RESEARCH ARTICLE

Evaluation of the clinical application of multiple tumor marker protein chip in the diagnostic of lung cancer

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Background: The early diagnostic of lung cancer plays an important role in the prognosis of surgical treatment among lung cancer patients. To evaluate the clinical application of multi-tumor markers protein biochip in the diagnosis of lung cancer, 12 tumor markers were detected in patients with different stages of lung cancer.

Methods: Serum CA125, CA19-9, Ferritin, CA15-3, CA242, CEA, AFP, NSE, PSA, f-PSA, HGH, and β -HGH were assessed in 506 patients, with 224 patients with lung cancer (including 123 cases of adenocarcinoma, 30 squamous cell carcinoma, 54 small-cell carcinoma, and 17 non classification), 159 patients with benign lung disease and 90 healthy people control by the C-12 multiple tumor protein-chip detective system.

Results: The positive rate of C-12 (77.23%) in lung cancer was significantly higher than that of benign lung disease (13.84%) and healthy people (9.76%) ($P < .01$). In lung cancer, the positive rate of CA199, NSE, CEA, CA242, Ferritin, f-PSA, and CA125 were significantly higher than that of benign lung disease and healthy people. In adenocarcinoma, the positive rate of CA125 (73.53%) was significantly higher than that of squamous cell carcinoma (36.67%) and small-cell carcinoma (56.62%).

Conclusion: The C-12 multiple tumor protein-chip detective system has acceptable sensitivity in the diagnostic of lung cancer.

KEYWORDS

lung cancer, multi-tumor markers protein biochip, tumor markers

1 | INTRODUCTION

One of the most common malignant cancers namely lung cancer, with an estimated 1.8 million new cases merely occurring in 2012, accounts for about 13% of total cancer diagnoses throughout the world.¹ In China, lung cancer accounts for most of the emerging cancers and is the leading cause of mortality of all cancers.² Majority of patients have been diagnosed in the middle stage or late stage, and thus are unable to receive surgical treatment. Five years relative survival rate of lung cancer is <15% and patients with symptoms are even lower.³ However, the survival rate of patients with lung cancer may increase and the prognosis is obviously improved when surgical treatment is proceeding at early stage.⁴

Therefore, early diagnosis is essential for the improvement of the survival rate of lung cancer patients. There are several methods to make the diagnosis for lung cancer, such as chest radiographs, tumor markers, and pathological examinations. Radiographic inspection is an important test method for the diagnosis of lung cancer, but it is difficult to distinguish benign pulmonary nodules from malignant tumors.⁵ Pathological examination needs to take biopsy samples, which is invasive and has the risk of injury. Despite high sensitivity of pathological examination, false-negative results may be obtained due to the limitation for the position from where the samples were taken.⁶

Tumor markers, commonly used as auxiliary index for diagnosing cancer, have been widely applied in clinical practice

FIGURE 1 Typical layout of each chip and the designs of the subarrays. A, Layout of 1 agarose-modified glass slide containing 16 physically isolated subarrays. B, B and A were one-to-one correspondence, and Samples 1-16 were the serum samples to be tested, with SDs0-4 as calibrator and Control as quality control serum in each chip. C, All the initial probes with 4 lines $\times$ 6 rows, including 12 antibodies (namely, anti-HGH antibody, anti-f-PSA antibody, anti-NSE antibody, anti-CA125 antibody, anti-Ferritin antibody, anti-CA199 antibody, anti-AFP antibody, anti-CEA antibody, anti-CA242 antibody, anti- β -HCG antibody, and anti-CA153 antibody) were printed onto agarose-modified glass slides in 2 replicates of a row and blocked, which were completed by the manufacturer before selling

FIGURE 2 Procedure of the protein chip-chemiluminescence method for simultaneous and rapid detection of 12 multi-tumor markers and results interpreted

for many years.⁷ Compared with radiographs and pathological examination, tumor markers detections are convenient and inexpensive with fewer invasions. Several tumor markers,

including carcinoembryonic antigen (CEA), cytokeratin fragment 19 (CYFRA21-1), human epididymis protein 4 (HE4), have been identified with considerable clinical significance for diagnosis

TABLE 1 Positive rate of total and C-12 tumor markers in the 3 groups (%)

Group	Number	Total positive rate (positive cases)	CA199	NSE	CEA	CA242	Ferritin	β -HCG	AFP	f-PSA	PSA	CA125	HGH	CA153
Lung cancer	224	77.23 (180)	37.05 ^a	9.82 ^a	61.16 ^a	21.07 ^a	17.41 ^a	3.13	4.46	4.91 ^a	3.13	70.98 ^a	0.45	10.71 ^a
Hospitalized	159	13.84 (22)	18.87 ^b	3.77 ^b	35.22 ^b	9.43 ^b	22.01 ^b	1.26	2.52	5.66 ^a	4.40	74.21 ^a	0	5.66 ^b
Control	123	9.76 (12)	4.76 ^c	0 ^c	2.38 ^c	0 ^c	0 ^c	0	0	0 ^c	2.38	2.38 ^c	0	0 ^c

In the same column, different number (eg, a and b, b and c, a and c) showed there was significant difference between the lung cancer, hospitalized and control group ($P < .05$). In the same column, the same number (eg, a and a, b and b, c and c) showed there was no significant difference between the lung cancer, hospitalized and control group ($P > .05$).

and prognosis of lung cancer.⁸⁻¹¹ In actual fact, the sensitivity and specificity of single tumor marker detection is lower than the combined measurement of multiple tumor markers. The principal objective of the present study is to evaluate the predictive value of C-12 multiple tumor marker protein-chip detective system in lung cancer screening of apparently healthy populations and cancer patients.

2 | MATERIALS AND METHODS

2.1 | Patients and serum samples

Three groups of people were selected between January 2014 and December 2015 from the No. 150 Central Hospital of PLA. The first group included 224 lung cancer patients, with 151 men and 73 women. The ages of the 224 patients ranged from 23 to 92 years old, and the median age was 64.00 ± 12.62 years old. The diagnosis of each patient was confirmed by cytological diagnosis and/or immunohistochemistry (IHC) test. Patients with recurrence lung cancer were excluded. A total of 123 cases were adenocarcinoma (additionally including adenosquamous carcinoma), 30 cases were squamous cell carcinoma, 54 cases were small-cell carcinoma, and 17 non classification. The second group was composed of 159 patients with benign pulmonary diseases, including pneumonia, tuberculosis, acute bronchitis, chronic bronchitis, bronchiectasis, and bronchial asthma that were confirmed by routine standard diagnostic methods or histological examination. Patients with a history of malignant disease, digestive or kidney disease, or 2 or more concomitant lung diseases were excluded. Of the 159 patients, 124 were men and 35 were women. The ages of the 159 patients ranged from 17 to 93 years old, and the median age was 62.01 ± 16.91 years old. The third group contained 123 people (78 men and 45 women) who attended in physical examination in outpatient during the same period, except for those with a family history of lung cancer. The ages of the 123 people ranged from 23 to 93 years old, and the median age was 60.12 ± 17.34 years old. This study was approved by the Ethics Committee of the No. 150 Central Hospital of PLA.

2.2 | Sample collection and detection

A 2 mL fasting venous blood sample was collected from each patient in the morning into a sterile tube. After centrifugation at 716 *g* for 5 minutes, the supernatants were aspirated and then applied into detection or stored at 4°C within 7 days. The concentration of the multi-tumor markers was quantified by a diagnostic kit (Huzhou HealthDigit corp.). The kit consisting of protein chip, concentrated washing buffer, reaction solutions, calibration solutions, control, solvent complex, and Test solution A & B. In particular, each of protein chips (namely, the reacting chips) includes 16 physically isolated subarrays whose layout is shown in Figure 1A. The samples were performed following the manufacturer's instructions with some

modifications shown in Figure 2. The results were read by HD-2001A/LU-07 biochip reader (SHMY HealthDigit Biochips Co., Ltd. Shanghai, China).

2.3 | Detection indicator and normal reference range

Detection indicator and normal reference range were as follows: CA125 < 35 U/mL, CA199 < 35 U/mL, Ferritin < 219 ng/mL (female) and 322 ng/mL (male), CA153 < 35 U/mL, CA242 < 20 U/mL, CEA < 5 ng/mL, AFP < 20 ng/mL, NSE < 13 ng/mL, PSA < 5 ng/mL, f-PSA < 1 ng/mL, HGH < 7.5 ng/mL, and β -HGH < 3 mIU/mL. Except Ferritin, if the tested result of any other tumor markers is higher than the normal reference range, the detected tumor marker can be defined as positive, otherwise negative.

2.4 | Statistical methods and data analysis

Statistical analysis was performed using SPSS Statistics 19.0 (SPSS, Inc., Chicago, IL, USA). The difference of rate between groups was calculated by Chi-square, and the level of serum markers between different lung cancers characteristics was compared by Student's *t* test. *P* < .05 was considered significant.

3 | RESULTS

3.1 | Comparison of the positive detection rates among the 3 groups

Positive rates of tumor markers among the 3 groups (namely lung cancer, hospitalized patients, and control) were included in the present study (shown in Table 1). The positive rate of the lung cancer group was 77.23%, the hospitalized patients' group was

13.84% and the control group was 9.76%. Compared with the hospitalized patients and control group, the positive rate of the lung cancer group was significantly higher and calculated by χ^2 test ($\chi^2 = 239.1$, *P* < .01; $\chi^2 = 300.0$, *P* < .01). The positive rate of CA199, NSE, CEA, CA242, Ferritin, and CA153 were significantly higher in patients with lung cancer than those with benign disease or apparently healthy people (*P* < .05), but not in β -HCG, AFP, PSA, and HGH (*P* > .05). In addition, there was no significant difference in f-PSA or CA125 between the cancer group and the hospitalized patients (*P* > .05).

3.2 | Serum level of C-12 tumor markers among the 3 groups

Serum levels of the tumor markers are shown in Table 2. Compared with the hospitalized patients and control, serum levels of CA199, CEA, CA242, β -HCG, AFP, CA125, and CA153 in cancer patients were significantly higher (*P* < .05). There was no significant difference in the concentration of β -HCG, PSA, or HGH among the 3 groups (*P* < .05).

3.3 | Positive rates in different lung cancer based on cytological diagnosis and/or IHC

Positive rates of tumor markers among different lung cancer based on cytological diagnosis and/or IHC are shown in Table 3. In detail, the positive rates of adenocarcinoma, squamous cell carcinoma, and small-cell carcinoma with joint detection were successively 69.92%, 60.00%, and 70.37%. Calculated by χ^2 test, the positive rates of CEA, CA242, CA125, and CA153 in adenocarcinoma were apparently higher than that of squamous cell carcinoma, and the positive rate of CA125 was obviously higher than that of squamous cell carcinoma (*P* < .05).

TABLE 2 Serum level of C-12 tumor markers in the 3 groups ($\bar{x} \pm s$)

Group	Lung cancer (n = 224)	Hospitalized (n = 159)	Control (123)
CA199 (U/mL)	91.84 $\pm$ 176.45 ^a	36.06 $\pm$ 87.11 ^b	11.17 $\pm$ 6.93 ^c
NSE (ng/mL)	8.65 $\pm$ 14.96 ^a	6.00 $\pm$ 5.33 ^a	3.74 $\pm$ 2.34 ^c
CEA (ng/mL)	38.12 $\pm$ 58.34 ^a	16.69 $\pm$ 33.75 ^b	2.02 $\pm$ 0.99 ^c
CA242 (U/mL)	28.39 $\pm$ 51.91 ^a	12.09 $\pm$ 30.05 ^b	4.39 $\pm$ 2.47 ^c
Ferritin (ng/mL)	172.29 $\pm$ 125.4 ^a	130.06 $\pm$ 129.20 ^a	92.64 $\pm$ 59.34 ^c
β -HCG (ng/mL)	1.95 $\pm$ 12.72	2.47 $\pm$ 21.68	0.35 $\pm$ 0.13
AFP (ng/mL)	9.46 $\pm$ 28.93 ^a	4.29 $\pm$ 7.76 ^b	2.26 $\pm$ 1.12 ^c
f-PSA (ng/mL)	0.28 $\pm$ 0.52 ^a	0.69 $\pm$ 4.46 ^c	0.22 $\pm$ 0.21 ^a
PSA (ng/mL)	1.71 $\pm$ 7.37	1.48 $\pm$ 3.48	1.23 $\pm$ 1.96
CA125 (U/mL)	158.91 $\pm$ 226.06 ^a	112.01 $\pm$ 132.56 ^b	12.74 $\pm$ 6.40 ^c
HGH (ng/mL)	0.62 $\pm$ 4.92	0.46 $\pm$ 0.77	0.28 $\pm$ 0.27
CA153 (U/mL)	14.84 $\pm$ 22.42 ^a	10.67 $\pm$ 13.15 ^b	8.14 $\pm$ 4.45 ^c

In the same row, different number (eg, a and b, b and c, a and c) showed there was significant difference between the lung cancer, hospitalized and control (*P* < .05). In the same row, the same number (eg, a and a, b and b, c and c) showed there was no significant difference between the lung cancer, hospitalized and control (*P* > .05).

TABLE 3 Positive rate of total and C-12 tumor markers in the group of the lung cancer (%)

Group	Number	Total positive rate (%)	CA199	NSE	CEA	CA242	Ferritin	β -HCG	AFP	f-PSA	PSA	CA125	HGH	CA153
Adenocarcinoma	123	69.92	44.12	8.82	61.76 ^a	35.29 ^a	32.35	5.88	2.82	2.94	2.94	73.53 ^a	0	17.65 ^a
Squamous cell carcinoma	30	60.00	22.22	0	40.56 ^b	21.11 ^b	26.67	0	0	0	0	36.67 ^b	0	10.23 ^b
Small-cell carcinoma	54	70.37	40.00	8.33	46.67 ^b	25.00 ^b	23.16	4.32	0	4.33	0	56.62 ^c	0	8.33 ^b

In the same column, different number (eg, a and b, b and c, a and c) showed there was significant difference between the adenocarcinoma, squamous cell carcinoma and small cell carcinoma group ($P < .05$). In the same column, the same number (eg, a and a, b and b, c and c) showed there was no significant difference between the adenocarcinoma, squamous cell carcinoma and small cell carcinoma group ($P > .05$).

3.4 | Serum level of C-12 tumor markers among 3 different lung cancers

Concentration of C-12 tumor markers of 3 different lung cancer is shown in Table 4. It showed a significant increase in the concentration of CA199, CEA, CA242, and CA125 in adenocarcinoma compared with squamous cell carcinoma and small-cell carcinoma ($P < .05$). In squamous cell carcinoma, the level of CA153 was higher than small-cell carcinoma ($P < .05$).

4 | DISCUSSION

Lung cancer has been the most common and lethal malignant worldwide. Characteristic of the lung cancer patients presents with inoperable, advanced disease entailing a poor prognosis. Early diagnosis of lung cancer could significantly improve the response rate and prolong survival time of patients. Tumor markers are biochemical substances produced by tumor cells and present as intracellular substances in tissues or may be released into blood or body fluids.¹² Detection of tumor markers has been widely used in cancer screen, evaluating the effectiveness of treatments and predicting prognostic information. In this study, the levels of tumor markers in serum were detecting using C-12 multiple detection system. The positive rate of CA199, NSE, CEA, CA242, Ferritin, and CA153 was significantly higher in patients with lung cancer than those with benign disease or apparently healthy people. Ferritin is an iron-storage protein and normally present in the serum and other body fluids. The level of Ferritin increased had been found in several malignancies, such as breast cancer, lymphoma, and metastatic disease.¹³⁻¹⁵ Hence, the level of ferritin may not be used to distinguish the lung cancer from the benign pulmonary. NSE is a subunit of enolase enzyme found mainly in neurons and neuroendocrine cells and is a marker of SCLC.¹⁶ CA125 is a glycoprotein found in epithelia ovarian carcinoma but not in normal adult ovarian tissues.¹⁷ In the diagnostic of lung cancer, it was reported that the sensitivity of CA125 was 44.25%.¹⁸ In the present study, the positive rate of CA125 was 70.98% in the lung cancer group, but in hospitalized patients, a high positive rate 74.21% was also observed. So, the CA125 may not be used as a valuable diagnostic marker for the screen of lung cancer with false positive. CEA is a glycoprotein that normally produced during fetal development but is not present in the blood of healthy people. Usually, CEA is considered to be a tumor marker in colorectal cancer or be associated with the prognostic of lung cancer.⁹ Recently, CEA has become the marker of choice for lung adenocarcinomas.¹⁹ In this study, the sensitivity of CEA was 61.16% in the lung cancer and 61.76% in lung adenocarcinomas. CA199 is a glycoprotein and exists as a ganglioside on tumor cell. CA199 is primarily used for the detection of cancer pancreas and gastrointestinal cancer.^{20,21} It was reported that combination of CEA and CA199 can reach a sensitivity of 91.5% in the diagnostic of lung adenocarcinoma-associated malignant pleural effusions (LAMPEs).²² In the present study, the sensitivity of the combination

TABLE 4 Serum level of C-12 tumor markers in the 3 groups ($\bar{x} \pm s$)

Group	Adenocarcinoma (n = 123)	Squamous cell carcinoma (n = 30)	Small-cell carcinoma (n = 54)
CA199 (U/mL)	137.11 $\pm$ 196.70 ^a	68.88 $\pm$ 146.20 ^b	37.23 $\pm$ 28.55 ^b
NSE (ng/mL)	8.75 $\pm$ 12.57	5.51 $\pm$ 2.90	9.11 $\pm$ 6.02
CEA (ng/mL)	40.96 $\pm$ 54.10 ^a	22.68 $\pm$ 29.37 ^b	19.93 $\pm$ 25.81 ^b
CA242 (U/mL)	46.87 $\pm$ 66.57 ^a	14.40 $\pm$ 20.11 ^b	13.41 $\pm$ 12.04 ^b
Ferritin (ng/mL)	174.64 $\pm$ 133.65	223.66 $\pm$ 126.07	185.73 $\pm$ 97.71
β -HCG (ng/mL)	3.16 $\pm$ 10.64	0.50 $\pm$ 0.40	8.21 $\pm$ 53.63
AFP (ng/mL)	9.28 $\pm$ 16.69	7.28 $\pm$ 6.56	4.64 $\pm$ 4.66
f-PSA (ng/mL)	0.24 $\pm$ 0.20	0.12 $\pm$ 0.07	0.50 $\pm$ 0.69
PSA (ng/mL)	4.01 $\pm$ 17.76	0.55 $\pm$ 0.39	0.99 $\pm$ 0.41
CA125 (U/mL)	179.41 $\pm$ 227.12 ^a	80.46 $\pm$ 83.88 ^b	61.15 $\pm$ 81.03 ^b
HGH (ng/mL)	0.27 $\pm$ 0.32	0.35 $\pm$ 0.33	0.21 $\pm$ 0.11
CA153 (U/mL)	21.62 $\pm$ 37.43 ^a	19.85 $\pm$ 16.78 ^a	10.82 $\pm$ 13.47 ^b

In the same row, different number (eg, a and b, b and c, a and c) showed there was significant difference between the adenocarcinoma, squamous cell carcinoma and small cell carcinoma ($P < .05$). In the same row, the same number (eg, a and a, b and b, c and c) showed there was no significant difference between the adenocarcinoma, squamous cell carcinoma and small cell carcinoma ($P > .05$).

of CEA and CA199 was 69.64% in the lung cancer and 71.43% in adenocarcinoma.

In summary, the C-12 multiple tumor protein-chip detective system has acceptable sensitivity in the early screen for lung cancer, but cannot be used as a confirmed method.

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