

Clinical Value of Peripheral Blood Circulating Tumor Cells and Cell-Free DNA Combined Detection in Triple-Negative Breast Cancer

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Abstract: *Objective:* To determine the clinical value of combined detection of circulating tumor cells (CTCs) and cell-free DNA (cfDNA) in peripheral blood of patients with triple-negative breast cancer. *Method:* 41 patients with breast cancer admitted to the First Central Hospital of Baoding from January 2020 to December 2021 were selected and recruited into the experimental group, 42 patients with benign breast cancer admitted during the same period were recruited into the conditional control group, and 41 healthy patients admitted during the same period were recruited into the blank control group. The positive rate of peripheral blood CTCs, the level of cfDNA, and the diagnostic efficacy of peripheral blood CTCs, cfDNA alone and the combination thereof for breast cancer were analyzed. *Result:* The positive rates of peripheral blood CTCs in the experimental group, the conditional control group, and the blank control group were 43.90%, 11.90%, and 9.74%, respectively, and there was significant difference among the groups. The levels of cfDNA in peripheral blood of the experimental group, the conditional control group, and the blank control group were 0.26 ± 0.08 bp, 0.17 ± 0.03 bp, and 0.15 ± 0.04 bp, respectively, which were statistically significant. The detection levels of 100 bp hTERT/ng·ml⁻¹ and 241 bp hTERT/ng·ml⁻¹ in the experimental group were significantly higher than those in the conditional control group and the blank control group. The accuracy of peripheral blood CTCs detection in the three groups was 66.21%, the accuracy of cfDNA 241 bp / 100 bp hTERT detection was 80.41%, and the accuracy of combined detection of peripheral blood CTCs and cfDNA was 94.03%. *Conclusion:* The clinical application of peripheral blood CTCs combined with cfDNA level detection can increase detection accuracy, provide data support for clinicians, and improve the clinical diagnostic effect of triple-negative breast cancer.

Keywords: Peripheral blood circulating tumor cells; Cell-free DNA; Triple-negative breast cancer

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1. Introduction

According to global statistics, the total number of global cancer cases in 2020 has reached 19.29 million, including 2.26 million cases of breast cancer, which surpassed the incidence rate of lung cancer and topped the world's cancer incidence rate. Breast cancer is a serious threat to women's health. Breast cancer is heterogeneous, and there are significant differences in clinical case characteristics, cancer molecular characteristics and cell biological behavior. Triple-negative breast cancer accounts for 15%–20% of the incidence of all breast cancer cases. It is characterized by strong drug resistance, strong invasion ability, and high recurrence and metastasis rates. At present, traditional endocrine therapy and molecular targeted therapy are used in the treatment of triple-negative breast cancer. The effect of these two treatments on

triple-negative breast cancer is not obvious. The patient mortality rate is still as high as 25%, and the survival rate of triple-negative breast cancer patients is only 17%, which is very low^[1-10]. B-ultrasound and X-ray are commonly used in the screening of breast cancer, but the detection effect is not high enough to meet the clinical needs. Peripheral blood circulating tumor cells (CTCs)^[11] and circulating blood extracellular fragment gene sequence (cell-free DNA, cfDNA)^[12] have been proposed and applied to the clinical detection of cancer. This study investigates application value of clinical use of CTCs and cfDNA combined detection in triple negative breast cancer.

2. Data and methods

2.1. General information

41 patients with breast cancer admitted to the First Central Hospital of Baoding from January 2020 to December 2021 were recruited into the experimental group, 42 benign breast patients admitted during the same period were recruited into the conditional control group, and 41 healthy breast patients admitted during the same period were recruited into the blank control group. There was no statistical difference in general data between the three groups.

2.2. Methods

Peripheral blood CTCs and cfDNA were tested and measured in individuals of all three groups. 7.5 ml of blood from the three groups was taken for peripheral blood CTCs test. After the blood was mixed with 6.5 ml buffer, it was centrifuged at the speed of 3000 rpm for 10 minutes, and then the supernatant was taken for test. If ≥ 1.0 CTCs were detected in every 7.5 ml of peripheral blood, the CTC status was considered positive. 4.5 ml fasting blood was taken from the three groups in the morning. Cetyltrimethylammonium bromide was used for DNA extraction, and quantitative polymerase chain reaction was used for DNA detection.

2.3. Observation indicators

The positive rate of peripheral blood CTCs^[13], cfDNA level^[14], and the single and combined detection of peripheral blood CTCs and cfDNA for breast cancer has diagnostic efficacy.

2.4. Statistical methods

SPSS19.0 software was used for statistical analysis of quantitative data. ANOVA was used for inter-group comparison of normally distributed data. *t*-test was used for pairwise comparison of two independent samples. Non-parametric rank sum test was used for inter-group comparison of non-normally distributed data. The statistical test level was $p < 0.05$. For the measurement data, the normal distribution test was carried out. The data in line with the normal distribution are expressed as mean \pm standard deviation. $p < 0.05$ is considered statistically significant.

3. Results

3.1. Comparison of positive rates of CTCs in peripheral blood among the three groups

The positive rates of peripheral blood CTCs in the experimental group, the conditional control group, and the blank control group were 43.90%, 11.90%, and 9.74% respectively, and there was significant difference among the groups, as shown in **Table 1**.

3.2. Comparison of cfDNA levels among the three groups

The levels of cfDNA in peripheral blood circulating blood in the experimental group, the conditional control group, and the blank control group were 0.26 ± 0.08 bp, 0.17 ± 0.03 bp, and 0.15 ± 0.04 bp, respectively,

which were statistically significant. The detection levels of 100 bp hTERT/ng·ml⁻¹ and 241 bp hTERT/ng·ml⁻¹ in the experimental group were significantly higher than those in the conditional control group and the blank control group, as shown in **Table 2**.

3.3. Diagnostic efficacy of peripheral blood CTCs, cfDNA, and their combination for breast cancer

The sensitivity, specificity, and accuracy of peripheral blood CTCs were 43.90%, 82.25%, and 66.21%, respectively. The sensitivity, specificity, and accuracy of cfDNA 241bp/100bp hTERT were 79.12%, 79.89%, and 80.41%, respectively. The sensitivity, specificity, and accuracy of the combined detection of peripheral blood CTCs and cfDNA were 94.25%, 92.02%, and 94.03%, respectively. It is obvious that the combined detection of peripheral blood CTCs and cfDNA has better effect and higher accuracy.

Table 1. Comparison of positive rates of CTCs in peripheral blood among the three groups

Group	Number of cases	Number of positive cases	Positive detection rate (%)
Experimental group	41	18	43.90
Conditional control group	42	5	11.90
Blank control group	41	4	9.74

Note: Compared with the experimental group, $\chi^2 = 10.6040$, $P = 0.0011$; compared with the blank control group, $\chi^2 = 12.1758$, $P = 0.0005$; compared with the blank control group, $\chi^2 = 0.0015$, $P = 0.9691$.

Table 2. Comparison of cfDNA levels among the three groups

Group	100 bp hTERT/ng·ml ⁻¹	241 bp hTERT/ng·ml ⁻¹	241 bp/100bp hTERT
Experimental group (n = 41)	35.12±6.25 ^a	8.45±0.56 ^a	0.26±0.08 ^a
Conditional control group (n = 42)	24.36±5.88 ^b	5.23±0.87 ^b	0.17±0.03 ^b
Blank control group (n = 41)	20.69±5.26	2.99±0.51	0.15±0.04

Note: ^a $P < 0.05$ compared with the conditional control group; ^b $P < 0.05$ compared with the blank control group.

Table 3. Single and combined detection of CTCs and cfDNA in peripheral blood

Index	Sensitivity	Specificity	Accuracy
CTCs	43.90%	82.25%	66.21%
241 bp/100bp hTERT	79.12%	79.89%	80.41%
Combined detection of cfDNA and CTCs in peripheral blood	94.25%	92.02%	94.03%

4. Discussion

Breast cancer is one of the most common malignant tumors in women, and accounts for 23% of the total number of female cancer patients in the world [8,15]. As a special subtype of breast cancer, triple-negative breast cancer has an incidence rate of 23.8% in China. It is characterized by insufficient or null expression of estrogen receptor (ER) and progesterone receptor (PR) and null expression of human epidermal growth factor receptor 2 (HER-2) [16-18]. Traditional endocrine and targeted therapy has no effect on triple-negative breast cancer lacking receptor expression. Therefore, standard cytotoxic chemotherapy combined with surgical resection is still the preferred method for systematic treatment of triple-negative breast cancer, but patients with triple-negative breast cancer may still have a high risk of recurrence and metastasis within 3–5 years after the first treatment. Compared with other breast cancer subtypes, triple-negative breast cancer has strong drug resistance, high recurrence rate, strong tissue invasion and invasion ability, which make

treatment difficult, and there are no effective biomarkers and predictive indicators for this cancer subtype [19, 20]. Therefore, it is very important to study the value of combined detection of peripheral blood CTCs and cfDNA in the clinical diagnosis of triple negative breast cancer.

The molecular fragments of free DNA in peripheral blood is the focus of the current study. It is found that the length of free DNA in peripheral blood in cancer patient is shorter than that in normal people, which may be caused by the proliferation of necrotic cells or tumor cells. The content of cfDNA fragments in the peripheral blood of normal people is low, mainly because the cells have been cleared by the immune system. The cancer cells in the patients undergo rapid proliferation, short growth cycle, and rapid apoptosis. Therefore, the content of cfDNA fragments in the peripheral blood of patients is relatively high, and the autoimmunity of cancer patients is reduced. The peripheral blood CTCs and cfDNA can be used for early diagnosis of breast cancer and evaluation of treatment curative effect.

In this study, we found that both the positive detection rate of peripheral blood CTCs and cfDNA level in the experimental group were significantly higher than those in the conditional control group and the blank control group. The accuracy rate of peripheral blood CTCs combined with cfDNA level in the experimental group was 94.03%, which was significantly higher than that of peripheral blood CTCs alone (66.21%) and cfDNA alone (80.41%).

Therefore, the clinical application of combined detection of peripheral blood CTCs and cfDNA level can increase detection accuracy, provide data support for clinicians, and improve the clinical diagnostic rate of triple-negative breast cancer.

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Disclosure statement

The authors declare no conflict of interest.

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