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Performance Evaluation of Combined Detection of Serum CEA, CYFRA21-1, CA125, and NSE in Patients with Lung Cancer by Fluorescence Flow Cytometry

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Abstract: *Objective:* To investigate the effect of combined detection of serum carcinoembryonic antigen (CEA), cytokeratin 19 fragment (CYFRA21-1), cancer antigen 125 (CA125), and neuron-specific enolase (NSE) in patients with lung cancer by fluorescence flow cytometry. *Methods:* From August 2019 to July 2022, 200 patients with lung cancer diagnosed by pathology in our hospital were retrospectively analyzed. 2 mL venous blood was collected in a fasting state and centrifuged to separate the serum (containing human chorionic gonadotropin antibody [anti-hCG antibody], hepatitis B surface antibody [anti-HBs antibody], and CEA). *Results:* The sensitivities of CEA and CYFRA21-1 detected via enzyme-linked immunosorbent assay (ELISA) were 100%, and the detection limits were 0.5 ng/mL and 0.1 ng/mL, respectively; the sensitivities of CA125 and NSE detected via flow cytometry were 100%, and the detection limits were 10 U/mL and 2 ng/mL, respectively. Compared with ELISA, the sensitivities of CA125 and NSE detected via flow cytometry were higher. When the concentration of CEA was 10–40 ng/mL, the sensitivities of the three markers CYFRA21-1, CA125, and NSE showed no significant changes ($P > 0.05$); when the concentration of CEA was 40–80 ng/mL, the sensitivity of CEA significantly decreased ($P < 0.01$), but the sensitivities of the three markers CYFRA21-1, CA125, and NSE showed no significant changes ($P > 0.05$); when the concentration of CEA was 80–200 ng/mL, the sensitivities of all four markers showed no significant changes ($P > 0.05$). *Conclusion:* Compared with the double-antibody sandwich ELISA, fluorescence flow cytometry has certain advantages, including high sensitivity, good precision, short detection time, low sample usage, and low medical cost; thus, it is worthy of clinical promotion.

Keywords: Fluorescence flow cytometry; Lung cancer; Serum

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1. Introduction

Lung cancer is a malignant tumor with the highest global incidence. The incidence and mortality of lung cancer are on the rise globally. Lung cancer is one of the most common malignant tumors in China among which its morbidity and mortality rank first in our country. Lung cancer has surpassed liver cancer as the leading cause of death from malignant tumors. It has always been one of the malignant tumors that seriously threatens human health and life. There are about 450,000 new lung cancer cases and about 500,000 deaths in our country every year. Therefore, it is particularly important to raise awareness of the symptoms of lung cancer and its early diagnosis, considering the cruciality of lung cancer prevention and screening. Studies

have shown that 80%–90% of lung cancer patients have elevated serum carcinoembryonic antigen (CEA). CEA is related to the prognosis of lung cancer [1]. At present, commonly used tumor marker detection methods include immunochromatography, enzyme-linked immunosorbent assay (ELISA), colloidal gold immunochromatography assay [2], chemiluminescence immunoassay [3,4], *etc*. However, these methods have varying degrees of limitations, especially for the detection of markers, such as CEA, cytokeratin 19 fragment (CYFRA21-1), cancer antigen 125 (CA125), and neuron-specific enolase (NSE) [5-8] . Therefore, in our study, serum CEA, CYFRA21-1, CA125, and NSE in patients with lung cancer were detected, and a comparative analysis was conducted based on commonly used clinical tumor markers. Five serum samples from patients with lung cancer were measured in parallel, and the results were compared and analyzed by double-antibody sandwich ELISA and fluorescence flow cytometry to verify the accuracy and stability of the methods. In this study, 200 serum samples from lung cancer patients were collected, including 70 single-positive serum samples and 130 double-positive serum samples. Fluorescence flow cytometry was used to detect CEA, CYFRA21-1, CA125, and NSE in lung cancer patients.

2. Materials and methods

2.1. Specimen collection

From August 2019 to July 2022, 200 patients with lung cancer in our hospital, including 128 males and 72 females, were retrospectively analyzed. All cases were confirmed by histopathological examination: 27 cases of stage I cancer, 67 cases of stage II cancer, 30 cases of stage III cancer, and 76 cases of stage IV cancer. Patients with vital organ involvement, including the heart, liver, and kidney, were excluded. 2 mL of venous blood were collected under fasting state and centrifuged to separate the serum (including human chorionic gonadotropin antibody [anti-hCG antibody], hepatitis B surface antibody [anti-HBs antibody], and CEA).

2.2. Reagents and instruments

The assay reagents were provided by Beijing Wantai Biotechnology Co., Ltd., and the fluorescence flow cytometry method was provided by Beijing Beckman Technology Co., Ltd. The instrument used was Hitachi 7020 automatic biochemistry analyzer; ELISA was provided by Shanghai Anpu Analytical Instrument Co., Ltd., and the double-antibody sandwich ELISA kit was manufactured by Hangzhou Haide Biotechnology Co., Ltd.

2.3. Methods

Five portions of fresh serum (containing CEA, CYFRA21-1, CA125, and NSE) were taken in 1–2 mL each and placed in 2 mL Eppendorf (EP) tubes. The sample-containing tubes were placed in a refrigerator at 4°C overnight and subsequently taken and placed on plate A. Parallel samples were made on plate A with 5×10^4/L alkaline phosphatase (ALP)-labeled gold secondary antibody (20 μL in each well). The results were determined by double-antibody sandwich ELISA and flow cytometry, respectively, and the result of the double-antibody sandwich ELISA were taken as the standard.

2.4. Statistical analysis

All data were processed and analyzed by SPSS 19.0. The measurement data were expressed as mean ± standard deviation (s), and t-test was used; the count data were expressed as percentages, and chi-squared (χ^2) test was used; $P < 0.05$ in one-way analysis of variance (ANOVA) was considered statistically significant.

3. Results

Under ELISA, the sensitivities of CEA and CYFRA21-1 were 100%, and the detection limits were 0.5 ng/mL and 0.1 ng/mL, respectively; the sensitivities of CA125 and NSE were 78.96% and 81.39%, respectively, with detection limits of 10 U/mL and 2 ng/mL, respectively. Compared with ELISA, for detection by flow cytometry, the sensitivities of CA125 and NSE were 100.00%, and the detection limits were 10 U/mL and 2 ng/mL, respectively; the sensitivities of CEA and CYFRA21-1 were 100%, with detection limits of 0.5 ng/mL and 0.1 ng/mL, respectively. The sensitivities of CA125 and NSE detected via flow cytometry were higher. When the concentration of CEA was 10–40 ng/mL, the sensitivities of the four markers showed no significant changes ($P > 0.05$); when the concentration of CEA was 40–80 ng/mL, the sensitivity of CEA significantly decreased ($P < 0.01$), but the sensitivities of CYFRA21-1, CA125, and NSE showed no significant changes ($P > 0.05$); when the concentration of CEA was 80–200 ng/mL, the sensitivities of all four markers showed no significant changes ($P > 0.05$), as shown in **Table 1**.

Table 1. Sensitivity of CEA, CYFRA21-1, CA125, and NSE detected by flow cytometry

Concentration	CEA	CYFRA21-1	CA125	NSE
10–40 ng/mL	87.12%	89.21%	85.36%	84.69%
40–80 ng/mL	67.98%	89.29%	86.23%	88.99%
80–200 ng/mL	81.36%	84.36%	87.21%	87.23%

4. Discussion

In recent years, the detection of tumor markers has been a common clinical practice, as people's understanding of the occurrence and development of tumors has grown. Since tumor cells or tissues in the human body have special structures and functions, different from normal cells, their metabolites or active substances are significantly different from normal tissues, so the detection of tumor markers is of great significance for clinical diagnosis and treatment. Currently, commonly used clinical tumor markers include CA125, CYFRA21-1, NSE, cytokeratin 7 (CK7), fibroblast growth factor 13 (FGF13), and tumor necrosis factor receptor superfamily 2. In the present study, CEA, CYFRA21-1, CA125, and NSE in the serum of patients with lung cancer were detected by flow cytometry. This method has a good correlation with ELISA; the linear range of CEA is 0.20–1.00 μg/L, the linear range of CYFRA21-1 is 0.40–1.00 μg/L, the linear range of CA125 is 0.40–1.00 μg/L, and the linear range of NSE is 0.40–1.00 μg/L.

Lung cancer serum is a relatively special tumor marker. By detecting lung cancer cells in the serum, we can determine if the patient is suffering from lung cancer. The diagnosis with lung cancer serum has certain similarities with that of other tumors, but its specificity is not high; hence, it is not a good diagnostic method. Lung cancer serum is a special type of tumor marker that has certain value in the diagnosis of lung cancer. In order to determine whether a particular type of tumor marker will affect the diagnosis, the first step is to determine which other tumors this tumor marker shares features with. For instance, both liver cancer and pancreatic cancer share common features of hepatocellular carcinoma; epithelial cell carcinoma shares a common feature with bowel cancer; and squamous cell carcinoma shares common features with both breast cancer and cervical cancer. Only in this way can its diagnostic value be determined according to the characteristics of different tumors. Lung cancer, like other tumors, is characterized by abnormal changes in cell differentiation, proliferation, or apoptosis. In terms of pathological types, lung cancer not only shares the same characteristics as adenocarcinoma, but also shares similar changing patterns with squamous cell carcinoma and small cell carcinoma. Lung cancer serum, as a special type of tumor marker, can reflect the possibility of a patient suffering from lung cancer and whether the patient will deteriorate after suffering from this cancer.

With more tumor cells in the serum, the patient's condition will be more severe. In clinical diagnosis, the tumor cell content in serum has a certain relationship with lung tumors. If tumor metastasis occurs in a patient with lung cancer, the tumor cell content in serum will show a sharp rise. Therefore, if there is an abnormal increase in the patient's serum, it is necessary to be alert of the likelihood that the cancer has metastasized. However, it may also be related to other problems, such as cancer recurrence. Therefore, tumor cell content in serum cannot be used as a basis for diagnosis. When a patient is diagnosed with lung cancer, related examinations and surgical treatment should be performed in time. If the condition worsens, it should be controlled and treated. The current state of medicine cannot determine whether a patient is suffering from lung cancer; rather, it can only determine the extent of the condition through relevant markers. Proteins in serum often change over time. Some of these proteins may show abnormalities even in normal conditions, such as molecules and other biologically active substances on the surface of cells that change over time. Therefore, if a patient's serum protein is abnormal, it indicates that the patient may be suffering from lung cancer.

The method of using serum to detect lung cancer is increasingly used in clinical practice, especially in the diagnosis of early lung cancer. This detection method of lung cancer has high application value. Serum testing for different cancers yields different data that can be used to determine the need for treatment. This method can be used for the prognosis of lung cancer, in addition to the diagnosis of early lung cancer. By analyzing the number and size of tumor cells in serum, it is possible to determine whether a patient is suffering from lung cancer. However, it is impossible to determine whether treatment is required solely based on the number of cancer cells in serum, mainly because malignant tumors have strong genetic characteristics. In many cases, the number of cancer cells in serum does not show obvious changes. In addition, even if an increase in number of cancer cells in serum is detected, but the patient does not show any symptoms or obvious discomfort, then no treatment is required.

Lung cancer is one of the most common malignant tumors in clinical practice. According to statistics, its incidence rate and mortality rate rank first among all malignant tumors, showing an increasing trend year by year. Patients with early lung cancer generally have no obvious symptoms, so most patients are already at an advanced stage when they are diagnosed, thus missing the best time for treatment. Therefore, early detection and early diagnosis are particularly important. At present, the tumor markers commonly used in clinical practice mainly include CEA, carbohydrate antigen 19-9 (CA19-9), glycosyltransferase (CAGT), cytokeratin-18, *etc*. These tumor markers have high value in early screening, but their specificity and sensitivity are limited. In recent years, with the development of China's medical and health services, tumor markers have been widely used in clinical practice, but the accuracy and stability of the test require further improvement. In recent years, as a high-sensitivity and high-specificity detection technology [9], fluorescence flow cytometry has garnered widespread attention in the field of tumor marker detection. CEA is generally elevated in the serum of lung cancer patients [10-12], and NSE, which is a highly specific nucleoprotein factor comprising a small molecular protein composed of 26 amino acids, is significantly elevated in lung cancer tissues [13-15].

In conclusion, compared with double-antibody sandwich ELISA, fluorescence flow cytometry has certain advantages, including high sensitivity, good precision, short detection time, less sample consumption, and low medical cost; thus, it is worthy of clinical promotion.

Disclosure statement

The authors declare no conflict of interest.

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