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Clinical Application of Circulating Tumor Cells in Evaluating the Effect of Chemotherapy in Advanced Non-Small Cell Lung Cancer

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Abstract: *Objective:* To explore the value of circulating tumor cells in evaluating the effect of chemotherapy for advanced non-small cell lung cancer. *Methods:* Sixty-two patients with advanced non-small cell lung cancer who received chemotherapy in the Affiliated Hospital of Hebei University from January 2018 to December 2021 were selected as the research subjects. The positive rate of CTCs after two weeks of chemotherapy and four weeks of chemotherapy as well as the evaluation of imaging efficacy were observed and analyzed. *Results:* Based on the clinical data of the patients, the positive rate of CTCs in male patients was 77.78%, that in female patients was 80.77%, that in patients ≥ 60 years old was 78.13%, that in patients < 60 years old was 76.67%, that in squamous cell carcinoma was 79.31%, that in adenocarcinoma was 78.79%, and that in highly differentiated, moderately differentiated, and poorly differentiated + undifferentiated CTCs was 66.67%, 84.21%, and 90.91%, respectively; there was no statistical difference in the general data. The positive rate of CTCs was 88.71% before chemotherapy and 66.13% after two weeks of chemotherapy, in which the difference was statistically significant; the positive rate of CTCs four weeks after chemotherapy was 59.68%, which was statistically significant compared with that before chemotherapy; however, there was no significant difference between two weeks after chemotherapy and four weeks after chemotherapy. After chemotherapy, 35 patients had CR+PR, 19 patients had SD, and 8 patients had PD. The proportions of CR+PR, SD, and PD in the imaging evaluation results were 56.45%, 30.65%, and 12.90%, respectively. After kappa consistency test, it was found that the consistency was good. *Conclusion:* CTCs can be used as one of the indicators to evaluate the effect of chemotherapy for advanced non-small cell lung cancer. The results are consistent with those of imaging evaluation. The detection of CTCs can be widely used as one of the clinical indicators.

Keywords: Circulating tumor cells; Non-small cell lung cancer; Chemotherapy

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1. Introduction

Lung cancer can be divided into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) based on the pathology, biological characteristics, therapeutic efficacy, and prognosis. NSCLC accounts for 85% of lung cancer cases, wherein 50% of them are lung adenocarcinoma and 30% are lung squamous cell carcinoma [1-5]. In the past, the difference in pathological type and stage is the main basis for formulating the treatment plan for lung cancer. However, since the human genome project was launched in 1990, it has been found that there are completely different abnormal gene expressions in different pathological types of lung cancer. In 2004, Paez et al. found that EGFR mutation in patients with NSCLC is closely related to the efficacy of gefitinib [6]. In recent years, a new method called “liquid biopsy” has garnered attention in

the treatment of lung cancer. Liquid biopsy, as a non-invasive detection method, is considered the best method. Just by collecting blood, urine, and other body fluid samples from patients and detecting circulating tumor cells (CTC), circulating tumor DNA (ctDNA), and exosomes in these samples, the occurrence and development of tumors can be identified, thus assisting in the diagnosis and treatment of tumors. Direct clinical applications of liquid biopsy include the following: early detection of cancer, estimation of overall tumor heterogeneity (primary and metastatic), real-time tracking of tumor dynamics, effective mapping of tumor molecular circuit for targeted therapy, early evaluation of treatment response, effective monitoring of minimal residual disease, and real-time quantification of treatment resistance and disease evolution [7,8].

2. Methods

2.1. Study population

Sixty-two patients who received chemotherapy for advanced non-small cell lung cancer in the Affiliated Hospital of Hebei University from January 2018 to December 2021 were selected as the research subjects. Patients who met the diagnostic criteria for advanced non-small cell lung cancer and with complete clinical data were included in the study, whereas patients with incomplete clinical data, other major diseases, and mental disorders were excluded.

2.2. Study design

CTC collection, detection, and positive standard: NSCLC patients received advanced first-line treatment, including chemotherapy, radiotherapy, targeted therapy, and others (mainly zoledronic acid, an anti-bone metastasis therapy); blood samples were collected within the first day (baseline) before the commencement of the late first-line treatment and within one month after the end of the last treatment (after treatment), which was defined as failure of first-line treatment and disease progression; the requirements for sample collection included a volume of 7.5 ml of peripheral blood on an empty stomach in the morning; the CTC detection was based on the CellSearch system; several literatures [9,10] were referred to in order to identify the relevant reagent consumables, CTC detection methods, and judgment standards; the positive standard of CTC in this study was considered as ≥ 1 CTC under microscope.

The main follow-up methods were outpatient and telephone follow-ups. As of December 31, 2021, all 62 patients in the group completed their follow-ups, with a follow-up rate of 100%. The end point of the follow-up was determined as PD or death. The median follow-up time was 16 months.

2.3. Indicators

The positive rate of CTCs after two weeks of chemotherapy and four weeks of chemotherapy as well as the evaluation of imaging efficacy.

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. A p value of less than 0.05 showed that the difference was statistically significant.

3. Results

3.1. Comparison of clinical data

Based on the clinical data of the patients, the positive rate of CTCs in male patients was 77.78%, that in female patients was 80.77%, that in patients ≥ 60 years old was 78.13%, that in patients < 60 years old was 76.67%, that in squamous cell carcinoma was 79.31%, that in adenocarcinoma was 78.79%, and that in

highly differentiated, moderately differentiated, and poorly differentiated + undifferentiated CTCs was 66.67%, 84.21%, and 90.91%, respectively. There was no statistical difference in the general data, as shown in **Table 1**.

Table 1. Comparison of clinical data

Group		CTCs		Positive rate (%)	χ^2	*p*
		Positive	Negative			
Gender	Male (n=36)	28	8	77.78	0.0815	0.7753
	Female (n=26)	21	5	80.77		
Age	≥ 60 years old (n=32)	25	7	78.13	0.0188	0.8988
	< 60 years old (n=30)	23	7	76.67		
Pathological type	Squamous cell carcinoma (n=29)	23	6	79.31	0.0025	0.9601
	Glandular cancer (n =34)	26	7	78.79		
Degree of pathological differentiation	Highly differentiated (n=12)	8	4	66.67	–	–
	Medium differentiation (n=38)	32	6	84.21		
	Poorly differentiated + undifferentiated (n=11)	10	2	90.91		

3.2. Comparison of CTC positive rates before and after chemotherapy

The positive rate of CTCs was 88.71% before chemotherapy and 66.13% after two weeks of chemotherapy, which was statistically significant compared with that before chemotherapy. At four weeks after chemotherapy, the positive rate of CTCs was 59.68%, which was statistically significant compared with that before chemotherapy. However, there was no significant difference between two weeks after chemotherapy and four weeks after chemotherapy, as shown in **Table 2**.

Table 2. Comparison of CTC positive rates before and after chemotherapy

Group	CTCs		Positive rate (%)	χ^2	*p*
	Positive	Negative			
Before chemotherapy	55	7	88.71		
Two weeks after chemotherapy	41	21	66.13	9.0417	0.0026
Four weeks after chemotherapy	37	25	59.68	13.6467	0.0002

3.3. Evaluation of image efficacy after chemotherapy

After chemotherapy, 35 patients had CR+PR, 19 patients had SD, and 8 patients had PD. The proportions of CR+PR, SD, and PD in the imaging evaluation results were 56.45%, 30.65%, and 12.90%, respectively. After kappa consistency test, it was found that the consistency was good, as shown in **Table 3**.

Table 3. Evaluation of image efficacy after chemotherapy

CTC change	Imaging evaluation results			Total	Kappa consistency test	*p*
	CR+PR	SD	PD			
Decrease (Effective)	30	5	0	35	0.578	< 0.001
Constant (Stable)	5	11	3	19		
Increase (Progress)	0	3	5	8		
Total	35	19	8			

4. Discussion

CTC refers to tumor cells that have separated from the primary or metastatic tumor and entered the blood circulation. Studies have shown that the formation of CTC is the premise of tumor blood metastasis [11], which is closely related to the pathological stage and prognosis of tumor [12]. In previous studies, it was reported that the CTC count in the peripheral blood of patients with stage IV NSCLC was significantly higher than that of patients with stage III NSCLC, and the CTC count was related to distant metastasis [13,14]. The correlation between baseline CTC count and patient prognosis has been confirmed in several studies using CellSearch [15,16]. Therefore, CTC detection is far superior to the current widely used clinical detection method as it has great application potential in the auxiliary diagnosis, curative effect evaluation, and prognosis of malignant tumors [7,8].

Circulating cell-free DNA (ccfDNA) is a type of DNA that exists in plasma or serum body fluid. It is free outside the cell and has no cellular state. It may be derived from apoptosis or necrosis and can be separated from plasma [8]. It can be detected in the blood of normal people, patients with malignant tumors, those with non-malignant diseases, such as systemic lupus erythematosus, rheumatoid arthritis, and pulmonary embolism, pregnant women, or even those receiving invasive treatment measures [9]. However, according to several research reports, the amount of cfDNA in healthy people is very small, about 1-100 μg/L, with 30 μg/L on average. The amount of cfDNA in patients with tumor is as high as 1000 μg/L, with an average of 180 μg/L. The amount of cfDNA in the blood increases when the human body is in a state of tumor, autoimmune disease, inflammatory reaction, trauma, exercise, myocardial infarction, end-stage renal failure, and so on [10-14].

As a type of cfDNA, ctDNA is a free DNA fragment in human blood. It carries a lot of information about tumors, including gene mutation, deletion, insertion, rearrangement, copy number abnormality, methylation, and others. It also reveals the genetic information of tumors and accurately reflects the heterogeneity of tumor tissue and tumor load. Furthermore, ctDNA has potential long-term longitudinal monitoring ability as it can be sampled repeatedly in the same patient. It is secreted and released by tumor cells following necrosis and apoptosis. The genomic information it carries is consistent with the tumor tissue, and it integrates the genetic material information from the primary tumor and after tumor metastasis. Its fragment size is about 166 bp [13]. When a large number of cancer cells proliferate, apoptosis and necrosis are bound to happen. The released nucleic acid exceeds its own clearance capacity, and thus the amount of ctDNA in the body increases. Hence, in cancer patients, the concentration of free ctDNA is higher than that in normal healthy people. Existing research shows that the abnormal increase of cfDNA in patients with breast cancer, liver cancer, gastric cancer, colorectal cancer, ovarian cancer, and other cancers is higher than that in normal healthy people [14]. Some scholars believe that the abnormal increase of cfDNA in the plasma of these cancer patients is attributed to the apoptosis and necrosis of cancer cells or the active release of tumor cells. This part of cfDNA is called circulating tumor DNA (ctDNA), which accounts for 0.01%–90% of cfDNA [15]. In a study, the ctDNA concentration in the plasma of NSCLC patients who received radical surgical resection, chemotherapy, radiotherapy, and other different treatment methods was dynamically monitored; it was found that the change of tumor load in early or late NSCLC patients can be reflected by the concentration of ctDNA in the plasma [16]. Therefore, CT DNA can be used as a new molecular biomarker for early diagnosis of lung cancer and evaluation of therapeutic efficacy [17-21].

In conclusion, CTCs can be used as an indicator to evaluate the effect of chemotherapy for advanced non-small cell lung cancer. Since the results are consistent with the imaging evaluation results, CTC detection can be widely used in clinical practice as one of the clinical indicators.

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Disclosure statement

The authors declare no conflict of interest.

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