Diagnostic and Prognostic Significance of Serum Tumor Markers in Patients With Lung Cancer

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Abstract

Background: Tumor markers CEA, CYFRA21-1, NSE, proGRP, and SCC-Ag were routinely used for lung cancer. The objective of this study was to evaluate the diagnostic and prognostic value of these markers.

Methods: The levels of 5 serum tumor markers were measured in 255 patients with lung cancer (44 squamous cell carcinoma, 183 adenocarcinoma, 28 small cell lung cancer), 103 patients with benign lung disease, and 120 healthy controls. The relevant clinical data of the patients were collected to analyze the expression of CEA, CYFRA21-1, NSE, proGRP, and SCC-Ag in the serum of lung cancer and their correlation with prognosis.

Results: The positive rates and expression levels of CEA, CYFRA21-1, NSE, proGRP, and SCC-Ag in the lung cancer group were higher than those in patients with benign lung diseases and healthy controls. CYFRA21-1 and SCC-Ag were highly expressed in lung squamous cell carcinoma, and NSE and proGRP were highly expressed in small cell lung cancer. The expression levels of CYFRA 21-1, NSE, and proGRP in small cell lung cancer were higher than those in non-small cell lung cancer. The levels of CEA, CYFARA21-1, NSE, and proGRP were linearly correlated with the occurrence of lung cancer, while the level of SCC-Ag was not significantly correlated with the occurrence of lung cancer. What's more, the levels of CYFAR21-1 and NSE in the death group of lung cancer patients were higher than those in the survival group.

Conclusion: CEA, CYFRA21-1, NSE, proGRP, and SCC-Ag played a good role in diagnosing lung cancer. CYFRA21-1 and SCC-Ag can be used as markers for the diagnosis of squamous cell carcinoma, and NSE and proGRP can be used as markers for small cell lung cancer. Meanwhile, the combined detection of CEA, CYFARA21-1, NSE, and proGRP had the highest diagnostic accuracy for lung cancer. Furthermore, CYFAR21-1 and NSE can be used to evaluate the prognosis of lung cancer patients.

1. Introduction

Lung cancer is one of the most common respiratory malignancies, with high global morbidity and mortality (1). Recently, an estimation by the American Cancer Society suggested that lung cancer would continue to be the leading cause of cancer-related deaths, and the number of new lung cancer cases will be the second-highest among all types of cancer in the United States (2). In China, lung cancer is the cancer with the highest morbidity and mortality (3). It is estimated that more than 20% of Chinese cancer patients died of lung cancer (4). Lung cancer has been pathologically subdivided into two subsets: small cell lung cancer or SCLC and non-small cell lung cancer or NSCLC. NSCLC is further classified into three groups: squamous cell carcinoma (25%), adenocarcinoma (40%), and large cell carcinoma (10%) (5). The treatment of SCLC is mainly palliative treatment, while for NSCLC, conventional surgery is curative in the early stage (6). Despite advances in modern diagnostic, staging, and therapeutic modalities, the overall 5-year survival rate is 18%. Such a low survival rate is mainly due to diagnosis in advanced stages, since the survival rates can be higher (55%) in early-stage disease but much lower (4%) in advanced stages (7). Imaging examinations such as computed tomography (CT), chest X-ray, positron emission tomography-CT (PET-CT), and magnetic resonance imaging (MRI), cannot be performed frequently and are expensive, whereas blood-based biomarker tests are economically acceptable and can be performed easily and quickly (8). Tumor marker detection is an effective, convenient, and simple auxiliary method that can be used for lung cancer diagnosis and can minimize trauma. What's more, it plays an important role in the early diagnosis of lung cancer, assessing pathological types, tumor staging, monitoring recurrence or metastasis, evaluating curative effect, and guiding prognosis (9). Commonly used primary lung cancer markers currently recommended by the American Society for Clinical Biochemistry and the European Tumor Marker Group include carcinoembryonic antigen (CEA), neuron-

specific enolase (NSE), cytokeratin fragment (CYFRA21-1), progastrin releasing peptide precursor (proGRP), and squamous cell carcinoma antigen (SCC-Ag) (10). In this study, we detected the levels of five tumor markers in patients with lung cancer, patients with benign lung diseases, and healthy controls, and analyzed their diagnostic value in different types of lung cancer and their relationship with prognosis.

2. Materials And Methods

2.1 Patients and control subjects

Medical records, laboratory, and histopathological data (from the hospital information system) were retrospectively collected from patients with lung cancer, benign lung diseases, and healthy subjects who visited the Second Affiliated Hospital of Wenzhou Medical University from April 2016 to May 2020. All patients with lung cancer were histopathologically or cytologically confirmed and had no other malignancies. Patients with benign lung diseases were diagnosed with pulmonary nodules, pulmonary infection, bronchiectasis with infection, bronchial asthma, acute exacerbation of chronic obstructive pulmonary disease, pulmonary tuberculosis, tuberculous pleurisy, silicosis, and pleural effusion by standard diagnostic methods or histological examination, and patients often had one or more benign lung diseases. The Examination Center of the Second Affiliated Hospital of Wenzhou Medical University obtained data of healthy people who did not show a history of tumors, did not show tumors in imaging, and had normal liver and kidney function.

2.2 Sample collection and measurement

Routine venous blood sampling collected about 5 mL, which was placed in an orange-cap vacuum blood collection tube containing separation gel, and transported to the laboratory within 2 hours. After the samples were received, they were centrifuged at 3500 rpm for 10 min to separate into the serum. The whole process was strictly controlled to avoid hemolysis. The sera were stored at -80 ℃ until analysis.

The concentration of CEA, CYFRA21-1, NSE, and proGRP was measured on a COBAS e602 analyzer by electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany). The concentration of SCC-Ag was determined on the Abbott Architect I1000 Luminescence Analyzer by the chemiluminescence method (Abbott Diagnostics, Abbott Park, IL, USA). All the reagents used were instrument kit reagents, and all operations were performed strictly according to the instrument and kit instructions.

2.3 Data analysis and definition

The cut-off value suggested by the kit was used as the criterion for negative or positive. When the serum concentration exceeded this reference range, the result was positive. The normal ranges of CEA, CYFRA21-1, NSE, proGRP, and SCC-Ag were 0-4.70 ng/mL, 0.10-3.30 ng/mL, 0-16.30 ng/mL, 0-69.2 pg/mL, and 0-1.50 ng/mL, respectively.

2.4 Statistical analysis

Statistical analyses were performed using SPSS 26.0 (SPSS Inc., Chicago, IL, USA). If the measurement data obeyed the normal distribution, they were expressed in the form of mean ± standard deviation. The differences between two groups were compared by t-test, and the differences among three groups were compared by analysis of variance. If data did not obey the normal distribution, median and upper and lower quartiles were used for expression. The Mann-Whitney U test was used to compare differences between two groups, and the Kruskal-Wallis H test was used to compare differences among three groups. The count data were expressed in terms of the number of cases and

the composition ratio, and the composition ratio of two or more groups was compared using the chi-squared test. The analysis of influencing factors adopted the unconditional logistic regression model. The diagnostic value of each index was evaluated by drawing receiver operating characteristic (ROC) curves. Statistical significance was set at $p < 0.05$, and all reported p-values were two-sided.

3. Results

3.1 Classification and composition of lung cancer patients

This study included 255 lung cancer patients (53.4%), 103 patients with benign lung diseases (21.5%), and 120 healthy controls (25.1%). Among lung cancer patients, 44 had lung squamous cell carcinoma (17.3%), 183 had lung adenocarcinoma (71.8%), and 28 had small cell lung cancer (SCLC) (11.1%) (Table 1).

Table 1
The sample size in this study

Group	Number of cases (n)	Composition ratio (%)
Lung cancer group	255	53.4
Benign group	103	21.5
Healthy control group	120	25.1
Total	478	100.0
Sample size of different types of lung cancer		

3.2 Comparison of tumor markers in lung cancer, benign lung diseases, and healthy controls

The positive rates, expression levels and the median concentrations of serum CEA, CYFRA21-1, NSE, proGRP, and SCC-Ag in patients with lung cancer were significantly higher than those in patients with benign lung diseases and healthy controls ($p< 0.001$). Besides, the positive rates of CYFRA21-1, CEA, NSE, SCC-Ag and proGRP were 58%, 43.5%, 31.3%, 19.8%, and 17.3%, respectively, and were not related to the patient's sex, ALT, and AST levels ($p>0.05$) (Table 2). What's more, there was a difference in age among the three groups ($p<0.001$), which might have been caused by confounding factors. Therefore, it's necessary to be corrected in a multivariate model.

Table 2
Comparison of various indicators in different groups

Index	Lung cancer group (n=262)	Benign group (n=103)	Healthy control group (n=120)	Statistics	P value
Gender					
Male	167(63.7)	53(51.5)	68(56.7)	5.113	0.078
Female	95(36.3)	50(48.5)	52(43.3)		
Age [M(P25，P75)	63.00 (56.00,71.00) ##**	60.00 (47.00,69.00) **	42.00 (36.00,50.00)	152.395	<0.001
Hb(±s)	127.45±18.15**	130.21±18.74**	144.87±14.98	41.354	<0.001
CEA					
Normal	148(56.5) ##**	99(96.1) *	120(100.0)	114.332	<0.001
Abnormal	114(43.5)	4(3.9)	0		
CEA[M(P25，P75)	3.75(2.02,12.83) ##**	1.82(1.34,2.79) **	1.35(0.88,2.01)	135.906	<0.001
CYFRA211					
Normal	110(42.0) ##**	90(87.4)	108(90.0)	114.032	<0.001
Abnormal	152(58.0)	13(12.6)	12(10.0)		
CYFRA211[M(P25，P75)	3.77(2.33,8.36) ##**	2.08(1.40,2.84)	1.99(1.55,2.47)	120.804	<0.001
NSE					
Normal	180(68.7) ##**	88(85.4) **	116(96.7)	42.145	<0.001
Abnormal	82(31.3)	15(14.6)	4(3.3)		
NSE[M(P25，P75)	13.29(11.01,18.23) ##**	11.15(9.74,14.03)	11.27(10.28,13.04)	44.803	<0.001
proGRP					
Normal	161(82.1) ##**	103(100.0)	120(100.0)	43.451	<0.001
Abnormal	35(17.9)	0	0		
proGRP[M(P25，P75)	46.51(36.66,62.64) ##**	36.65(28.15,44.66) *	38.05(32.92,47.07)	56.704	<0.001
SCC					
Normal	178(80.2) #**	90(90.0)	113(95.8)	17.408	<0.001
Abnormal	44(19.8)	10(10.0)	5(4.2)		
SCC[M(P25，P75)	0.90(0.60,1.30) ##*	0.70(0.50,1.00) *	0.80(0.60,1.00)	17.781	<0.001

Index	Lung cancer group (n=262)	Benign group (n=103)	Healthy control group (n=120)	Statistics	P value
ALT[M(P25，P75)	16.00(12.00,24.00) *	19.00(12.25,27.75)	19.00(12.75,28.00)	6.027	0.049
AST[M(P25，P75)	19.00(16.00,24.00)	20.00(16.00,26.00)	20.50(17.00,24.00)	1.816	0.403
BUN[M(P25，P75)	5.45(4.30,6.50) #**	4.97(3.90,6.08)	4.70(4.00,5.70)	17.284	<0.001
CREA[M(P25，P75)	61.20(53.00,72.00) ##	54.60(47.55,65.00) **	62.00(52.45,72.00)	17.697	<0.001

3.3 The diagnostic value of tumor markers in different types of lung cancer

Patients with different pathological types of lung cancer showed differences in serum CYFRA21-1, NSE, proGRP, and SCC-Ag levels and positive rates. CEA levels were not statistically significant among the three groups (p>0.05). The level of CYFRA21-1 in lung squamous cell carcinoma (median 6.26) was higher than that in lung adenocarcinoma (median 3.60) and small cell lung cancer (median 5.64) (p<0.05), while the levels of NSE and proGRP in small cell lung cancer (median 535.85) were significantly higher than those in lung squamous cell carcinoma (median 42.30) and lung adenocarcinoma (median 44.34) (p <0.001). The level of SCC-Ag in the squamous cell carcinoma group (median 2.20) was higher than that in the lung adenocarcinoma (median 0.80) and small cell carcinoma (median 0.75) groups (p<0.001) (Table 3), while compared to the small cell lung cancer group, there was no statistically significant difference in the level of SCC-Ag in the non-small cell lung cancer group (p>0.05) (Table 4). The levels of CYFRA 21-1, NSE, and proGRP levels in the small cell lung cancer group (median: 5.64, 32.50, 535.85, respectively) were significantly higher than those in the non-small cell lung cancer group (median: 3.71, 13.20, 44.33, respectively) (p <0.05) (Table 4).

Table 3
Comparison of various indexes of different lung cancer pathological types

Index	Squamous cell carcinoma (n=44)	Adenocarcinoma (n=183)	Small cell lung cancer (n=28)	Statistics	P value
Gender					
Male	39(88.6)	98(53.6)	25(89.3)	27.851	<0.001
Female	5(11.4)	85(46.4)	3(10.7)		
Age[M(P25，P75)	65.00 (58.00,71.75)	63.00 (55.00,71.00)	65.00 (61.00,70.00)	2.349	0.309
Smoking					
Yes	33(75.0)	56(30.8)	18(64.3)	34.772	<0.001
No	11(25.0)	126(69.2)	10(35.7)		
CEA					
normal	31(70.5)	96(52.5)	16(57.1)	4.678	0.096
abnormal	13(29.5)	87(47.5)	12(42.9)		
CEA[M(P25，P75)	3.58(1.86,4.99)	4.09(2.02,19.25)	3.53(2.07,10.27)	4.037	0.133
CYFRA211					
normal	14(31.8)	84(45.9)	7(25.0)	6.303	0.043
abnormal	30(68.2)	99(54.1)	21(75.0)		
CYFRA211[M(P25，P75)	6.26(2.57,18.90)	3.60(2.15,6.87)	5.64(3.26,12.60)	12.420	0.002
NSE					
normal	32(72.7)	135(73.8)	7(25.0)	27.144	<0.001
abnormal	12(27.3)	48(26.2)	21(75.0)		
NSE[M(P25，P75)	13.24(11.31,16.66)	13.19(10.92,16.79)	32.50(15.37,83.28)	21.885	<0.001
proGRP					
normal	29(90.6)	123(88.5)	5(25.0)	50.031	<0.001
abnormal	3(9.4)	16(11.5)	15(75.0)		
proGRP[M(P25，P75)	42.30(36.36,54.36)	44.34(36.33,57.80)	535.85(66.53,2924.25)	24.969	<0.001
SCC					
normal	15(40.5)	136(88.3)	23(95.8)	48.001	<0.001
abnormal	22(59.5)	18(11.7)	1(4.2)		
SCC[M(P25，P75)	2.20(0.95,3.60)	0.80(0.60,1.10)	0.75(0.60,0.98)	35.922	<0.001

Table 4
Comparison of non-small cell lung cancer group (combination of squamous cell carcinoma and adenocarcinoma) and small cell lung cancer group

Index	Non-small cell lung cancer group(n=227)	Small cell lung cancer group(n=28)	Statistics	P value
Gender				
Male	137(60.4)	25(89.3)	9.006	0.003
Female	90(39.6)	3(10.7)		
Age[M(P25，P75)	63.00(56.00,71.00)	65.00(61.00,70.00)	-1.231	0.218
Smoking				
Yes	137(60.6)	10(35.7)	6.338	0.012
No	89(39.4)	18(64.3)		
CEA				
Normal	127(55.9)	16(57.1)	0.014	0.904
Abnormal	100(44.1)	12(42.9)		
CEA[M(P25，P75)	3.78(2.02,14.26)	3.53(2.07,10.27)	-0.257	0.797
CYFRA211				
Normal	98(43.2)	7(25.0)	3.398	0.065
Abnormal	129(56.8)	21(75.0)		
CYFRA211[M(P25，P75)	3.71(2.28,7.87)	5.64(3.26,12.60)	-2.038	0.042
NSE				
Normal	167(73.6)	7(25.0)	27.127	<0.001
Abnormal	60(26.4)	21(75.0)		
NSE[M(P25，P75)	13.20(10.96,16.72)	32.50(15.37,83.28)	-4.675	<0.001
proGRP				
Normal	152(88.9)	5(25.0)	49.950	<0.001
Abnormal	19(11.1)	15(75.0)		
proGRP[M(P25，P75)	44.33(36.33,56.39)	535.85(66.53,2924.25)	-5.912	<0.001
SCC				
Normal	151(79.1)	23(95.8)	3.888	0.049
Abnormal	40(20.9)	1(4.2)		
SCC[M(P25，P75)	0.90(0.60,1.30)	0.75(0.60,0.98)	-1.834	0.067

3.4 The relationship between the level of markers and the occurrence of lung cancer

The quartile range of the independent variable were divided into four groups and introduced into the model as a grade variable (Table 5). After quartiles were divided, a non-conditional logistic regression model was constructed. The higher the levels of CEA, CYFRA21-1, NSE, and proGRP, the higher the incidence of lung cancer; on the contrary, the level of SCC-Ag was not correlated with the occurrence of lung cancer (p>0.05) (Table 6).

Table 5
Comparison of CEA, CYFRA211, NSE, proGRP, SCC between lung cancer group and benign group (indices are grouped according to quartiles)

Index	Group	n (%)	Lung cancer group (n=262)	Benign group (n=103)	Statistics	P value
CEA	Q1(<=1.70)	92(25.2)	49(18.7)	43(41.7)	58.695	<0.001
	Q2(1.71-2.86)	91(24.9)	54(20.6)	37(35.9)		
	Q3(2.87-7.94)	91(24.9)	68(26.0)	23(22.3)		
	Q4(>7.94)	91(24.9)	91(34.7)	0		
CYFRA211	Q1(<=1.97)	92(25.2)	44(16.8)	48(46.6)	60.738	<0.001
	Q2(1.98-3.04)	92(25.2)	59(22.5)	33(32.0)		
	Q3(3.05-6.10)	90(24.7)	70(26.7)	20(19.4)		
	Q4(>6.10)	91(24.9)	89(34.0)	2(1.9)		
NSE	Q1(<=10.45)	91(24.9)	47(17.9)	44(42.7)	28.843	<0.001
	Q2(10.46-12.76)	92(25.2)	72(27.5)	20(19.4)		
	Q3(12.77-16.74)	91(24.9)	65(24.8)	26(25.2)		
	Q4(>16.74)	91(24.9)	78(29.8)	13(12.6)		
proGRP	Q1(<=34.19)	75(25.1)	31(15.8)	44(42.7)	42.534	<0.001
	Q2(34.20-42.35)	75(25.1)	47(24.0)	28(27.2)		
	Q3(42.36-53.98)	75(25.1)	50(25.5)	25(24.3)		
	Q4(>53.98)	74(24.7)	68(34.7)	6(5.8)		
SCC	Q1(<=0.60)	102(31.7)	57(25.7)	45(45.0)	14.237	0.003
	Q2(0.61-0.80)	65(20.2)	44(19.8)	21(21.0)		
	Q3(0.81-1.20)	84(26.1)	65(29.3)	19(19.0)		
	Q4(>1.20)	71(22.0)	56(25.2)	15(15.0)		

Table 6
Correlation model of CYFRA211, NSE, proGRP, SCC and lung cancer

Model	OR(95%CI)				P Value for Trend
	Q1	Q2	Q3	Q4	
Model one *: CEA	1.000		4.319 (2.451-7.610)		<0.001
P Values			<0.001		
Model two: CYFRA211	1.000	1.883 (0.980-3.620)	3.036 (1.509-6.108)	34.256 (7.744-151.535)	<0.001
P Values		0.058	0.002	<0.001	
Model three: NSE	1.000	3.431 (1.695-6.946)	2.288 (1.165-4.495)	4.629 (2.155-9.944)	<0.001
P Values		0.001	0.016	<0.001	
Model four: proGRP	1.000	2.253 (1.119-4.536)	2.469 (1.194-5.103)	12.328 (4.283-35.479)	<0.001
P Values		0.023	0.015	<0.001	
Model five: SCC	1.000	1.188 (0.591-2.385)	1.679 (0.823-3.425)	1.463 (0.682-3.139)	0.193
P Values		0.629	0.155	0.328	

Note: The models have been corrected for age, BUN and CREA. *The index control group is <=median, and the comparison group is> median.

3.5 The combination of biomarkers in diagnosis of lung cancer

The ROC curve was used to analyze the diagnostic efficacy of these four risk factors alone or in combination with the diagnosis of lung cancer (Figures 1-4). The areas under the curves of CEA, CYFARA21-1, NSE, and proGRP were arranged from largest to smallest: CEA (0.746) = CYFARA21-1 (0.746) > proGRP (0.734) > NSE (0.631). We confirmed the critical value according to the principle of the largest Youden index, and then calculated the sensitivity and specificity: CEA 3.53 ng/mL, diagnostic sensitivity 51.50%, specificity 89.30%; CYFARA21-1 3.24 ng/mL, diagnostic sensitivity 55.10%, specificity 87.50%; NSE 10.86 ng/mL, diagnostic sensitivity 75.50%, specificity 47.60%; proGRP 47.91 pg/mL, diagnostic sensitivity 49.50%, specificity 84.50%. Among the single tumor markers, CYFRA21-1 had the highest diagnostic value in the lung cancer group, with a sensitivity of 55.10% and a Youden index of 0.426. The CEA+CYFRA21-1 lung cancer group had the highest diagnostic value in the combination of two tumor markers, with a sensitivity of 59.50% and a Youden index of 0.550. Among the three tumor markers, the CEA+CYFRA21-1+proGRP lung cancer group had the highest diagnostic value, with a sensitivity of 61.20% and a

Youden index of 0.583. When the four tumor markers were combined, the sensitivity was 64.30%, the specificity was 93.20%, and the Youden index was 0.575 (Table 7).

Table 7 The area under the ROC curve of the diagnostic value of various indicators for lung cancer

Index	AUC(95%CI)	Cutoff	Sensitivity	Specificity	Youden
CEA	0.746(0.692-0.800)	3.53	51.50%	89.30%	0.408
CYFRA211	0.746(0.692-0.801)	3.24	55.10%	87.50%	0.426
NSE	0.631(0.565-0.697)	10.86	75.50%	47.60%	0.231
proGRP	0.734(0.677-0.791)	47.91	49.50%	84.50%	0.340
CEA+CYFARA211	0.796(0.748-0.845)	0.78	59.90%	95.10%	0.550
CEA+NSE	0.764(0.712-0.817)	0.67	63.00%	83.50%	0.465
CEA+proGRP	0.822(0.777-0.868)	0.83	51.00%	99.00%	0.500
CYFARA211+NSE	0.749(0.695-0.803)	0.72	57.30%	87.40%	0.447
CYFARA211+proGRP	0.819(0.773-0.865)	0.72	61.20%	90.30%	0.515
NSE+proGRP	0.746(0.690-0.801)	0.79	41.80%	94.20%	0.360
CEA+CYFARA211+NSE	0.799(0.751-0.847)	0.78	60.70%	94.20%	0.549
CEA+CYFARA211+proGRP	0.848(0.807-0.890)	0.76	61.20%	97.10%	0.583
CEA+NSE+proGRP	0.826(0.782-0.871)	0.83	53.10%	100.00%	0.531
CYFARA211+NSE+proGRP	0.819(0.774-0.865)	0.72	58.70%	91.30%	0.500
CEA+CYFARA211+NSE+proGRP	0.850(0.809-0.892)	0.74	64.30%	93.20	0.575

Abbreviations: ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval. There is no diagnostic value when AUC<0.5, the diagnostic value is low when AUC is 0.5-0.7, the diagnostic value is moderate when AUC is 0.7-0.9, and the diagnostic value is higher when AUC>0.9. Sensitivity: number of true positive cases / (number of true positive cases + number of false negative cases), specificity: number of true negative cases / (number of true negative cases + number of false positive cases); Youden index: (sensitivity + specificity)- 1.

3.6 The combination of biomarkers in the diagnosis of small cell lung cancer

We used the ROC curve to analyze the diagnostic efficacy of a single index or combined detection for small cell lung cancer (Figures 5-6). The diagnostic value of CYFARA21-1 combined with proGRP, NSE combined with proGRP, and the three combined detections of small cell lung cancer (AUC of 0.928, 0.925, and 0.924, respectively) was better than that of proGRP alone (AUC of 0.904) (Table 8).

Table 8
The area under the ROC curve of indicators for the diagnosis of small cell carcinoma

Index	AUC(95%CI)
CYFRA211	0.665(0.555-0.775)
NSE	0.782(0.636-0.927)
proGRP	0.904(0.827-0.982)
CYFARA211+NSE	0.786(0.650-0.922)
CYFARA211+proGRP	0.928(0.855-1.002)
NSE+proGRP	0.925(0.840-1.010)
CYFARA211+NSE+proGRP	0.924(0.838-1.010)

Abbreviations: ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval. There is no diagnostic value when AUC<0.5, the diagnostic value is low when AUC is 0.5-0.7, the diagnostic value is moderate when AUC is 0.7-0.9, and the diagnostic value is higher when AUC>0.9.

3.7 Tumor markers play an important role in guiding the prognosis

The postoperative follow-up results of lung cancer patients undergoing surgery (excluding those who lost follow-up) showed that a total of 59 lung cancer patients survived and 9 died. There was no significant difference in gender, age, CEA, proGRP, and SCC-Ag between the survival group and the death group (p>0.05), indicating that the survival of lung cancer patients after surgery was not related to these factors. However, we found that the levels of CYFAR21-1 and NSE in the survival group were significantly lower than those in the death group. (p<0.05), indicating that CYFAR21-1 and NSE had a certain value in prognostic evaluation (Table 9).

Table 9
Comparison of various indicators of lung cancer survival group and death group

Index	Survival group (n=59)	Death group (n=9)	Statistics	P value
Gender				
Male	32(54.2)	7(77.8)	1.890	0.282
Femal	27(45.8)	2(22.2)		
Age[M(P25，P75)	60.00(53.00,67.00)	65.00(61.00,73.00)	-1.675	0.094
CEA[M(P25，P75)	2.28(1.68,3.53)	1.99(1.02,40.57)	-0.299	0.765
CYFRA211[M(P25，P75)	2.49(2.00,3.61)	5.72(3.06,6.60)	-2.706	0.007
NSE[M(P25，P75)	12.23(10.54,14.42)	17.67(13.76,24.80)	-2.669	0.008
proGRP[M(P25，P75)	41.20(34.63,51.98)	68.00(29.49,1025.93)	-0.633	0.527
SCC[M(P25，P75)	0.80(0.60,1.03)	1.40(0.75,2.95)	-1.882	0.060

Discussion

In recent years, with the deterioration of the environment and the aging of the population, the occurrence of various respiratory diseases is increasing. Lung cancer is a common respiratory malignant tumor that occurs in the bronchial mucosal epithelium and has become one of the main diseases endangering human life (11). Serum tumor markers are considered to be biological indicators detected from the serum or plasma of patients with suspicious tumors, which are used for early diagnosis, treatment monitoring, and prognostic evaluation(12). However, due to the insufficient sensitivity and specificity of tumor markers, the combined detection of multiple tumor markers has greater significance in tumor diagnosis (13).

CEA is a serum glycoprotein, which was first discovered by Gold and Freedman in gastrointestinal cancer cells in 1965 (14). A great number of studies have shown that the expression of CEA is elevated in patients with lung cancer, especially lung adenocarcinoma(15, 16). Our study showed that the positive rate and level of CEA were significantly higher in patients with lung cancer than in patients with benign lung diseases and healthy controls. However, there was no statistically significant difference in CEA levels among the three types of lung cancer ($p>0.05$).

CYFRA 21-1, a member of the keratin family, is a protein encoded by the KRT19 gene (17). Studies have shown that CYFRA21-1 is the most sensitive biomarker in NSCLC, especially for squamous cell carcinoma (18). Our results showed that the positive rate and level of CYFRA21-1 were significantly higher in patients with lung cancer than in those with benign lung diseases and healthy controls. We also found that the level of CYFRA21-1 in the lung squamous cell carcinoma group was higher than that in the lung adenocarcinoma and lung small cell carcinoma groups.

NSE, a form of glycolytic enolase isoenzyme, is considered to be a multifunctional protein (19). Serum NSE level is associated with melanoma (20), seminoma (21), renal cell carcinoma (22), immature teratoma (23) and malignant pheochromocytoma (24), particularly SCLC (25). Therefore, NSE is generally considered a diagnostic and therapeutic marker of SCLC(26). ProGRP is composed of three isoforms expressed at the mRNA level and is the neuropeptide gastrin-releasing peptide produced in SCLC cells (27). Besides, serum ProGRP elevations are specific to SCLCs, pulmonary carcinoid tumors, and several types of neuroendocrine tumors(28). Combined with NSE, proGRP plays an important role in the diagnosis of tumors and follow-up of small cell lung cancer (29). In this study, the positive rates and expression levels of serum NSE and proGRP in patients with lung cancer were significantly higher than those in patients with benign lung diseases and healthy controls. The levels of NSE and pro-GRP in the lung small cell carcinoma group were higher than those in the lung squamous cell carcinoma and lung adenocarcinoma groups, similar to the results reported by Cavalieri, S. et al. (30). In addition, our research showed that the diagnostic value of CYFARA21-1 combined with proGRP, NSE combined with proGRP, and the three indicators combined to detect small cell lung cancer was better than that of proGRP alone.

SCC-Ag is a tumor-associated protein used as a biomarker for a variety of human squamous cell carcinomas, including oral cancer (31), cervical cancer (32), esophageal cancer (33), and lung cancer (34). We found that the positive rate and level of SCC-Ag in patients with lung cancer were higher than those in patients with benign lung diseases and healthy controls, and the level of SCC-Ag in patients with squamous cell carcinoma was higher than in those with lung adenocarcinoma and lung squamous cell carcinoma; thus, SCC-Ag can be used as a diagnostic marker for lung squamous cell carcinoma.

This study analyzed the influence factors of lung cancer through an unconditional logistic regression model, and found that after quartiles, the higher the level of CEA, CYFRA21-1, NSE, and proGRP, the higher the incidence of lung cancer. This indicates that the levels of CEA, CYFARA21-1, NSE, and proGRP were linearly related to the occurrence

of lung cancer, while the level of SCC-Ag was not significantly related to the occurrence of lung cancer. We then evaluated the diagnostic value of these four tumor markers alone and combined detection for lung cancer through the ROC curve, and found that the combined detection of CEA, CYFARA21-1, NSE, and proGRP had the highest diagnostic accuracy for lung cancer, and had certain practical value in clinical practice.

In addition to comprehensive diagnostic analysis of diagnostic markers, we also compared and analyzed the data of lung cancer survival and death groups. We found that the levels of CYFAR21-1 and NSE in the death group were higher than those in the survival group. At the same time, this study shows that the levels of CYFAR21-1 and NSE in patients with lung cancer are higher than those in patients with benign lung diseases and healthy controls. Therefore, it is obvious that CYFAR21-1 and NSE can be used to assess the prognosis of lung cancer patients and are regarded as indicators for predicting the prognosis of lung cancer patients.

In summary, tumor markers are important for the diagnosis of lung cancer. The combined detection of CEA, CYFARA21-1, NSE, and proGRP had the highest diagnostic accuracy for lung cancer. CYFAR21-1 and NSE can be used to evaluate the prognosis of lung cancer patients. Further research is required to identify new circulating biomarkers with sufficient specificity and sensitivity for clinical applications.

Abbreviations

SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; CT, computed tomography; PET-CT, positron emission tomography-CT; MRI, magnetic resonance imaging; CEA, carcinoembryonic antigen; NSE, neuron-specific enolase; CYFRA21-1, cytokeratin fragment; proGRP, progastrin releasing peptide precursor; SCC-Ag, squamous cell carcinoma antigen; ROC, receiver operating characteristic; AUC, area under the curve

Declarations

Ethics approval and consent to participate: The study was approved by the Ethical Committee of the Second Affiliated Hospital of Wenzhou Medical University (Approval No: 2021-K-10-01). Written informed consent of patients was obtained from the patients, and this study was conducted in accordance with the Declaration of Helsinki.

Consent for publication: Not applicable.

Availability of data and material: The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: Neither this paper nor any similar paper has been or will be submitted to or published in any other scientific journal. All authors are aware and agree to the content of the paper and to their being listed as an author on the manuscript. There is no conflict of interest or competing financial interests for all authors.

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Authors' contributions: XQ conceived and designed the study; CS, HS and KM collected data; HS, LJ, ZT and CY had a phone follow-up; XQ, KM and WH conducted the statistical analyses. CS, KM and XQ drafted the manuscript. All authors read and approved the final version of the manuscript.

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Figures

Figure 1

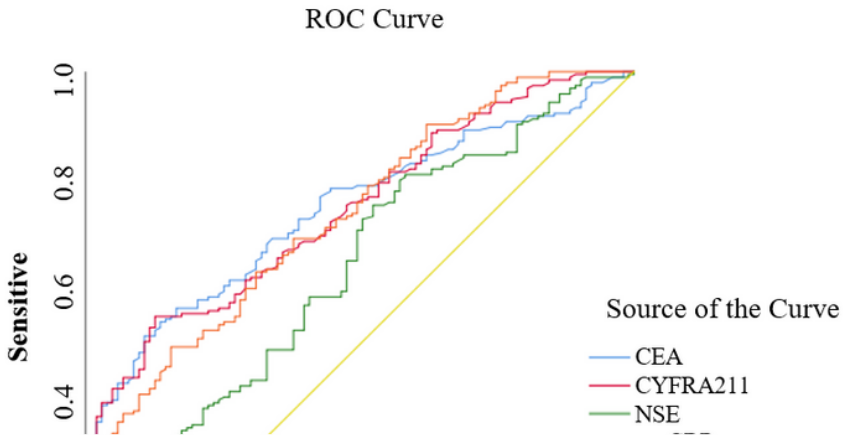

Figure 1

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Figure 2

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Figure 3

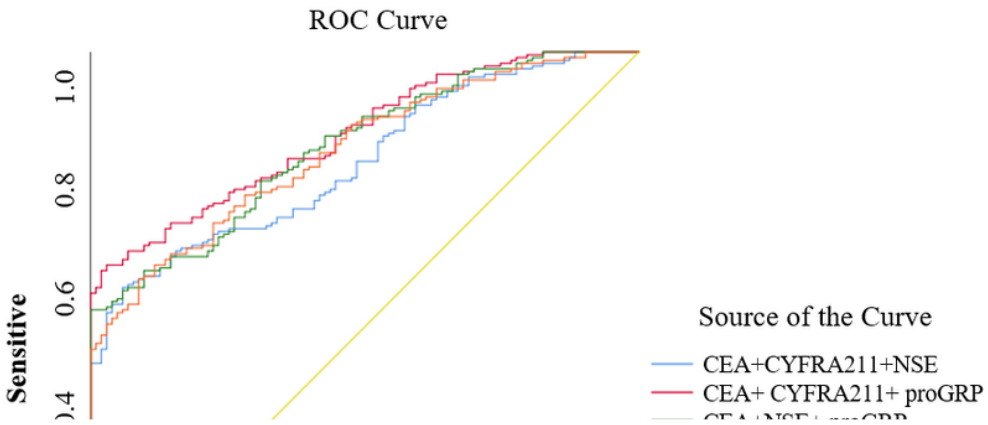

Figure 3

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