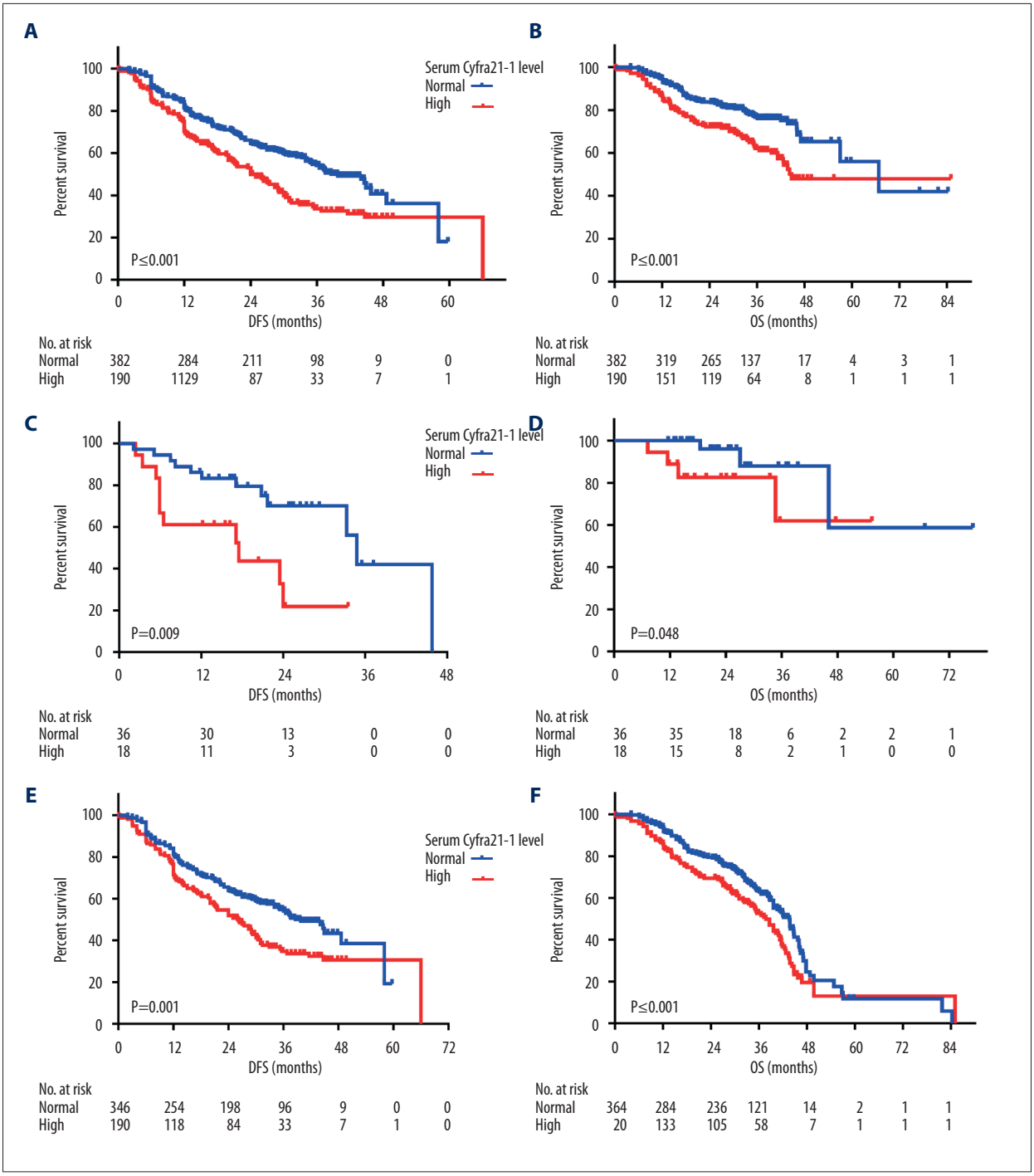

Supplementary Figure 2. Kaplan-Meier survival curves of DFS (**A**) and OS (**B**) according to Cyfra21-1 level in all patients. Kaplan-Meier survival curves of DFS (**C**) and OS (**D**) according to Cyfra21-1 level in ALK-positive patients. Kaplan-Meier survival curves of DFS (**E**) and OS (**F**) according to Cyfra21-1 level in ALK-negative patients.

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