Prognostic Value of Combined Detection of Serum and Pleural Fluid Tumor Markers in Non-small Cell Lung Cancer With Malignant Pleural Effusion

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Research

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Abstract

Background: Serum tumor markers are considered a negative prognostic factor in non-small cell lung cancer (NSCLC). Combined detection of serum and pleural fluid (PF) tumor markers is rarely reported. The aim of this study was to analyze the relationship between combined detection of serum and pleural fluid (SPF) tumor markers and prognosis in advanced NSCLC.

Results: High levels of SPF CEA ($P = 0.001$), SPF CYFRA21-1 ($P = 0.001$) and SPF CA-125 ($P = 0.023$) were adversely prognostic factors for overall survival (OS). High levels of SPF CEA ($P = 0.003$), SPF CYFRA21-1 ($P = 0.001$) and SPF NSE ($P = 0.019$) were related to worse progression-free survival (PFS). In multivariate analysis high levels of SPF CYFRA21-1 was a independent predictor of OS and high levels of SPF CEA and SPF CYFRA21-1 were independent predictors of PFS.

Conclusions: High levels of SPF CEA and SPF CYFRA21-1 are correlated with worse survival in advanced NSCLC patients with MPE. The identification of prognostic factors maybe useful in stratifying high-risk populations and will assist the choice of treatment planning.

Background

Non-small cell lung cancer (NSCLC) remains a leading cause of cancer-related death worldwide, representing about 80% of lung cancer [1,2]. Despite significant advances in public awareness and medical care, more than 50% of patients diagnosed with NSCLC have distant metastases and only 16% of cases are diagnosed at early stages [3]. Several prognostic factors of patients with NSCLC have been reported; however, many factors require invasive examination or surgery [4]. The measurement of serum or pleural fluid (PF) tumor markers are inexpensive method and routinely available.

For lung cancer diagnosis, the related markers are carcinoembryonic antigen (CEA), soluble fragment of cytokeratin 19 (CYFRA21-1) and neuron-specific enolase (NSE). Recent studies have reported that high levels of CEA, CYFRA21-1, NSE and carbohydrate antigen (CA)-125 are associated with poor prognosis in NSCLC [5-7]. CEA is a glycoprotein in carcinoembryonic cell surface, and it has been a widely applied tumor marker in serum and PF. In NSCLC, CEA has been considered a marker for the adenocarcinoma histological subtype [8]. CYFRA21-1 is a soluble fragment of cytokeratin 19 and a primitively expressed epithelial cytokeratin. CA-125 is a mucinous glycoprotein originated from fetal lumen epithelium and it is used for the follow-up of ovarian cancer [9]. NSE is an isozyme of enolase, existed in neuroendocrine tumors. NSE is a sensitive marker in the diagnosis of small cell lung cancer (SCLC), but increased serum NSE has been reported in 11.7% to 28% of patients with NSCLC [5].

However, the findings regarding the prognosis of serum tumor markers have been studied in lung cancer with conflicting results and the relationship between combined detection of serum and PF tumor markers and prognosis in advanced NSCLC is unclear [10-12]. Therefore, the aim of this study is to assess the prognostic value of combined detection of serum and PF tumor markers CEA, CYFRA21-1, CA-125 and NSE in advanced NSCLC.

Methods

Patients

Two hundred and forty four patients diagnosed with NSCLC by thoracoscopy for malignant pleural effusion (MPE) between 2005 and 2017 at a tertiary hospital were retrospectively reviewed. Inclusion criteria was as follows: (i). Inpatients who voluntarily signed informed consent for specimen collection histologically confirmed NSCLC. (ii). Survival status could be determined. The duration of OS was studies from the date of diagnosis to the date of death. Progression-free survival (PFS) was calculated from lung cancer diagnosis to first event (progression or death from any reason). (iii) All clinical data were available. (iv) Patients underwent active anticancer treatment. Docetaxel, gemcitabine, pemetrexed or paclitaxel combined with carboplatin/cisplatin as systemic conventional chemotherapy regimens for NSCLC. Patients with positive epidermal growth factor receptor (EGFR) mutations were treated with gefitinib, erlotinib or afatinib. Patients with positive anaplastic lymphoma kinase (ALK) translocations were treated with crizotinib. Exclusion criteria: (i). Patients with heart, kidney, liver and other disease were at high risk of chemotherapy. (ii). The clinical data were incomplete. (iii). Patients did not receive anticancer treatment. The local ethics committee approved this study.

Sample collection and detection

As part of the routine tests for suspicion of lung cancer, tumor markers were acquired initially at the first visit to the hospital. CEA, CYFRA21-1, CA-125 and NSE were collected in all populations.

PF samples were obtained via thoracocentesis before thoracoscopy and were collected in dry tubes. Venous blood sample were acquired from patients. The samples were centrifuged for 10 min at 1500 g, and stored at -20°C until assayed. The levels of tumor markers were measured with electrochemiluminescence in both PF and serum. The optimal cutoff values of tumor markers were used the cut-off value of hospital routine use. The upper limit of the normal values were at 10.0ng/ml for CEA, 6.0ng/ml for CYFRA21-1, 39.0 U/ml for CA-125 and 16.3 ng/ml for NSE, respectively.

Data collection

Clinicopathological data including age, sex, histology (adenocarcinoma and squamous carcinoma), smoking status and first line treatment programme were assessed for all enrolled patients. From laboratory data, serum tumor markers CEA, CYFRA21-1, CA-125 and NSE and pleural fluid tumor markers CEA, CYFRA21-1, CA-125 and NSE were evaluated.

Statistical analysis

Statistical analysis were performed using statistical software (SPSS version 17.0, Chicago, IL). Continuous variables were presented as means $\pm$ standard deviations or medians. The levels of tumor markers in serum and PF were compared using t-tests. The Kaplan-Meier univariate analysis was employed to determine the significance of variables for OS and PFS. The Cox proportional hazard regression model

was performed for multivariate survival analysis. Log-rank test and Kaplan-Meier analysis were used to determine survival differences. For all analyses, statistical significance was accepted for *P* values less than 0.05.

Results

Baseline characteristics of patients

A total of 335 patients with MPE were histologically confirmed NSCLC. Ninety-one patients were excluded for some reasons, including 45 patients incomplete data, 18 patients lost to follow-up and 28 patient no treatment. Finally, 244 patients were enrolled in this study (Fig 1).

Characteristics of the patients are outlined in Table 1. Their median age was 60 years (31-79 years). Among all patients, 132 (54.1%) were females and 201 (82.4%) were diagnosed with adenocarcinoma. For first line anticancer treatment, 194 (79.5%) patients received traditional systemic chemotherapy and 50 (20.5%) patients underwent targeted therapy. Median PFS and OS were 6.1 months and 12.7 months, respectively (Fig 2). Univariate analyses for OS and PFS are shown in Table 1.

Serum CEA was elevated in 155 of the 244 patients analyzed (63.5%), serum CYFRA21-1 in 137 of 244 patients (56.1%), serum CA-125 in 145 of 244 patients (59.4%), and serum NSE in 135 of 244 patients (55.3%). PF CEA was elevated in 176 of the 244 patients analyzed (72.1%), PF CYFRA21-1 in 175 of 244 patients (71.7%), PF CA-125 in 177 of 244 patients (72.5%), and PF NSE in 138 of 244 patients (55.3%). Serum and pleural fluid (SPF) CEA was elevated in 138 of the 244 patients analyzed (56.6%), SPF CYFRA21-1 in 135 of 244 patients (55.3%), SPF CA-125 in 134 of 244 patients (54.9%), and SPF NSE in 114 of 244 patients (46.7%).

In our population, 44 patients (18.0%) presented the 4 serum tumor markers elevated, 62 patients (25.4%) 3 serum tumor markers elevated, 88 patients (36.1%) 2 serum tumor markers elevated and 35 patients (14.3%) 1 serum tumor markers elevated.

Levels of tumor markers in serum and PF

Table 3 describes the levels of serum and PF tumor markers. Serum levels of CEA, CYFRA21-1, CA-125 and NSE were 79.15 ± 186.36 ng/mL, 9.63 ± 29.36 ng/mL, 156.98 ± 251.65 U/mL and 19.71 ± 34.99 ng/mL, respectively. PF levels of CEA, CYFRA21-1, CA-125 and NSE were 280.96 ± 358.16 ng/mL, 136.85 ± 175.19 ng/mL, 1103.26 ± 1340.92 U/mL and 38.15 ± 75.10 ng/mL, respectively, and were significantly higher than those in serum (all $P < 0.001$).

CEA analysis

High levels of serum CEA was adversely prognostic factors for PFS and OS. The PFS was 6.6 months versus 5.0 months in patients with normal and elevated level of serum CEA ($P = 0.003$) and the OS was 15.0 months versus 12.0 months in patients with normal and high levels of tumor marker ($P = 0.030$). We

analyzed the prognostic value of combination of serum and pleural fluid CEA. High levels of SPF CEA were related to worse PFS and OS. The PFS was 6.6 months versus 5.0 months in patients with normal and elevated level of SPF CEA ($P = 0.003$) and the OS was 15.3 months versus 10.6 months in patients with normal and high levels of tumor marker ($P = 0.001$) (Fig 3).

CYFRA21-1 analysis

We found that abnormal levels of serum CYFRA21-1 and SPF CYFRA21-1 were negative prognostic factors. The PFS was 6.5 months versus 5.3 months in patients with normal and elevated level of serum CYFRA21-1 ($P < 0.001$) and the OS was 16.1 months versus 11.7 months in patients with normal and high levels of tumor marker ($P = 0.004$). The PFS was 6.5 months versus 5.3 months in patients with normal and elevated level of SPF CYFRA21-1 ($P = 0.001$) and the OS was 15.8 months versus 11.1 months in patients with normal and high levels of tumor marker ($P = 0.001$) (Fig 4).

CA-125 analysis

Serum CA-125 and SPF CA-125 were negative prognostic factors for OS. The OS was 15.0 months versus 12.0 months in patients with normal and elevated level of serum CA-125 ($P = 0.043$) and 12.7 months versus 12.0 months in patients with normal and high levels of SPF CA-125 ($P = 0.023$) (Fig 5).

NSE analysis

High levels of SPF NSE was a negative prognostic factor for PFS. The PFS was 6.4 months versus 5.5 months in patients with normal and elevated level of SPF NSE ($P = 0.019$) (Fig 6).

Serum CEA, CYFRA21-1, CA-125 and NSEcombination

We analyzed the prognostic value of combination of serum tumor markers. For patients with 4 tumor markers elevated PFS was 4.0 months versus 5.5 months in patients with 3 tumor markers, 6.4 months in patients with 2 tumor markers, 7.0 months in patients with 1 tumor marker and 6.8 months in patients with all the tumor markers negative ($P = 0.001$). We found the same results for OS in patients with a combination of serum tumor markers ($P = 0.018$) (Fig 7).

Significant variables from univariate analysis were evaluated by multivariate analysis. High levels of SPF CYFRA21-1 (HR 1.581, 95% CI 1.138-2.199; $P = 0.006$) was a independent predictive variable for shorter OS. High levels of SPF CEA (HR 1.426, 95% CI 1.007-2.018; $P = 0.045$) and SPF CYFRA21-1 (HR 1.597, 95% CI 1.181-2.158; $P = 0.002$) were independent predictive variables for shorter PFS. Results are shown in Table 2.

Dissusion

With the progress of molecular biology in recent years, the role of tumor markers in predicting outcome and detecting recurrence of patients with lung cancer has been the subject of extensive researches [13,14]. The results of our study indicated that high levels of SPF CEA and SPF CYFRA21-1 may be used as biomarkers

to predict outcomes in NSCLC with MPE; this conclusion was based on the significant differences in OS and PFS observed in these patients. To the best of our knowledge, this study is the first to analyze the relationship between combined detection of SPF tumor markers and prognosis in advanced NSCLC.

Tumor marker CEA and CYFRA21-1 has been reported as poor prognostic factors in NSCLC. In patients with stage IIIB-IV NSCLC, Arrieta *et al.* showed that patients with elevated serum CEA had shorter OS than patients who had normal levels [15]. In patients with stage I-III NSCLC, Tomita *et al.* observed that elevated CEA in pleural lavage fluid (PLF) was a significant prognostic factor for OS [16]. In the meta-analysis of Zhang *et al.* with 1990 patients, serum CYFRA21-1 and CEA were independent prognosis factors of NSCLC patients [6]. We found that high levels of serum CEA and CYFRA21-1 were unfavorable prognostic factors for survival, but this results were inconsistent with Tokito *et al.* [13]. Interestingly, when combining use of serum and pleural fluid tumor markers, we found that SPF CEA and CYFRA21-1 correlated with prognosis in a significant and independent manner. In other words, high levels of SPF CEA and CYFRA21-1 may be useful noninvasiveness markers to identify advanced NSCLC risk. It is not surprising that the relationship between elevated CEA or CYFRA21-1 levels and prognosis remains controversial. In fact, many studies reporting on the prognosis of tumor markers levels were based on different stages or subtypes. Further prospective validation of our data would be necessary.

Previously, Muley *et al.* introduced the use of a Tumor Marker Index (TMI), based on the combined use of serum CEA and CYFRA21-1 [17]. Muley *et al.* showed that TMI was the prognostic significance in operate early stage of NSCLC, and this is substantiated by Tomita *et al.* [18]. Barlèsi *et al.* analyzed the prognostic value of combination of CEA, CYFRA21-1 and NSE in stage IIIB-IV NSCLC and found that combination of the three markers was a poor prognostic factor [19]. In contrast, Blankenburg *et al.* reported that TMI was not associated with a worse outcome in stage I NSCLC [20]. In the present study, the use of tumor marker indexes including serum CEA, CYFRA21-1, CA-125 and NSE evaluated the prognosis and progression of patients with stage IV NSCLC. We found that combining of the four markers was a poor prognostic factor. The simultaneous use of several serum tumor markers may increase the reliability of prognostic value.

Several reports have been published about the prognostic value of serum CA-125 and NSE. In a random survival forest prognostic model, serum CA-125 was related to a poor outcome in lung adenocarcinoma patients with brain metastasis [21]. Li *et al.* reported that high levels of serum NSE was correlated with worse survival in resected lung adenocarcinoma patients harboring anaplastic lymphoma kinase (ALK) fusion gene rearrangements [7]. We found that serum CA-125 and SPF CA-125 were prognostic risk factors for OS. In our study, serum NSE was not a prognostic factor, but SPF NSE was associated with worse PFS.

We also found that PF levels of CEA, CYFRA21-1, CA-125 and NSE were significantly higher than those in serum. Because of the large molecular weight of tumor markers, they are easier to accumulate in the pleural cavity than the blood. Also, tumor markers in the pleural cavity are not easily inactivated by liver. Hackner *et al.* showed that ratio of CEA in PF and serum was a useful tool for diagnosis of MPE [22].

There are several limitations in our retrospective study. The populations enrolled in the study were suitable for undergoing thoracoscopy and the selection bias could not be completely avoided. In addition, we did

not perform the trials evaluating the use of consecutive measurement of tumor markers during treatment and follow-up. Finally, further prospective study in this area are warranted.

Conclusions

In conclusion, high levels of tumor markers at baseline are correlated with worse survival in advanced NSCLC patients with MPE. The identification of prognostic factors maybe useful in stratifying high-risk populations and will assist the choice of treatment planning.

Availability of data and materials

Abbreviations

NSCLC: non-small cell lung cancer

PF: pleural fluid

SPF: serum and pleural fluid

OS: overall survival

PFS: progression-free survival

CEA: carcinoembryonic antigen

CYFRA21-1: soluble fragment of cytokeratin 19

NSE: neuron-specific enolase

MPE: malignant pleural effusion

EGFR: epidermal growth factor receptor

ALK: anaplastic lymphoma kinase

Declarations

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Contributions: Li-Xu Xie conceived and designed this study and wrote the manuscript. Shi-Chang Jiang and Zi Chen participated in data collection and analysis. Shu-Juan Jiang, Xing-Guang Wang and Lin-Fu Zhou critically guided the revision of the manuscript. All authors read the manuscript and approved it for publication.

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Conflict of interest: The authors report no conflicts of interest.

Ethical approval: This study was approved by the Ethics Committee of the Shandong Provincial Hospital.

All data generated by this study are included in the manuscript.

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Tables

Table 1. Univariate analysis on OS and PFS in NSCLC

Variables	Value	n(%)	Median	95% CI	Median	95% CI	p ^a	p ^b
			OS (months)	OS	PFS (months)	PFS		
NSCLC		244	12.7	10.56-14.84	6.1	5.73-6.87	0.146	0.294
	Adenocarcinoma	201	15.0	12.61-17.39	5.6	4.94-6.26		
	Squamous	43	11.8	9.24-14.36	6.8	5.00-8.60		
Sex							0.069	0.739
	Male	112	11.7	9.54-13.86	5.6	4.72-6.48		
	Female	132	14.5	11.99-17.00	6.4	5.46-7.14		
Age, Median							0.751	0.712
	≤60	114	11.1	7.59-14.61	5.6	4.79-6.41		
	≥60	130	14.2	12.07-16.33	6.3	5.64-6.96		
Smoking							0.196	0.309
	No	136	14.5	12.09-16.91	5.5	4.78-6.22		
	Yes	108	11.7	8.90-14.50	6.5	6.14-6.86		
Treatment							0.136	0.261
	Conventional	194	14.2	11.65-16.75	5.5	4.82-6.18		
	Targeted	50	11.8	8.92-14.69	6.7	5.89-7.51		
Serum CEA							0.030	0.003
	Normal	89	15.0	12.13-17.88	6.6	6.10-7.10		
	Elevated	155	12.0	8.98-15.02	5.0	4.49-5.51		
PF CEA							0.078	0.089

	Normal	68	13.5	9.46-17.54	6.5	5.60-7.40		
	Elevated	176	12.0	8.72-15.29	5.5	4.79-6.21		
Elevated SPF CEA							0.001	0.003
	No	106	15.3	12.95-17.65	6.6	6.18-7.02		
	Yes	138	10.6	7.39-13.81	5.0	4.51-5.49		
Serum CA-125							0.043	0.609
	Normal	99	15.0	12.08-17.93	6.4	5.69-7.11		
	Elevated	145	12.0	8.83-15.17	5.5	5.76-6.24		
PF CA-125							0.829	0.517
	Normal	67	12.5	10.55-14.45	6.5	4.90-7.90		
	Elevated	177	13.5	10.78-16.22	6.1	5.43-6.77		
Elevated SPF CA-125							0.023	0.265
	No	110	12.7	10.30-15.10	6.4	5.59-7.21		
	Yes	134	12.0	8.44-15.57	5.5	4.76-6.24		
Serum CYFRA21-1							0.004	<0.001
	Normal	107	16.1	12.60-19.60	6.5	6.11-6.89		
	Elevated	137	11.7	10.49-12.91	5.3	4.48-6.12		
PF CYFRA21-1							0.620	0.157
	Normal	69	14.5	11.65-17.35	5.0	4.06-5.94		

Elevated	175	12.0	9.53-14.48	6.3	5.74-6.86		
Elevated SPF CYFRA21-1						0.001	0.001
No	109	15.8	13.12-19.08	6.5	6.11-6.89		
Yes	135	11.1	9.90-12.30	5.3	4.49-6.11		
Serum NSE						0.445	0.075
Normal	109	12.7	10.82-14.58	6.4	5.53-7.27		
Elevated	135	13.5	10.21-16.79	5.5	4.48-6.52		
PF NSE						0.312	0.129
Normal	106	12.5	9.22-15.78	6.3	5.33-7.27		
Elevated	138	13.5	10.74-16.26	5.6	4.45-6.75		
Elevated SPF NSE						0.235	0.019
No	130	12.7	10.65-14.75	6.4	5.52-7.28		
Yes	114	15.0	11.97-18.03	5.5	4.51-6.42		
Number of Elevated Serum Tumor Markers						0.018	0.001
0	15	12.7	11.58-13.82	6.8	3.64-9.96		
1	35	16.1	14.75-17.45	7.0	5.84-8.16		
2	88	14.5	11.47-17.53	6.4	5.64-7.16		
3	62	11.8	9.48-14.12	5.5	4.12-6.88		

4	44	6.5	2.17-10.83	4.0	3.14-4.86
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^a The value of overall survival.

^b The value of progression-free survival.

OS, overall survival; PFS, progression-free survival; NSCLC, non-small cell lung cancer; CI, confidence interval; PF, pleural fluid; SPF, serum and pleural fluid.

Table 2. Multivariate analysis on OS and PFS in NSCLC

Predictors	HR	95%CI	HR	95%CI	p ^a	p ^b
	OS	OS	PFS	PFS		
SPF CEA	1.399	0.988-1.980	1.426 0.045	1.007-2.018	0.058	
SPF CYFRA21-1	1.581	1.138-2.199	1.597 0.002	1.181-2.158	0.006	
SPF CA-125	1.233	0.868-1.753		0.242		
SPF NSE			1.279 0.127	0.933-1.752		
Number of elevated serum tumor markers	1.018	0.851-1.219	1.024 0.810	0.844-1.242	0.845	

^a The value of overall survival.

^b The value of progression-free survival.

OS, overall survival; PFS, progression-free survival; NSCLC, non-small cell lung cancer; HR, hazard ratio; CI, confidence interval; SPF, serum and pleural fluid.

Table 3. Levels of tumor markers in serum and pleural fluid

Types	CEA (ng/mL)	CA-125 (U/mL)	CYFRA21-1 (ng/mL)	NSE (ng/mL)
Serum (Mean ± SD)	79.15±186.36	156.98±251.65	9.63±29.36	19.71±34.99
Pleural fluid (Mean ± SD)	280.96±358.16□	1103.26±1340.92□	136.85±175.19□	38.15±75.10□

□ $p < 0.001$, vs serum tumor markers.

Figures

Figure 1

Flowchart of patient numbers

Figure 2

Kaplan-Meier curve demonstrating the survival of the non-small cell lung cancer patients. (a) The median survival for progression-free survival (PFS) was 6.1 months. (b) The median survival for overall survival (OS) was 12.7 months

Figure 3

Kaplan-Meier curves demonstrating the relationship of CEA with survival in non-small cell lung cancer patients. (a) Progression-free survival (PFS) probability in patients with elevated vs. normal serum CEA (P = 0.003). (b) Overall survival (OS) probability in patients with elevated vs. normal serum CEA (P = 0.030). (c) PFS probability in patients with elevated vs. normal combined detection of serum and pleural fluid (SPF) CEA (P = 0.003). (d) OS probability in patients with elevated vs. normal SPF CEA (P = 0.001)

Figure 4

Kaplan-Meier curves demonstrating the relationship of CYFRA21-1 with survival in non-small cell lung cancer patients. (a) Progression-free survival (PFS) probability in patients with elevated vs. normal serum CYFRA21-1 ($P < 0.001$). (b) Overall survival (OS) probability in patients with elevated vs. normal serum CYFRA21-1 ($P = 0.004$). (c) PFS probability in patients with elevated vs. normal combined detection of serum and pleural fluid (SPF) CYFRA21-1 ($P = 0.001$). (d) OS probability in patients with elevated vs. normal SPF CYFRA21-1 ($P = 0.001$)

Figure 5

Kaplan-Meier curves demonstrating the relationship of CA-125 with survival in non-small cell lung cancer patients. (a) overall survival (OS) probability in patients with elevated vs. normal serum CA-125 ($P = 0.043$). (b) OS probability in patients with elevated vs. normal combined detection of serum and pleural fluid (SPF) CA-125 ($P = 0.023$)

Figure 6

Kaplan-Meier curves demonstrating the relationship of combined detection of serum and pleural fluid (SPF) NSE with progression-free survival (PFS) in non-small cell lung cancer patients ($P = 0.019$)

Figure 7

Kaplan-Meier curves demonstrating the relationship of number of elevated serum tumor markers with progression-free survival (PFS) (a, $P = 0.001$) and overall survival (OS) (b, $P = 0.018$)