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# Diagnostic value of joint detection of serum tumor markers in different pathological types of lung cancer

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► ***Original article***

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## ABSTRACT

***Background*:** To evaluate the diagnostic potential of joint testing of serum tumor markers containing cytokeratin fragment antigen 21-1 (CYFRA21-1), progastrin-releasing peptide (ProGRP), carcinoembryonic antigen (CEA) as well as neuron-specific enolase (NSE) in a variety oflung cancer (LC). ***Materials and Methods*:** The LC group comprised 150 LC patients who were diagnosed for the first time and were not treated. During the same period, 120 patients harbored benign lung diseases and 120 healthy subjects were respectively designated as the benign group and control group. Immunochemiluminescence assay was implemented to detect tumor markers in serum from three groups and different types of LC. ***Results:*** Levels of four serum tumor markers in LC group were elevated relative to control group. CYFRA21-1 level in lung squamous cell carcinoma (LSCC) group was elevated compared to lung adenocarcinoma (LUAD) and small cell lung cancer (SCLC) groups. ProGRP and NSE levels in SCLC group increased compared to LSCC and LUAD groups. CEA level in LUAD group was higher compared to LSCC and SCLC groups. The highest positive rate of LSCC was CYFRA21-1, the highest positive rate of LUAD was CEA and the highest positive rates of SCLC were ProGRP and NSE. In terms of single tumor marker detection, CYFRA21-1 possessed the best diagnostic efficiency for LSCC, CEA possessed the best diagnostic efficiency for LUAD, NSE and ProGRP possessed the best diagnostic efficiency for SCLC, respectively. ***Conclusion:*** The individual determination of CYFRA21-1, ProGRP, CEA, and NSE has certain clinical application value for the pathological classification of LC.

## INTRODUCTION

Lung cancer (LC) belongs to the major cause of tumor-linked death all over the world. In spite of advances have achieved in modern diagnosis together with treatment methods, early patients have mild symptoms and not obvious clinical presentations, and most patients have entered the advanced stage when they are found, with a low 5-year survival time and a high mortality rate [1]. According to histology, LC is classified into two types: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), with NSCLC accounting for around 80-85% of LC patients [2]. NSCLC majorly includes two histological subtypes: lung adenocarcinoma (LUAD, about 50%) as well as lung squamous cell carcinoma (LSCC, about 30%) [3]. Different pathologic types of LC have different treatment methods. The early diagnosis and determination of the pathological classification of LC is critical to promoting the survival rate and prognosis of LC patients [4]. Over the past few years, radiotherapy has been utilized to treat early-stage lung cancer and has achieved promising efficacy, reducing the complication rate and mortality caused by surgery. Therefore, the early diagnosis of lung cancer is to gain more survival time for patients, and there is an urgent need to seek accurate and effective early diagnosis methods [5].

At present, LC is mainly found through imaging examination in clinical practice, and the gold standard for diagnosis is still histopathological examination, which is characterized by greater trauma and poor patient compliance [6]. Radiographic screening for lung cancer mainly includes chest X-rays and spiral CT, among which CT can collect data continuously and has high diagnostic accuracy. It is crucial to highlight, however, that individuals who have frequent CT scans have an increased risk of cancer. In addition, a higher false-positive rate requires patients to undergo more invasive tests, such as biopsies and surgeries, to eliminate abnormalities, resulting in additional intraoperative and postoperative risks and complications [7]. Tumor markers are specific products produced during the proliferation and differentiation of tumor cells, and their detection methods are economical, effective, accurate, and highly reproducible, with a wide variety [8]. Serum tumor markers are identified as an effective means for the diagnosis of LC and are not invasive and biohazardous [9]. Cytokeratin fragment antigen 21-1 (CYFRA21-1), progastrin-releasing peptide (ProGRP), carcinoembryonic antigen (CEA),



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as well as neuron-specific enolase (NSE) have been reported to be highly expressed in LC, and are commonly applied tumor markers in clinical studies of LC, showing important diagnostic value and potential prognostic indicators, which are conducive to the monitoring of systematic treatment [10, 11]. However, during the early diagnosis of LC, the sensitivity together with specificity of a single detection index are often poor [12]. Therefore, in this study, we explored the diagnostic value of joint detection of these serum tumor markers in distinct pathogenic kinds of LC. This study seeks a more precise diagnosis method for different types of lung cancer, which will reduce the discomfort of patients and the burden on patients and society, so as to carry out early intervention and treatment, and improve survival and prognosis.

## MATERIALS AND METHODS

### *General data*

This LC group comprised 150 newly diagnosed LC patients (45 LSCC cases, 40 SCLC cases, and 65 LUAD cases) who did not receive any treatment between January 2021 and December 2022. During the same period, 120 patients with benign lung illnesses and 120 healthy people were divided into the benign and control groups. Benign lung lesions include bronchiectasis, bronchitis, and lung infections. The LC group contained 100 men and 50 women, aged 40-72 years, and the age distribution of the benign group was 53.48±5.92 years. The benign group contained 78 men and 42 women, ranging in age 40-71 years, and the age distribution of the benign group was 53.40±5.87 years. The control group contained 79 men and 41 women, ranging in age 41-71 years, and the age distribution of the benign group was 53.45±5.89 years. The three groups' overall data was equivalent (P>0.05). Inclusion criteria: (1) Approved by the Medical Board and patients confirmed by histopathologic classification. (2) The patient's clinical data was complete, and compliance was high. (3) Informed consent was signed by patients and their families. Exclusion criteria: (1) Patients with other malignant tumors. (2) With heart, liver, lung, kidney and other major diseases. (3) Those with other chronic diseases.

### *Detection method*

All individuals who had an empty stomach in the morning had their venous blood taken in 3 milliliters. Serum was centrifuged after blood coagulation, and the levels of markers CYFRA21-1 (Biolegend, USA), ProGRP (CUSABIO, China), CEA (Wako, Japan), and NSE (CUSABIO, China) were detected using ELISA kits by immunochemiluminescence. The Roche COBASE601 luminescent chemical immunoassay analyzer and matching kit were used.

### *Normal reference range*

The normal reference range was CYFRA21-1 < 3.2 μg/L, ProGRP > 65 pg/mL, CEA < 4.3 μg/L, NSE < 13 μg/L, and exceeding the normal reference range was judged as positive.

### *Observation indicators*

(1) Serum tumor markers' levels were compared among the three groups. (2) The positive determination criteria of tumor markers are shown in the reference range. The proportion of marker positives of serum markers detection results in the LC was calculated as follows: positive cases/LC cases (%). (3) Diagnostic efficacy of single and joint testing of tumor markers in different types of LC.

### *Statistical analysis*

The measurement results were presented as mean ± standard deviation, and the SPSS 10.0 software was used to perform a t-test analysis. The statistical data were exhibited as a percentage and $\chi^2$ test was implemented for analysis. The diagnostic utility of tumor markers in various LC varieties was examined using the ROC curve.

## RESULTS

### *The level of serum tumor markers in the LC, benign, and control group*

The immunochemiluminescence was used to measure the serum content of tumor markers in the LC, benign, and control group. Figure 1 illustrates the results, which demonstrated that the LC group's levels of CYFRA21-1, ProGRP, CEA, and NSE were considerably higher (P<0.05) than those of the benign and healthy control groups. However, there was no difference seen in the levels of these serum indicators between the benign and the healthy control group (P>0.05).

### *Serum tumor markers' levels in patients with different types of lung cancer*

In order to explore the trend of Serum tumor markers in different types of lung cancer, we used immunochemiluminescence to detect the level of four markers in LSCC, LUAD and SCLC groups. It was displayed in Figure 2 that, serum CYFRA21-1 level in LSCC group was elevated compared to LUAD and SCLC groups (P<0.05). The SCLC group had greater levels of NSE and ProGRP in their serum relative to the LUAD and LSCC groups (P<0.05). In comparison to the LSCC and SCLC groups, the LUAD group's serum CEA level was higher (P<0.05).

### *Positive rate of tumor markers in different groups*

The positive detection rates of tumor markers in LC group were promoted in contrast to the benign and control groups (P<0.05, table 1).



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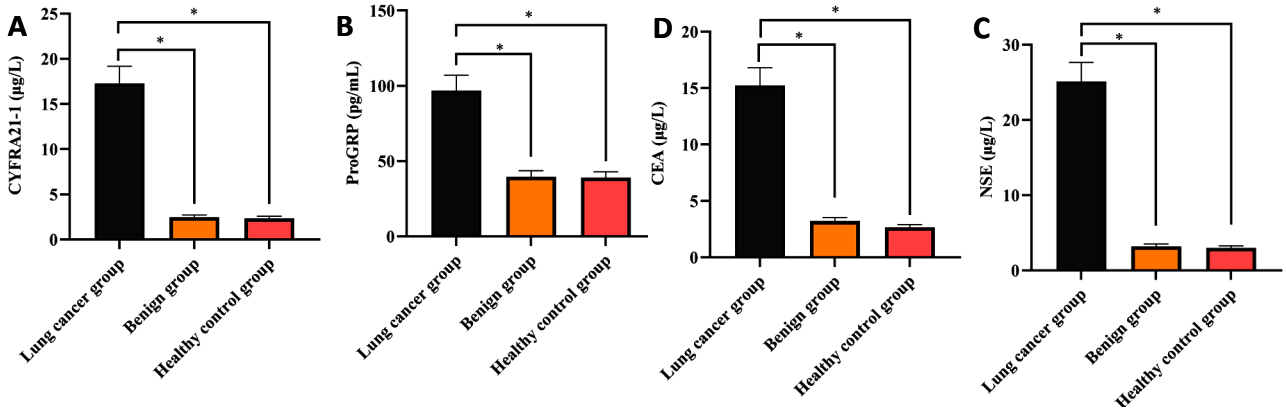


**Figure 1.** Serum tumor markers' levels in three groups. **(A)** The immunochemiluminescence detected the level of CYFRA21-1 in the LC, benign, and control group. **(B)** The immunochemiluminescence detected the level of ProGRP in the LC, benign, and control group. **(C)** The immunochemiluminescence detected the level of CEA in the LC, benign, and control group. **(D)** The immunochemiluminescence detected the level of NSE in the LC, benign, and control group. *P<0.05.

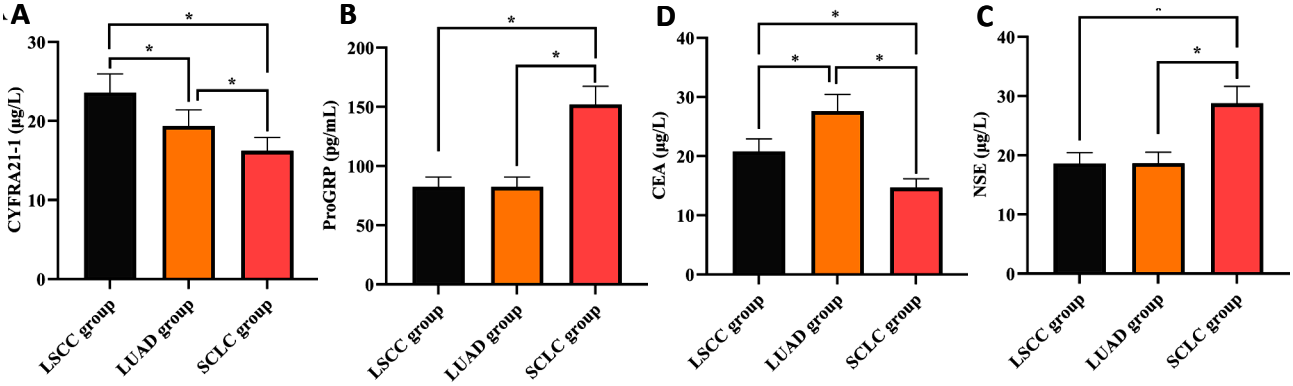


**Figure 2.** Serum tumor markers' levels in patients with different kinds of LC. **(A)** The immunochemiluminescence detected the level of CYFRA21-1 in patients with LSCC, LUAD and SCLC groups. **(B)** The immunochemiluminescence detected the level of ProGRP in patients with LSCC, LUAD and SCLC groups. **(C)** The immunochemiluminescence detected the level of CEA in patients with LSCC, LUAD and SCLC groups. **(D)** The immunochemiluminescence detected the level of NSE in patients with LSCC, LUAD and SCLC groups. *P<0.05.

**Table 1.** Positive rate of tumor markers in different groups.

| Groups | Total | Sex | | Age | Race | CYFRA21-1 | ProGRP | CEA | NSE |
|---|---|---|---|---|---|---|---|---|---|
| | | Male | Female | | Asian | Positive | Positive | Positive | Positive |
| Lung cancer group | 150 | 100 | 50 | 53.48±5.92 | 150 | 77 | 52 | 75 | 60 |
| Benign group | 120 | 78 | 42 | 53.40±5.87 | 150 | 1 | 2 | 2 | 0 |
| Control group | 120 | 79 | 41 | 53.45±5.89 | 150 | 1 | 0 | 1 | 0 |
| $\chi^2$ | | 0.08272 | | | | 82.76 | 45.38 | 137.12 | 61.71 |
| P | | >0.05 | | | | <0.05 | <0.05 | <0.05 | <0.05 |

**CYFRA21-1:** Cytokeratin fragment antigen 21-1, ProGRP: progastrin-releasing peptide, CEA: carcinoembryonic antigen, NSE: neuron-specific enolas.

### *Positive rate of tumor markers in patients with various forms of LC*

The highest positive rate of LSCC was CYFRA21-1, the highest positive rate of LUAD was CEA and the highest positive rates of SCLC were ProGRP and NSE (P<0.05, table 2).

**Table 2.** Positive rate of tumor markers in patients with various forms of LC.

| Groups | CYFRA21-1 | ProGRP | CEA | NSE |
|---|---|---|---|---|
| LSCC group | 36/45 | 13/45 | 20/45 | 15/45 |
| LUAD group | 23/65 | 12/65 | 42/65 | 17/65 |
| SCLC group | 18/40 | 27/40 | 13/40 | 28/40 |
| $\chi^2$ | 22.06 | 27.24 | 11.01 | 21.03 |
| P | <0.05 | <0.05 | <0.05 | <0.05 |

CYFRA21-1: Cytokeratin fragment antigen 21-1, ProGRP: progastrin-releasing peptide, CEA: carcinoembryonic antigen, NSE: neuron-specific enolas, LSCC: squamous cell carcinoma, LUAD: lung adenocarcinoma, SCLC: small cell lung cancer.

### *ROC curve showing the individual and combined diagnostic significance of tumor markers for LCSerum*

CYFRA21-1 had the highest diagnostic efficiency for LSCC (AUC = 0.932), serum CEA the highest diagnostic efficiency for LUAD (AUC = 0.811), serum NSE and ProGRP the highest diagnostic efficiency for SCLC (AUC = 0.805 and 0.802, respectively), and serum MDA the highest diagnostic efficiency for LUAD (AUC = 0.811). Significantly, with an AUC of 0.978, 0.959, and 0.911, respectively, the joint testing of the four markers had a higher diagnostic efficiency than the single detection in LSCC, LUAD, and SCLC (figure 3).



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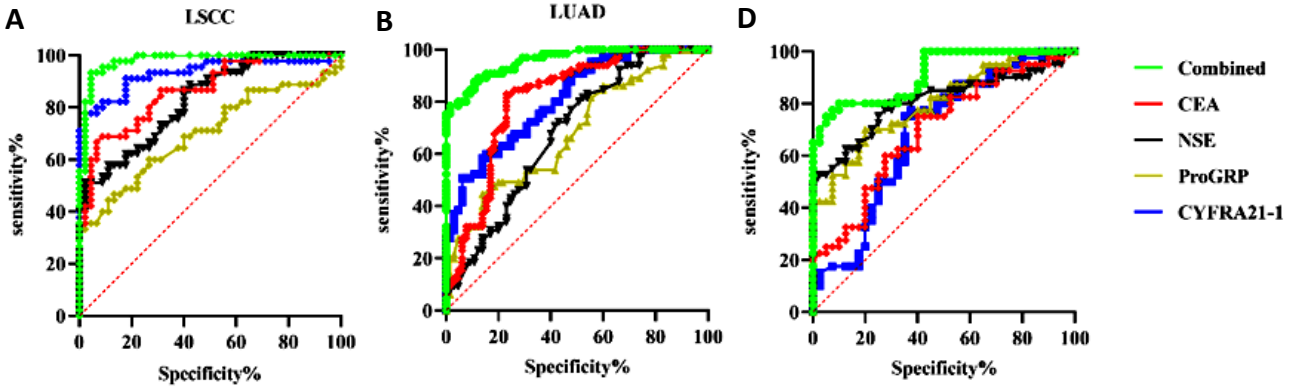


**Figure 3.** ROC curve showing the individual and combined diagnostic significance of tumor markers for LC **(A)** ROC curve for the separate and combination diagnostic value of CYFRA21-1, ProGRP, CEA, and NSE in the diagnosis of LSCC. **(B)** ROC curve for the separate and combination diagnostic value of 4 markers in the diagnosis of LUAD. **(C)** ROC curve for the separate and combination diagnostic value of 4 markers in the diagnosis of SCLC.

## DISCUSSION

LC belongs to a kind of the most frequent malignant tumors all over the world, and is considered to be the most dangerous malignant tumor to human health and life [13]. With the discovery of new tumor markers, the assessment of tumor markers has emerged as a crucial means for the early diagnosis of LC in recent years [7]. However, there is still a lack of a specific marker for LC, which cannot make a good differential diagnosis of LC. Therefore, in this study, four tumor markers (CYFRA21-1, ProGRP, CEA, and NSE) were jointly detected to assess their clinical value in the diagnosis of LC as well as provide a basis for their clinical application.

In previous studies, the level of CYFRA21-1, ProGRP, CEA, and NSE fluctuated abnormally in the serum of lung cancer patients and could be used as tumor markers to aid early diagnosis, respectively [14-17]. Consistently, in this study, we discovered that serum markers' levels in LC group were elevated relative to healthy control group and benign group, and the positive detection rates of tumor markers in LC group were increased in contrast to other two groups which implied these markers are of great value in the diagnosis of LC.

CYFRA21-1 is an acidic protein mainly present in the cytoplasm of epithelial origin tumor cells such as LC and esophageal cancer [18, 19]. When tumor cells dissolve or necrosis, CYFRA21-1 can be released into the blood [20]. Elevated CYFRA21-1 in the blood is more common in NSCLC, especially LSCC, and is considered to be the most sensitive indicator for LSCC [21]. Consistently, our study discovered that serum CYFRA21-1 level in LSCC group was higher compared to LUAD and SCLC groups, and the highest positive rate of LSCC was CYFRA21-1.

NSE is a glycolytic enzyme, which mainly exists in brain neurons, peripheral nerve tissues and endocrine tissue [22]. NSE is abundant in LC tissues, and its content in SCLC tissues is 3-35 times that of normal lung cells [23]. As a precursor structure of GRP, ProGRP can be statically present in serum and widely exist in neuroendocrine cells of nerve tissue and lung tissue [24] . ProGRP level represents the expression level of GRP, and is generally used for the diagnosis of SCLC [25]. In line with the above studies, our study discovered that serum ProGRP and NSE levels in SCLC group were higher compared to LSCC and LUAD groups, and the highest positive rates of SCLC were ProGRP and NSE.

CEA, as a tumor marker of adenocarcinoma, has been widely applied in the diagnosis of various tumors [26]. CEA exists in cell membrane and is easy to slip into body fluids. The increase of CEA in tumor patients may be related to the changes of oncogenes [27]. When a cell becomes cancerous, the genes on the corresponding chromosomes are inhibited, causing the originally inhibited genes to reactivate in the cancer tissue and produce CEA [28]. In addition to LC, malignant tumors of digestive tract, urogenital tract, thyroid cancer, cervix and breast cancer all have elevated CEA levels [29]. 52%-77% of serum CEA levels in LC patients are higher than normal values [30]. Most reports have suggested that serum CEA levels are related to histological types of LC, with LUAD being the highest, LSCC the second, and SCLC the lowest [31]. Likewise, our study revealed that serum CEA level in LUAD group was higher compared to LSCC and SCLC groups. Besides, the highest positive rate of LUAD was CEA.

In addition, our investigation also included The ROC curve was used to examine the tumor markers' diagnostic usefulness in various LC types. In terms of single tumor marker detection, serum CYFRA21-1 possessed the best diagnostic efficiency for LSCC, and the AUC was 0.932, serum CEA possessed the best diagnostic efficiency for LUAD, and the AUC was 0.811, and serum NSE and ProGRP possessed the best diagnostic efficiency for SCLC, and the AUC was 0.805 and 0.802, respectively. Importantly, the diagnostic efficiency of the joint testing of the four markers was elevated in contrast to the single detection in LSCC,


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LUAD and SCLC, with an AUC of 0.978, 0.959 and 0.911, respectively. It could be confirmed that the combined detection of serum CYFRA21-1, ProGRP, CEA, together NSE had high clinical value in the early diagnosis of LC, which was similar to previous studies [32].

## CONCLUSION

In conclusion, the individual determination of CYFRA21-1, ProGRP, CEA, and NSE has effective clinical application value for the pathological classification of lung cancer. The joint determination has high diagnostic efficiency and is suitable for screening of lung cancer.

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

***Ethical consideration***: All patients signed a documented, voluntarily informed consent form. All methods were carried out in compliance with the Helsinki Declaration criteria, and this study was authorized by our institution's Ethics Committee.

***Author contribution***: Dong Zang conceived and designed the experiments. Wenwang Liu contributed significantly to the experiments and arranging data. Wenwang Liu and Dong Zang performed data analyses. Wenwang Liu wrote the draft manuscript. Dong Zang revised the manuscript. All authors read and approved the final manuscript.

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