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Study on the application value of combined detection of multiple tumor markers in lung cancer classification diagnosis

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► ***Original article***

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ABSTRACT

Background: To evaluate the value of six tumor markers including squamous cell carcinoma antigen (SCCA), cytokeratin 19 fragment (CYFRA21-1), carcinoembryonic antigen (CEA), glycoconjugate antigen 125 (CA125), neuron-specific enolase (NSE), along with gastrin-releasing peptide precursor (ProGRP) in the diagnosis and pathological staging of lung cancer (LC) alone or in combination. ***Materials and Methods:*** A retrospective analysis was performed on 300 patients of which 62 cases were diagnosed as small cell lung cancer (SCLC), 105 adenocarcinoma (LUAD), 41 squamous cell carcinoma (LUSC) and 92 large cell lung cancer (LCLC) by histopathology. Another 364 patients with benign lung disease in the same period were chosen for the benign lung disease group. ***Results:*** The detection results of 6 tumor markers in serum of LC, SCCA, NSE and ProGP patients with different pathological types were higher compared with lung benign lesions group (P<0.01). LC can be separated into SCLC along with non-Small cell lung cancer (NSCLC) by NSE+ProGRP combination. The detection rate of lung squamous cell carcinoma by SCCA+ cytokeratin 19 fragment (CYFRA21-1) was higher than that by CEA+CA125 combination for adenocarcinoma. The combined determination of NSE and ProGRP was highly sensitive to SCLC. In NSCLC, the combined detection of SCC-Ag and CYFRA21-1 showed high sensitivity to LUSC. CEA+CA125 combined detection was highly sensitive to LUAD. ***Conclusion:*** The combined determination of NSE and ProGRP was highly sensitive to SCLC. CEA+CA125 combined detection was highly sensitive to LUAD, and SCCA+CYFR21-1+CEA+CA125 was the best combined detection analysis of multiple indexes.

INTRODUCTION

Lung cancer (LC) belongs to a malignant tumor of respiratory system. Its incidence and growth rate rank first among all malignant tumors, and it is also the most frequent malignancy with the largest number of deaths worldwide. The 5-year survival rate of patients with advanced LC is lower than 15%, so it is significant to improve the early diagnosis of LC [1]. The diagnostic tools currently in use are not sensitive enough to make a diagnosis in the early stages of the disease. Therefore, finding new methods for early and accurate diagnosis of lung cancer is essential for the effective treatment of lung cancer. Lung cancer is the result of multi-stage carcinogenesis, with a gradual increase in genetic and epigenetic changes. Screening for characteristic genetic markers can be used for early diagnosis of lung cancer [2]. Therefore, numerous research have examined serum tumor indicators in relation to LC early diagnosis [3, 4]. Tumor markers have the advantages of low trauma, high efficiency, convenience, high sensitivity and easy specimen acquisition. They are specific indicators mirroring the existence as well as growth of tumors, and are a group of substances produced or secreted by tumor cells during the process of tumor proliferation and released into cells, blood and body fluids [5]. As a result, screening, identifying, and detecting serum tumor markers is crucial for an early LC diagnosis.

Currently, there are mainly the following categories of significant markers of lung cancer:

(1) Enzyme antigens: mainly include isoenzymes, such as neuron enolase, lactate dehydrogenase, and gastrin-releasing peptide precursors.

(2) Glycoprotein antigens: mainly squamous cell antigen, carbohydrate antigen 199 (CA199), carbohydrate antigen 125 (CA125), along with carbohydrate antigen 153.

(3) Embryonic antigen: such as carcinoembryonic antigen (CEA).

(4) Keratin antigens: including tissue polypeptide specific antigen, and cell keratin 19 fragment antigen. The monitoring of single tumor markers generally has limited overall specificity and sensitivity to lung cancer, and only has high sensitivity to specific types

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of lung cancer. In order to determine which tumor markers can be adopted as follow-up monitoring indicators after cancer treatment, improve the auxiliary diagnostic value of serum tumor markers. This work was aimed to assess the value of six tumor markers including squamous cell carcinoma antigen (SCCA), cytokeratin 19 fragment (CYFRA21-1), CEA, CA125, neuron-specific enolase (NSE), and gastrin-releasing peptide precursor (ProGRP) in the diagnosis and pathological staging of LC alone or in combination.

In order to determine a new course for the diagnosis and treatment of lung cancer, our study assesses the usefulness of six tumor markers-SCCA, CYFRA21-1, CEA, CA125, NSE, and ProGRP-either alone or in combination for the pathological staging and diagnosis of lung cancer.

MATERIALS AND METHODS

General clinical data

A retrospective analysis was performed on 300 patients with lung cancer admitted to Gaozhou People's Hospital from January 2020 to December 2022, of which 62 cases were diagnosed as small cell lung cancer (SCLC), 105 adenocarcinoma (LUAD), 41 squamous cell carcinoma (LUSC) and 92 large cell lung cancer (LCLC) by histopathology. In addition, 364 patients with benign lung lesions during the same period were chosen as the group of benign lung lesions, containing 135 cases of pulmonary tuberculosis, 102 cases of pneumonia, 52 cases of pulmonary heart disease, 75 cases of pulmonary cyst. In addition, 100 healthy subjects in Gaozhou People's Hospital during the same period were collected as the control group, and no lung related diseases were confirmed.

Table 1. General clinical data of patients.

groups	Number	Type	Number
lung cancer	300	SCLC	62
		LUAD	105
		LUSC	41
		LCLC	92
benign lung lesions	364	pulmonary tuberculosis	135
healthy subjects	100	Pneumonia	102
		pulmonary heart disease	52
		pulmonary cyst	75

Collection of serum samples

Before treatment, 5ml of blood was drawn from the hospitalized patients, centrifuged at 3000 r/min for 10 min, and then the serum was separated for routine testing. Collect samples of no less than 800 µl and store them cryostead at 80 °C to facilitate further batch testing.

Detection of tumor markers

Six tumor markers including SCCA, CYFRA21-1, CEA, CA125, NSE and ProGRP were determined by magnetic particle chemiluminescence assay (Huamei Biological Engineering, Wuhan, China). All experimental procedures were operated according to the reagent instructions. Result judgment: If it exceeds the critical value provided in the instruction manual, it will be judged as positive, and record its detection value. The positive criteria (if an indicator is positive, it can be determined) were SCCA >1.5 ng/ml, CYFRA21-1 >3.3 ng/ml, CEA >5 ng/ml, CA125 >35 U/ml, NSE >20 ng/ml, ProGRP >65 pg/ml.

Statistical analysis

SPSS 19.0 software was implemented for statistical analysis of experimental data. Analysis of variance was used for significance test of multiple groups of measurement data. Sensitivity, specificity, and area under the ROC curve were used to assess the value of the combined detection of various markers, and the *t-test* was used to compare groups and the chi-square test was used to compare detection rates. $P<0.05$ was statistically significant.

RESULTS

Detection results of 6 tumor markers in serum of patients with different pathological types of LC and lung benign lesions

The detection results of 6 tumor markers in serum of LC patients with different pathological types were higher compared to lung benign lesions group ($P<0.05$). Patients with small cell lesion (SCLC) had greater serum levels of NSE and ProGRP than the non-small cell lesion group ($P<0.01$). LUSC patients had higher levels of CYFRA21-1 and SCCA than other groups ($P<0.01$). Patients with LUAD had higher levels of CEA and CA125 compared to other groups ($P<0.01$) (table 2).

Detection rate of tumor marker combination test

The independent and combined detection of 6 tumor markers can be helpful for the pathological classification of lung cancer patients. NSE and ProGRP together can be used to separate LC into NSCLC and SCLC. When SCCA+CYFRA21-1 was combined, the detection rate of lung squamous cell cancer increased. The combination of CEA+CA125 increased the rate of adenocarcinoma identification (table 3).

Diagnostic value of combined examination of tumor markers

The combined determination of NSE and ProGRP was highly sensitive to SCLC. In NSCLC group, the combined detection of SCC-Ag and CYFRA21-1 showed high sensitivity to LUSC. CEA + CA125 combined detection showed high sensitivity to LUAD (table 4). Consistently, the results of figure 1 indicated that SCC-Ag + CYFRA21-1 showed high sensitivity to LUSC, and EA + CA125 showed high sensitivity to LUAD.

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Table 2. Detection results of 6 tumor markers in serum of patients with different pathological types of LC and lung benign lesions.

Groups	Cases (n)	SCCA (ng/ml)	CYFRA21-1 (ng/ml)	CEA (ng/ml)	CA125 (U/ml)	NSE (ng/ml)	ProGRP (pg/ml)
Small cell lung cancer group	69	2.02±0.82	3.41±1.26	3.79±2.65	25.37±10.85	90.96±48.92	548.37±486.95
Non-small cell lung cancer group							
Adenocarcinoma	108	10.23±2.52	5.56±3.79	110.94±46.85	70.63±49.86	15.38±8.49	62.36±45.08
Squamous carcinoma	45	31.96±20.83	9.79±7.86	4.01±2.06	27.76±8.55	9.55±4.97	53.26±24.85
Large cell lung cancer	92	1.97±1.05	3.57±1.68	3.54±1.96	30.86±7.94	10.37±7.83	65.79±36.74
Lung benign lesion group							
Pulmonary tuberculosis	135	0.95±0.44	1.27±0.48	1.95±0.85	13.97±10.26	8.84±3.55	37.96±19.25
Pneumonic lesion	102	0.62±0.37	1.44±0.52	2.37±0.29	12.58±7.99	7.63±3.01	39.98±20.02
Pulmonary heart disease	52	0.77±0.59	1.02±0.24	1.05±0.84	17.83±8.28	9.95±4.38	25.03±10.28
Pulmonary cyst	75	0.84±0.37	1.21±0.12	2.96±1.52	10.47±2.95	9.03±2.36	19.94±20.58
Healthy control group	100	0.26±0.12	0.14±0.27	0.86±0.93	0.87±0.79	1.28±1.02	25.47±15.66

SCCA: squamous cell carcinoma antigen; CYFRA21-1: cytokeratin 19 fragment; CEA: carcinoembryonic antigen; CA125: glycoconjugate antigen; NSE: neuron-specific enolase; ProGRP: gastrin-releasing peptide precursor.

Table 3. Detection rate of different pathological types of LC by the combination of 6 tumor markers (%).

Detection groups	NSE+ProGRP	SCCA+CYFR21-1	CEA+CA125	SCCA+CYFR21-1+CEA+CA125
Small cell lung cancer	78.55	35.97	23.29	37.55
Lung squamous carcinoma	21.53	82.46	34.87	84.06
Lung adenocarcinoma	20.95	40.53	57.57	58.99
Non-small cell lung cancer	21.27	82.95	50.25	84.85

NSE: neuron-specific enolase; ProGRP: gastrin-releasing peptide precursor; SCCA: squamous cell carcinoma antigen; CYFRA21-1: cytokeratin 19 fragment; CEA: carcinoembryonic antigen; CA125: glycoconjugate antigen.

Table 4. Diagnostic value of the combined pattern of 6 tumor markers in different pathological types of lung cancer.

Combination detection modes	Detection groups	Sensitivity (%)	Specificity (%)	Accuracy (%)	Youden index	Area under ROC curve
NSE+ProGRP	Small cell lung cancer	89.95	87.53	84.06	63.48	0.794
SCCA+CYFR21-1	Lung squamous carcinoma	82.43	74.97	77.58	57.4	0.672
CEA+CA125	Lung adenocarcinoma	73.28	68.48	67.46	61.76	0.597
SCCA+CYFR21-1+CEA+CA125	Non-small cell lung cancer	92.54	94.68	95.96	87.22	0.821

NSE: neuron-specific enolase; ProGRP: gastrin-releasing peptide precursor; SCCA: squamous cell carcinoma antigen; CYFRA21-1: cytokeratin 19 fragment; CEA: carcinoembryonic antigen; CA125: glycoconjugate antigen.

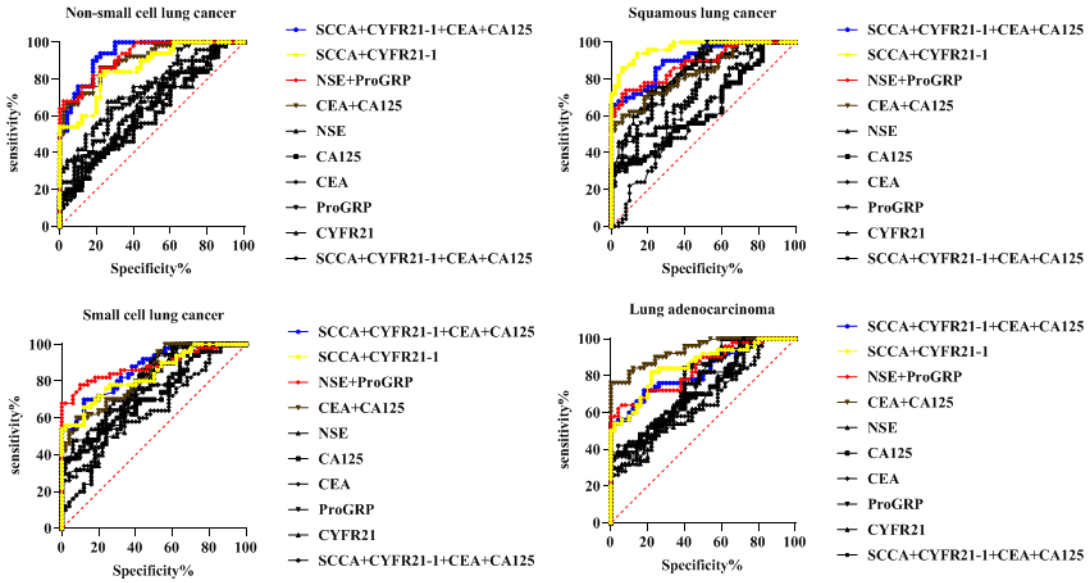

Figure 1. Diagnostic value of combined examination of tumor markers.

DISCUSSION

LC is a common malignancy in the world, and its diagnosis mainly depends on pathology and imaging examination. Clinical studies have shown that the cure rate of in-situ cancer is close to 100%, and the 5-year survival rate of stage I LC patients after appropriate treatment is 60%~90% [6]. However, most patients have lost the opportunity for radical treatment when diagnosed. Detection of serum tumor markers has a significant role in the early diagnosis, efficacy assessment and prognosis of LC, but single marker detection cannot fully meet the needs of clinical diagnosis and treatment. Therefore, sensitive

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and specific combination of markers is needed to improve the accuracy and precision of tumor diagnosis [7].

NSE is a γ dimer isoenzyme of enolase, which exists in neuroendocrine cells and neurogenic tumors. SCLC is a tumor with neurosecretion, so the level and positive rate of NSE in LC, especially in SCLC, are significantly increased, which is valuable for its diagnosis, efficacy assessment and prognosis [8, 9]. CYFRA21-1 is widely distributed on the surface of normal tissues, such as lamellar or squamous epithelium. In malignant epithelial cells, activation of protease accelerates cell degradation, resulting in the release of large amounts of cytokeratin fragments into the blood, which is believed to have good sensitivity together with specificity for NSCLC, especially LUSC. It has been reported in the literature that the sensitivity of CYFRA21-1 to the diagnosis of various types of LC is in the order of LUSC > LUAD > LCLC > SCLC. In particular, the sensitivity of CyFRA21 -1 to the detection of LUSC can reach more than 55%, which has good clinical application value [7, 10]. SCC-A is an antigen produced by squamous cells, which was first used in the diagnosis of squamous cell carcinoma, especially LUSC, when serum SCC-A is abnormally elevated, and its concentration increases with the progression of the disease [11, 12].

In our study, compared to other forms of lung cancer, patients with LUSC had a considerably higher detection rate of 82.46% for the CYFRA21-1+SCC-A combination test. CEA is a type of acidic glycoprotein on the surface of cells that has a specific determinant of human embryonic antigen. It is highly sensitive to lung adenocarcinoma and can assess the size and scope of tumor tissue, the progression of the disease, and the observation of curative effects. Some patients with digestive tract tumors, breast cancer, and lung cancer have abnormally elevated serum levels of CEA [13, 14]. CA125 is an ovaria-associated antigen with a molecular weight of 200000-1000000, which is a polymeric glycoprotein, but it shares common antigen with lung cancer cells. Its increase can be seen in ovarian cancer, lung adenocarcinoma, etc., and abnormal increase can also be seen in some non-tumor diseases such as hepatitis, pregnancy and some gynecological inflammation [15]. In this study, the detection rate of CA125+CEA combined test in lung adenocarcinoma patients was 57.57%, which was significantly higher than that of other types of lung cancer. ProGRP is an autonomous growth factor for small cell carcinoma [16], which makes up for the deficiency of NSE detection to some extent. The sensitivities and specificities of ProGRP and NSE in the determination of SCLC were 73% and 60%, and 98% and 92%, respectively [17]. In this study, the detection rate of NSE+ProGRP in serum patients with SCLC was 78.55%, which was consistent with the reported results [18, 19].

Due to the complex structure of lung cancer tissues, different tumor tissue types are often mixed, and different subtypes also exist in the same tumor tissue [20]. Therefore, the value of a single index for typing diagnosis is limited, so the combined detection becomes a trend. The single diagnostic value of CYFRA21-1, SCC-A, CA125, CEA, NSE, ProGRP showed high confidence in distinguishing tumor from benign disease, but could not distinguish SCLC from NSCLC well. Sensitivity and specificity, as two important efficacy indexes in the evaluation of tumor markers, were reversed by the influence of "boundary value." The area under ROC curve along with Youden index can comprehensively reflect the diagnostic ability of tumor markers [21].

Based on the ROC curve and the principle of maximum Youden index, this paper set the positive threshold and conducted the combined analysis of multiple indexes. The Youden index of NSE+ ProGRP, SCCA+CYFR21-1 and CEA+CA125 was 63.48, 57.40 and 61.76, respectively. The sensitivity, specificity, accuracy along with Youden index of the two tumor marker combination models for lung cancer typing were close. The specificity, accuracy along with Youden index was increased significantly, but the sensitivity was decreased slightly when multiple indexes were combined.

In our research, the study's findings showed that the combination detection of NSE and ProGRP was very sensitive to SCLC. The combination detection of CYFRA21-1 and SCC-Ag in the NSCLC group demonstrated a high sensitivity to LUSC. The best combined detection analysis of various indices was found to be SCCA+CYFR21-1+CEA+CA125. The combined detection of CEA+CA125 was very sensitive to LUAD.

CONCLUSION

In conclusion, the early detection of lung cancer and its pathological categorization are powerful indicators that increase patient survival rates, and the clinical application value of different lung cancer markers combined detection is increasingly recognized.

***Conflicts of interests*:** No potential conflict of interest was reported by the authors.

***Ethical consideration*:** All patients provided their written, voluntarily informed consent. All procedures were carried out in accordance with the guidelines outlined in the Helsinki Declaration and this study was approved by the Ethics Committee of our institution.

***Author contribution*:** Rongmei Zhou and Zhonghan Cai conceived and designed the experiments. Yinru Yuan, Zhiliang Zhou, Huo Ding, Yaoqiang Cheng contributed significantly to the experiments and arranging data. Liang Li and Zineng Tuo performed

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data analyses. Liang Li and Zineng Tuo wrote the draft manuscript. Liang Li and Zineng Tuo revised the manuscript. All authors read and approved the final manuscript.

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