Diagnostic Significance of Combined Detection of Serum Tumor Markers in Lung Cancer

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Abstract: Serum tumor marker (TM) is one of the major research topics of modern oncology, while the serum detection of lung cancer (LC) has become an important target for the diagnosis of malignancy. However, a single TM would not be satisfactory in both sensitivity and specificity for detection, which shows its limitation in diagnosing LC. Thus, for LC patients, we combined the examination of lung tumor-associated antigen (LTA), cytokeratin fragment antigen 21-1 (CYFRA21-1), carcinoembryonic antigen (CEA) and neuron specific enolase (NSE) in the diagnosis of this mortal disease. The aim of this study is to evaluate the role of the combined detection in the diagnosis of lung cancer. In our series, patients were divided into three groups named lung cancer (LC) group, benign lung disease (BLD) group and control group. The LC group was then subdivided into two subgroups named non-small cell lung cancer (NSCLC) subgroup and small cell LC(SCLC) subgroup. The NSCLE patients were further divided into squamous cell carcinoma subjects and conducted adenocarcinoma patients. The latex agglutination (LA) assay was performed to measure the LTA level of all the subjects. Radioimmunoassay (RIMA) was performed to detect CYFRA21-1, CEA and NSE. The significant data of contrasts among various groups were found through variance analysis and χ^2 was used to compare the positive rates. The positive rates of LTA in NSCLC, CYFRA21-1, NSE and CEA in LC patients were much higher than those in BLD patients and normal control with significant difference between them ($P<0.01$). The positive rates of LTA in NSCLC, CYFRA21-1 in squamous carcinoma, CEA in adenocarcinoma and NSE in SCLC were 75.6%, 84.6%, 81.4% and 82.3% respectively, with a significant difference between them ($P<0.01$). Obviously, there was a correlation between their positive rates and the pathological types of LC. The serum levels of LTA, CYFRA21-1, CEA and NSE in stage Ⅲ and stage Ⅳ patients were much higher than those in stage Ⅰ and stage Ⅱ with a significant difference ($P<0.01$). The positive rates of combined detection of LTA, CYFRA21-1, CEA and NSE were higher than detecting one or three of the above four items. We think LTA is sensitive in distinguishing LC from BLD, among which NSCLC is the highest. This may aid the diagnosis of LC. CYFRA21-1, NSE and CEA are valuable for LC diagnosis. Squamous carcinoma has the highest CYFRA21-1 positive rates, whereas pulmonary adenocarcinoma shows the highest CEA positive rates. For SCLC, the highest positive rates are showed in NSE. The united detection of LTA, CYFRA21-1, CEA and NSE is effective in raising LC detection rate. [Life Science Journal. 2006;3(1):35-39] (ISSN: 1097-8135).

Keywords: lung cancer; tumor-associated antigen (LTA); cytokeratin fragment antigen 21-1 (CYFRA21-1); carcinoembryonic antigen (CEA); neuron specific enolase (NSE)

1 Introduction

Lung cancer is one of the leading causes of cancer death throughout the world with more than one million death annually. The poor survival rates are due to the propensity for early spread, lack of effective tools for screening and early diagnosis, and the inability of systemic therapy to cure metastatic focuses. The discovery that many tumor markers were shed into the circulation led to great expectations that a serum tumor markers test could be developed to detect early lung cancer (Ando, 2003). Many studies evaluated tumor markers such as NSE, CEA, CYFRA21-1 and LTA, which were found through detection of NSCLC (non-small cell lung cancer) (Nackaerts, 1997). Unfortunately, none of these tumor markers proved to be useful or cost-effective in lung cancer screening. However, we have conducted combined detection of the LTA, CYFRA21-1, CEA and NSE in the diagnosis of this mortal disease in order to evaluate the clinical significance of combined detection in the diagnosis of lung cancer.

2 Materials and Methods

Subjects (n = 201) were divided into 3 groups: normal group (control, n = 33, male 13, female 20, age between 22 - 78 years); benign

lung disease group (BLD, $n=69$, male 37, female 32, age between 24 – 84 years; including acute bronchitis 9 cases, pneumonia 38 cases, pulmonary tuberculosis 10 cases, bronchial asthma 9 cases, bronchiectasis 3 cases) and lung cancer group ($n=99$, male 62, female 37, age between 36 – 78 years); according to pathology diagnosis: NSCLC 82, squamous carcinoma 39, adenocarcinoma 43, small cell lung cancer (SCLC) 17. The stages were sorted as UICC standard (1997), stage Ⅰ + Ⅱ: 17 cases (NSCLC 13 cases, SCLC 4 cases), stage Ⅲ: 47 cases (NSCLC 39 cases, SCLC 8 cases), stage Ⅳ: 35 cases (NSCLC 30 cases, SCLC 5 cases).

Serum were collected and stored at −20℃. The latex agglutination (LA) assay was performed to measure the LTA level. Radioimmunoassay (RIMA) was performed to detect the CYFRA21-1, CEA and NSE levels. Statistical analysis was carried out by SPSS 10.0 software.

3 Results

3.1 The levels of four tumor markers in lung cancer, BLD and control groups (Table 1)

According to the results of tumor marker levels from the normal control cases ($n=33$), we determined the normal ranges as LTA < 50 units, CEA < 15 μg/L, NSE < 20 μg/L, CYFRA21-1 < 3.3 μg/L.

3.2 The positive rates, specificity and sensitivity of four tumor markers in lung cancer, BLD and control groups (Table 2)

We detected 16 false positive cases in 69 BLD patients, which were consisted of LTA ($n=9$), CEA ($n=4$), NSE ($n=1$), CYFRA21-1 ($n=2$). The difference of LTA levels might be used to distinguish lung cancer from benign lung diseases.

3.3 Positive rates of tumor markers in different lung cancer groups (Table 3)

According to different types of lung cancer, there were different positive rates of tumor markers. The positive rates of LTA, CEA, CYRFA21-1 in NSCLC patients were significantly higher than those of SCLC patients. An even higher CEA positive rate (81.4%) was found in adenocarcinoma than in squamous carcinoma patients ($P<0.05$). The positive rate of CYRFA21-1 was 84.6%, which was much higher than the adenocarcinoma's level ($P<0.05$). The NSE positive level was 82.3%, markedly exceeding the NSCLC patients' level ($P<0.01$).

3.4 The positive rates of tumor markers in different stages of lung cancer (Table 4)

The LTA, CYFRA21-1, CEA and NSE levels in stage Ⅰ, Ⅱ were significant lower than those in stage Ⅲ, Ⅳ ($P<0.01$). Moreover, the tendency was increasing of four detected tumor markers accompanied by the disease development.

3.5 The positive rates in lung cancer that were determined by combinative detection of the four tumor markers can be seen from Table 5.

The positive rate of lung cancer has been up to 94.9%.

Table 1. Tumor marker levels in different groups ($\bar{\chi}\pm S$)

Group (cases)	LTA (units)	CYFRA21-1 (μg/L)	CEA(μg/L)	NSE(μg/L)
Lung cancer(99)	158.4±87.3*	13.9±11.4*	29.5±27.8*	21.0±29.2*
BLD(69)	60.3±12.5△	1.7±1.2△	10.4±2.2△	12.6±6.8△
Control(33)	30.0±12.6	1.3±0.9	11.4±1.8	11.1±4.5

*Comparisons between lung cancer, BLD, control groups, $P<0.05$; △Comparisons between BLD and control groups, $P>0.05$

Table 2. The positive rates, specificity and sensitivity of four tumor markers in different groups (%)

Lung cancer	LTA	CYFRA21-1	CEA	NSE
Positive rates				
NSCLC	75.6(62/82)	73.2(60/82)	74.4(61/82)	32.9(27/82)
SCLC	29.4(5/17)	29.4(5/17)	35.3(6/17)	82.3(14/17)
Specificity	89.2(91/102)	98.0(100/102)	96.1(98/102)	99.0(101/102)
Sensitivity	67.7(67/99)	65.7(65/99)	67.7(67/99)	41.4(41/99)

Specificity = control groups negative cases / BLD + normal groups cases; Sensitivity = Positive rates of cancer groups / cancer groups cases

Table 3. The positive rates of tumor markers in different types of lung cancers (%)

Group (cases)	LTA	CYFRA21-1	CEA	NSE
NSCLC(82)	75.6*(62/82)	73.2*(60/82)	74.4*(61/82)	32.9(27/82)
Squamous carcinomas(39)	79.5(31/39)	84.6#(33/39)	66.7(26/39)	38.5(15/39)
Adenocarcinomas(43)	72.1(31/43)	62.8(27/43)	81.4&(26/43)	27.9(12/43)
SCLC(17)	29.4(5/17)	29.4(5/17)	35.3(6/17)	82.3△(14/17)

*Comparisons between NSCLC、SCLC, $P<0.01$; △Comparisons between SCLC、NSCLC, $P<0.01$; # Comparisons between squamous carcinomas、adenocarcinomas, $P<0.05$; &Comparisons between adenocarcinomas、squamous carcinomas, $P<0.05$

Table 4. The positive rates of tumor markers in different stage of lung cancer (%)

Stage of lung cancer	Cases	LTA	CYFRA21-1	CEA	NSE
Ⅰ Ⅱ	17	41.2* (7/17)	35.3* (5/17)	35.3* (6/17)	17.6* (3/17)
NSCLC	13	46.2(6/13)	38.5(5/13)	46.2(6/13)	7.7(1/13)
SCLC	4	25.0(1/4)	0(0/4)	0(0/4)	50.0(2/4)
Ⅲ	47	68.1(32/47)	70.2 (33/47)	70.2 (32/47)	44.7(21/47)
NSCLC	39	76.9(30/39)	76.9(30/39)	76.9(30/39)	35.9(14/39)
SCLC	8	25.0(2/8)	37.5(3/8)	25.0(2/8)	87.5(7/8)
Ⅳ	35	80.0(28/35)	74.3(26/35)	77.1(27/35)	48.6(17/35)
NSCLC	30	86.7(26/30)	80.0(24/30)	83.3(25/30)	40.0(12/30)
SCLC	5	40.0(2/5)	40.0(2/5)	40.0(2/5)	100.0(5/5)

*Comparisons between Ⅰ, Ⅱ, Ⅲ, Ⅳ: $P<0.01$

Table 5. The comparison of positive rates by combinative detection of four tumor markers (%)

Combinative tumor makers	Positive rates
LTA + CEA + NSE	80.8 (80/99)
CEA + CYFRA21-1 + NSE	86.9 (86/99)
LTA + CYFRA21-1 + NSE	84.8 (84/99)
LTA + CYFRA21-1 + CEA	87.9 (87/99)
LTA + CYFRA21-1 + CEA + NSE	94.9* (94/99)

The positive rate of combinative detection: Two positive cases/cancer groups cases
*Comparisons between combined three detections, $P<0.05$

4 Discussion

Cancer of lung and bronchus indeed ranked top of all cancer death in both genders. The survival of patients with lung cancer is poor, primarily due to its early and widespread nature of metastases. By the time of diagnosis, lung cancer usually has already been disseminated, with only 20% – 30% patients having limited-stage disease (Nackaerts, 1997). Hence, developing new strategies of screening and early detection is critical. Serum tumor markers in lung cancer have long been studied in the hope of allowing early detection of the disease in asymptomatic individuals, improving diagnosis, as well as monitoring recurrence after treatments. Nonetheless, current serum biomarkers have turned out to be a non-effective clinical tool in screening and in early diagnosis.

Tumor markers of lung cancer, in general, can be classified into serum markers, tissue markers and sputum markers. Serum markers stand out as most attractive due to their easy accessibility over time. A number of serum tumor biomarkers have been studied in lung cancer in the past. Nonetheless, no practical serum tumor biomarkers exist for lung cancer. Here, we developed a method of combined detection of four tumor biomarkers in order to give a new light on screening and early diagnosis of lung cancer.

4.1 LTA and lung cancer diagnosis

Since 1970s, many researches have been focused on proteoglycan and observed that levels of proteoglycan in lung cancer were 1.7 – 3.5 times more than those of normal lung tissue. Such kind of proteoglycan has long fragment of proteoglycan, which is important in tumor's proliferation, metastasis, synthesis collagen. Chondroitin sulfate (CS) and hyaluronic acid (HA) are the basic component of proteoglycan, especially CS, which is related with the differentiation of lung cancer. Hence, detecting the level of CS might be helpful in diagnosing lung cancer (Kulpa, 2002). We have measured LTA levels in 201 cases and found that the positive rates were 6.06%, 13.0%, 75.6%, 29.4% in normal control, benign lung diseases, NSCLC and SCLC groups respectively. The specificity of LTA was 89.2% and the sensitivity was 67.7%.

4.2 Clinical applications values of CYFRA21-1, CEA, NSE in lung cancer

CYFRA21-1 lies in the cytoplasm of monolayer and polylayer tumor cells and is consisted of two monoclonal antibodies of keratin 19. The level of CYFRA21-1 will rise by the release of soluble fragments that were produced by dead tumor cell and the highest positive rate of CYFRA21-1 was found in squamous carcinomas (Brechot, 1997; Pujol, 2004). We have determined the positive rate as well as the specificity of CYFRA21-1 in lung cancer

as 65.7% and 98.0%; the positive rate in squamous carcinomas was 84.6% prior to that of NSE (38.5%) and CEA (27.9%).

CEA is one of the most widely used tumor associated markers in the diagnocancersis of lung cancer, and its diagnostic value in lung has be testified by clinical trials. CEA can been produced by lung cancer cells and it has been considered to be a better tumor marker to evaluate the patient's response to treatment, to monitor disease progression and to predict prognosis. The levels of CEA rose in about two thirds of NSCLC patients and one third of SCLC patients (Buccheri, 2003; Yoshimasu, 2003; Sakao, 2004). In our research the positive rate of CEA was 67.7% in lung cancer and 96.1% in pulmonary adenocarcinomas, in priority to CYFRA21-1 (62.8%) and NSE (27.9%).

NSE is a glycolytic enzyme and the predominant enolase was found in neural tissue (Fizazi, 1998). It has been recognized that SCLC patients frequently had increased levels of NSE at diagnosis compared with control cases (Buccheri, 2003). We observed the positive rate of NSE in lung cancer was 41.4%, specificity was 99.0%, and moreover the positive rate in SCLC patients was 82.3% with significant difference with other types of lung cancer, which was similar to the results of other researches.

Consistent with their locations of tumor markers such as CYFRA21-1 (lies in almost all epidemic cells), CEA (lies in adenoidal cells) and NSE (lies in normal neurocyte and neurosecretory cells), they are sensitive to pulmonary squamous carcinomas, pulmonary adenocarcinomas and SCLC, respectively. Our results were identical to the conclusions with the positive rate of 84.6%, 81.4%, and 82.3%.

4.3 Evaluation of combined determination of LTA, CYFRA21-1, CEA and NSE in lung cancer

In recent years tumor markers have been considered to be more and more important in diagnosis of malign diseases, but the applications of single tumor markers were limited by their low detective rates (Takamoch, 2004; Imura, 2003; Okada, 2003; Sawabata, 2002). As in lung cancer, current serum biomarkers have not been an effective clinical tool in screening or early diagnosing (Buccheri, 2003; Schneide, 2003; Pujol, 2003) Furthermore, there are different treatments for different types of lung cancer, such as pulmonary squamous carcinomas, pulmonary adenocarcinomas and SCLC. Here, we measured the combination of four tumor markers, LTA, CEA, NSE and CYFRA21-1 in order to evaluate the combined diagnostic value in lung cancer. We got the positive rates of LTA, CYFRA21-1, CEA and NSE in lung cancer that were 67.7%, 65.7%, 67.7%, and 41.4%; the specificities were 89.2%, 98.0%, 96.0%, and 99.0% respectively. The positive rates of combined detection of LTA, CYFRA21-1, CEA and NSE were higher than that detecting just one or three of the above four items. The combined detection increased the detection rates of lung cancer to 94.9%.

In conclusion, LTA, CEA, NSE and CYFRA21-1 are useful tumor markers in lung cancer diagnosis, and the united detection of LTA, CYFRA21-1, CEA and NSE is a valuable method in raising lung cancer detection rate and is especially advisable for the early diagnosis of LC and for its effective treatment.

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