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The Level of Circulating Tumor Cells in Patients with Non-Small Cell Lung Cancer and Its Relationship with Tumor Markers

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Abstract: *Objective:* To explore the level of circulating tumor cells in patients with non-small cell lung cancer and its relationship with tumor markers. *Methods:* Fifty patients with NSCLC admitted to a hospital from March 2019 to February 2022 were retrospectively selected as the research subjects; their clinical data were sorted out and analyzed. All patients were examined for CTCs. According to their levels, the patients were divided into a positive group (30 cases, ≥ 4%) and a negative group (20 cases, < 4%). The positive rate of peripheral CTCs in patients with different gender, age, and pathological types of NSCLC, the positive rate of peripheral CTCs in patients with different staging of NSCLC, and the relationship between serum CEA, CA125, CYFRA21-1, and peripheral CTCs were analyzed and observed. *Results:* There was no significant difference in gender, age, and pathological type between the positive group and the negative group. There was also no significant difference in the T staging, N staging, and M staging between the positive group and the negative group. However, there was significant difference in the clinical staging of the positive group and the negative group. The CEA, CA125, and CYFRA21-1 of the positive group were 7.45 ± 1.26, 38.56 ± 4.12, and 5.01 ± 1.36, respectively, whereas those of the negative group were 5.12 ± 1.22, 32.69 ± 4.01, and 3.87 ± 1.25, respectively. The comparison between the two groups was statistically significant. *Conclusion:* CTCs provide the possibility of detecting cancer before the use of imaging methods, guide treatment in combination with other tumor markers, monitor postoperative treatment, and predict patients' outcome.

Keywords: Circulating tumor cells; Tumor markers; Lung cancer

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1. Introduction

Lung cancer is the most common malignant tumor of the respiratory system. Its incidence and mortality rates rank first in the world, and its 5-year survival rate is only about 15% [1]. Local epidemiological studies have found that the incidence rate of lung cancer is increasing year by year, posing a great threat to people's physical and mental health. Among them, non-small cell lung cancer (NSCLC) accounts for more than 80% [2]. At present, it is generally believed that it originates from lung epithelial cells and encompasses a number of histological subtypes [3]. Pathological biopsy is still the only gold standard for diagnosing NSCLC at present; however, this procedure may cause trauma to patients and may even result in complications, such as bleeding and pneumothorax. Its clinical application is limited [4]. Therefore, it is particularly important to look for a non-invasive diagnostic method. Lung cancer has the highest incidence and mortality rates among malignant tumors, and non-small cell lung cancer (NSCLC) accounts for 85% of the cases. Although

there has been significant progress in the treatment of patients with NSCLC in recent years, the 5-year survival rate of patients is still only about 17%. Surgery is still the treatment of choice for patients with early lung cancer. Postoperative adjuvant chemotherapy may delay tumor recurrence and metastasis, but the latter remains the main cause of death in patients with lung cancer. A large number of studies have proved that circulating tumor cells (CTCs) play a key role in distant metastasis. CTCs are independent markers that can predict the survival of patients with tumors, and there is an association between the number of CTCs and tumor invasion, metastasis, as well as the time to disease recurrence. Therefore, the research on CTCs will not only promote further understanding of tumor metastasis, but also develop new clinical diagnostic methods to guide tumor treatment strategies, so that patients may benefit from quality-of-life improvement and survival-time prolongation.

2. Data and methods

2.1. General information

Fifty patients with NSCLC admitted to a hospital from March 2019 to February 2022 were retrospectively selected as the research subjects. Their clinical data were sorted out and analyzed. All the patients were examined for CTCs. According to their levels, the patients were divided into a positive group (30 cases, ≥ 4%) and a negative group (20 cases, < 4%). Patient data, including gender, age, pathological type, staging, tumor size, lymph node infiltration, tumor location, and chemotherapy type, were collected. The general data of the patients were not statistically significant.

2.2. Methods

All patients were tested for CTCs upon admission. After taking 5 ml of venous blood from each patient's median cubital vein, the tumor cells were enriched by the immunomagnetic bead negative enrichment method within 24 days; subsequently, the peripheral CTC count was detected by the CTC detection system (Lyell [Beijing] Medical Devices Co., Ltd.).

2.3. Observation indicators

The observation indicators were the positive rate of peripheral CTCs in NSCLC patients of different gender, age, and pathological type; the positive rate of peripheral CTCs in NSCLC patients with different staging; the relationship between serum CEA, CA125, CYFRA21-1, and peripheral CTCs.

2.4. Statistical analysis

SPSS 19.0 and GraphPad Prism 6.0 were used for data analysis. The experimental data were expressed in mean ± standard deviation ($\bar{x}$ ± s), one-way ANOVA was used to compare the mean of each group, and SNK-q test was used to compare the data. The difference was considered statistically significant when $p < 0.05$.

3. Results

3.1. The positive rate of peripheral CTCs in NSCLC patients of different gender, age, and pathological type

There was no statistical difference in terms of gender, age, and pathological type between the positive group and the negative group, as shown in **Table 1**.

Table 1. The positive rate of peripheral CTCs in NSCLC patients of different gender, age, and pathological type

Group		Positive group (n = 30)	Negative group (n = 20)	X^2	*p*
Gender	Male	14 (46.67)	11 (55.00)	0.3333	0.5637
	Female	16 (53.33)	9 (45.00)		
Age	< 60	28 (93.33)	17 (75.00)	0.2315	0.6304
	≥ 60	2 (6.67)	3 (15.00)		
Pathological type	Squamous cell carcinoma	13 (43.33)	9 (45.00)	0.0135	0.9075
	Adenocarcinoma	17 (56.67)	11 (55.00)		

3.2. The positive rate of peripheral CTCs in NSCLC patients with different staging

There was no statistical significance between the positive group and the negative group in terms of T staging, N staging, and M staging. In the positive group, the number of cases with clinical staging I, II, III, and IV was 2, 2, 5, and 21, accounting for 6.67%, 6.67%, 16.66%, and 70.00%, respectively. In the negative group, the number of cases with clinical staging I, II, III, and IV was 4, 1, 9, and 6, accounting for 20.00%, 5.00%, 45.00%, and 30.00%, respectively. The clinical stagings of the positive group and the negative group were statistically significant ($p < 0.05$), as shown in **Table 2**.

Table 2. The positive rate of peripheral CTCs in NSCLC patients with different staging

Group		Positive group (n = 30)	Negative group (n = 20)	X^2	*p*
Clinical staging	I	2 (6.67)	4 (20.00)	8.829	0.032
	II	2 (6.67)	1 (5.00)		
	III	5 (16.66)	9 (45.00)		
	IV	21 (70.00)	6 (30.00)		
T	T1	4 (13.33)	2 (10.00)	0.195	0.978
	T2	12 (40.00)	9 (45.00)		
	T3	6 (20.00)	4 (20.00)		
	T4	8 (26.67)	5 (25.00)		
N	N0	7 (23.33)	4 (20.00)	0.907	0.824
	N1	6 (20.00)	4 (20.00)		
	N2	10 (33.33)	9 (45.00)		
	N3	7 (23.33)	3 (15.00)		
M	M0	13 (43.33)	6 (30.00)	0.905	0.341
	M1	17 (56.77)	14 (70.00)		

3.3. The relationship between serum CEA, CA125, CYFRA21-1 and peripheral CTCs

The CEA, CA125, and CYFRA21-1 levels of the positive group were 7.45 ± 1.26, 38.56 ± 4.12, and 5.01 ± 1.36, respectively, whereas those of the negative group were 5.12 ± 1.22, 32.69 ± 4.01, and 3.87 ± 1.25, respectively. The comparison between the two groups was statistically significant, as shown in **Table 3**.

Table 3. The relationship between serum CEA, CA125, CYFRA21-1, and peripheral CTCs

Group	Positive group (n = 30)	Negative group (n = 20)	t	*p*
CEA (μg/L)	7.45±1.26	5.12±1.22	6.4866	0.0000
CA125 (U/ml)	38.56±4.12	32.69±4.01	4.9878	0.0000
CYFRA21-1 (μg/L)	5.01±1.36	3.87±1.25	2.9973	0.0043

4. Discussion

Liquid biopsy is a non-invasive, dynamic monitoring and analysis of body health and disease status through the detection of molecular or cellular markers in body fluid. Therefore, it has great potential in diagnosis, prognosis, monitoring of disease progress and efficacy, understanding disease mechanism, and drug target research [5-9]. Tumor liquid biopsy mainly refers to the analysis of cellular or non-cellular nucleic acids and proteins in the blood to provide timely and accurate dynamic progress information for cancer diagnosis and treatment, including early tumor diagnosis, risk assessment and staging, curative effect, prognosis, recurrence detection, and monitoring [10]. The main components of detection include CTCs (clusters) in the form of cells, circulating tumor DNA (ctDNA), microRNA and RNA in the form of non-cells, as well as extracellular vesicles or platelets assimilated by tumors [11]. Among them, CTCs, ctDNA, and extracellular vesicles represent the most developed and significant advancements of tumor liquid biopsy, and they show increasing value in tumor diagnosis and prognosis [12]. Although the separation of ctDNA and extracellular vesicles is simpler than that of CTCs, the applicable analysis of cfDNA and extracellular vesicles is relatively limited. For instance, ctDNA is mainly used for analyzing genomic mutations [13], whereas CTCs can be used to carry out various analyses, including cell morphology, immunophenotype, and important epitope analysis, tumor cell clone heterogeneity and evolution analysis, as well as epigenetic group, transcriptome, proteome, and metabolome analysis [6,14]. Additionally, patients suffer a great deal of discomfort and anguish with the existing puncture sampling method that is commonly used in clinical practice; even then, the required tumor cells may not be able to be collected. This invasive sampling process also increases the potential risk of cancer metastasis.

There have been a large number of studies on the clinical application value of CTCs, and the United States Food and Drug Administration has already approved the application of CTC detection in the diagnosis of breast cancer [8]. In recent years, it has been found that CTC is of great significance as a new marker in the diagnosis of liver cancer [9]. Therefore, CTC detection can be used for early diagnosis and screening of tumors. CTC PD-L1 expression detection, which is mainly used in disease monitoring and prognosis of metastatic tumors, has not been properly applied to early diagnosis. Therefore, PD-L1 expression in circulating tumor cells has a limited role in early diagnosis and screening [15-20].

Due to the limitations of tissue detection of PD-L1 expression, PD-L1(+) CTC can be used as a potential biomarker for patients with high risk of metastasis and poor prognosis. It can help to screen out the beneficiaries of immunotherapy and more accurately evaluate the prognosis and trend of disease change in patients.

In clinical examination, the aforementioned in vitro methods for detecting CTCs in blood samples are based on a basic assumption, that is, the count of CTCs in peripheral blood will not change significantly over time; however, recent studies have challenged this assumption [15]. They claimed that the distribution of CTCs from recirculation may not be uniform, and patients whom CTCs could not detected at a given point of time may not be without CTCs [11]. However, it is unrealistic to repeatedly draw patients' blood to determine the distribution of CTCs in accordance with time. Therefore, a feasible strategy is to monitor CTCs by in vivo detection. In vivo flow cytometry (IVFC) enables direct detection of mobile cells in animal models in a non-invasive, continuous manner. Based on different principles, IVFC can be divided into

fluorescent IVFC and photoacoustic flow cytometry (PAFC).

In conclusion, CTCs provide the possibility of detecting cancer before the use of imaging methods, guide treatment in combination with other tumor markers, monitor the postoperative management of patients, and predict patients' outcome.

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