Clinical Value of cfDNA Content in Peripheral Blood of Patients with Triple-Negative Breast Cancer

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Abstract: *Objective:* To explore the value of circulating free (cfDNA) content in the clinical diagnosis and treatment of triple-negative breast cancer (TNBC). *Methods:* A total of 39 TNBC patients, 45 non-TNBC patients, and 50 healthy individuals admitted to the Baoding First Central Hospital during 2019–2022 were recruited. The clinical data, peripheral blood cfDNA concentration, and clinicopathological indicators of the patients were observed and analyzed. *Results:* The difference in clinical indicators such as age, age range, tumor size, clinical stage, and lymph node metastasis between patients with TNBC and non-TNBC was insignificant ($P > 0.05$). The cfDNA concentrations (ng/mL) of the TNBC group, non-TNBC group, and healthy group were 24.12 ± 4.98, 15.36 ± 4.12, and 3.12 ± 1.02, respectively, and they are statistically different ($P < 0.05$). The difference in cfDNA concentration was insignificant between TNBC patients with tumors ≤ 2 cm and > 2 cm ($P > 0.05$) but was significant between TNBC patients with clinical stages I+II and III+IV ($P < 0.05$). The cfDNA concentration in TNBC patients with lymph node metastasis was significantly higher than those without lymph node metastasis ($P < 0.05$). *Conclusion:* cfDNA has an important application value in the diagnosis and treatment of breast cancer. By detecting the cfDNA level and its gene variation, valuable information about the progress and treatment effects of breast cancer can be obtained. This non-invasive detection method has a wide range of applications and can be used for early screening, auxiliary diagnosis, efficacy evaluation, and recurrence monitoring of breast cancer.

Keywords: Triple-negative breast cancer; cfDNA; Tumor cells

1. Introduction

Triple-negative breast cancer (TNBC) refers to three different types of breast cancer: human epidermal growth factor 2 (HER2) protein, estrogen receptor (ER), and progesterone receptor (PR) negative breast cancer [1-6]. TNBC is second only to ductal carcinoma in incidence in women, accounting for about 15% of all cancers in women. The clinical symptoms of TNBC lack specificity, and its diagnosis is mainly based on the tumor histological type, and most patients have no obvious clinical symptoms in the early stage. The treatment of TNBC patients is mainly surgery, but due to its high recurrence rate, high metastasis rate, and poor prognosis, the curative effect is dissatisfactory. In recent years, attention has turned to finding noninvasive biomarkers, with circulating free DNA (cfDNA) attracting widespread interest in its potential role in breast cancer. cfDNA is a kind of DNA in blood circulation, and its sources include tumor cells and normal cells [7-10]. In breast cancer, the level of cfDNA and its gene variation information may reflect tumor progression and treatment effect [11]. cfDNA plays an important role in the diagnosis, treatment, and

monitoring of TNBC and is a potential biomarker. An in-depth study of the relationship between cfDNA and TNBC allows a better understanding of the occurrence and development of TNBC and provides patients with more accurate and effective treatment strategies. This study aimed to investigate the correlation between peripheral blood cfDNA content and clinicopathological features in TNBC patients.

2. Materials and methods
2.1. General information
The clinicopathological data of 39 TNBC patients admitted to the Baoding First Central Hospital from 2019 to 2022 and 45 non-TNBC patients were retrospectively analyzed, and compared with 50 cases of normal physical examination population hospitalized during the same period.

Inclusion criteria: tumor located in lymph node metastasis, tumor diameter ≤ 3 cm, tumor infiltration depth ≤ 3 cm; patients without liver, lung, or bone metastasis; patients and their families gave informed consent and signed an informed consent form.

Exclusion criteria: history of other malignant tumors, radiotherapy, chemotherapy, or endocrine therapy within 1 year before surgery; preoperative chemotherapy time ≥ 1 cycle, or postoperative pathological examination showed local recurrence or distant metastasis. All patients underwent a modified radical mastectomy, and breast-enhanced CT or MRI before operation.

2.2. Methods
Three mL of venous blood were taken, centrifuged at 4°C for 10 min, and the upper plasma was utilized for detection. The cfDNA content in the peripheral blood of TNBC patients was detected using quantitative reverse transcription PCR (qRT-PCR). Reagents were provided by Shanghai Bioengineering Co., Ltd.

2.3. Observation indicators
Clinical data, cfDNA concentration, and clinicopathological indicators of patients were observed and analyzed.

2.4. Statistical methods
Statistical software SPSS 24.0 was used to analyze the data. Measurement data were expressed as mean ± standard deviation (SD), using a t-test; count data were expressed as %, using a χ^2 test, and $P < 0.05$ was considered statistically significant.

3. Results
3.1. Clinical analysis
There was no statistical difference in age, extent, tumor size, clinical stage, lymph node metastasis, and other clinical indicators between patients with TNBC and non-TNBC ($P > 0.05$), as shown in **Table 1**.

Table 1. Clinical data

Item		TNBC	Non-TNBC	t / χ^2	P
Number (case)		39	45	-	-
Age		51.21 ± 10.32	50.97 ± 11.23	0.1014	0.9195
Age range (years)		31–80	29–79	-	-
Tumor size	≤ 2 cm	21 (53.85)	21 (46.67)	0.4308	0.5116
	> 2 cm	18 (46.15)	24 (53.33)		

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Item		TNBC	Non-TNBC	t / χ^2	P
Clinical stage	Phase I+II	26 (66.67)	30 (66.67)	0.0000	1.0000
	Phase III+IV	13 (33.33)	15 (33.33)		
Lymph node metastasis	Have	17 (43.59)	20 (44.44)	0.0062	0.9372
	None	22 (56.41)	25 (55.56)		

3.2. Measurement results of peripheral blood cfDNA concentration of patients in each group

The peripheral blood cfDNA concentrations (ng/mL) of the TNBC group, non-TNBC group, and healthy group were 24.12 ± 4.98, 15.36 ± 4.12, and 3.12 ± 1.02, respectively. There are statistical differences ($P < 0.05$), as shown in **Table 2**.

Table 2. Measurement results of peripheral blood cfDNA concentration of patients in each group (mean ± SD)

Group	Number of cases	cfDNA concentration (ng/mL)
TNBC group	39	24.12 ± 4.98
Non-TNBC group	45	15.36 ± 4.12
Healthy group	50	3.12 ± 1.02

Note: $P < 0.05$ between the comparisons.

3.3. The relationship between the concentration of peripheral blood cfDNA and the clinicopathological indicators of TNBC

There was no statistical difference in the cfDNA concentration between TNBC patients with tumors ≤ 2 cm and > 2 cm ($P > 0.05$), and the cfDNA concentration of TNBC patients with clinical stages I+II and III+IV were statistically different ($P < 0.05$). The peripheral blood cfDNA concentration of TNBC patients with lymph node metastasis is significantly higher than that of TNBC patients without lymph node metastasis ($P < 0.05$), see **Table 3** shown.

Table 3. The relationship between the concentration of cfDNA in peripheral blood and the clinicopathological indicators of TNBC (mean ± SD)

TNBC		cfDNA concentration (ng/mL)	t	P
Tumor size	≤ 2 cm ($n = 21$)	24.12 ± 4.98	0.1285	0.8984
	> 2 cm ($n = 18$)	24.32 ± 4.68		
Clinical stage	Stage I+II ($n = 26$)	28.12 ± 5.98	3.1894	0.0029
	Stage III+IV ($n = 13$)	22.12 ± 4.48		
Lymph node metastasis	Have ($n = 17$)	27.12 ± 3.88	3.3492	0.0019
	None ($n = 22$)	23.42 ± 3.08		

4. Discussion

TNBC is a highly invasive disease with a poor prognosis. The current treatment of TNBC is mainly surgery, but the curative effect is dissatisfactory. The change of cfDNA content in tumor tissue is a complex process, which is related to many factors such as tumor growth, differentiation, metastasis, and treatment, and the cfDNA content in tumor tissue is closely related to its occurrence and development. Elevated cfDNA content in the peripheral blood of TNBC patients can be used as an independent prognostic factor,

suggesting that it may be an important clinical indicator of poor prognosis in TNBC patients [12-15].

cfDNA is DNA released into the blood circulation by a variety of cells, mainly including the following sources: normal cells will initiate apoptosis at the end of their life cycle, and after cell death, the DNA in them will be released into the blood circulation; Abnormal cells, such as tumor cells, may release a large amount of DNA into the blood circulation; under certain conditions, such as inflammation, trauma, etc., normal cells may actively release DNA into the blood circulation.

The characteristics of cfDNA are as follows: the concentration of cfDNA in the blood circulation is usually very low, generally between several to tens of nanograms per milliliter; the length of cfDNA is usually shorter than that of normal cell DNA because it will be degraded by enzymes during release; In some disease states, such as cancer, cfDNA may carry disease-related gene variations, such as mutations, methylation, etc.; the half-life of cfDNA in blood circulation is usually very short, generally between minutes and hours; due to the diversity of cfDNA sources, its concentration may be affected by various factors, such as diet, drugs, etc. Therefore, it is necessary to ensure the consistency of experimental conditions when performing cfDNA detection to obtain reproducible results. cfDNA is a non-invasive biomarker with a wide range of sources and diverse characteristics, which can reflect the health status and disease progression of the body to a certain extent. Detecting cfDNA can provide more information about the diagnosis, treatment, and monitoring of diseases such as breast cancer.

There is a certain association and influence between cfDNA and breast cancer. Breast cancer is a common malignant tumor, and its occurrence and development are related to changes in cfDNA. The cfDNA released by breast cancer cells into the blood circulation can reflect the status and progress of breast cancer to a certain extent. These genetic variations can be detected and analyzed by high-throughput sequencing and other technologies, providing important information for the diagnosis, treatment, and monitoring of breast cancer. The concentration and level of cfDNA can be used as an indicator of breast cancer prognosis. For example, a high concentration of cfDNA may indicate an increase in the proliferation and release of breast cancer cells, suggesting that the prognosis of patients may be poor. By detecting gene variations in cfDNA, breast cancer patients can be provided with personalized treatment recommendations and monitoring options. For example, if a mutation in a specific gene is detected in a patient's cfDNA, targeted drug therapy for the mutation can be selected. In addition to being used as a biomarker, cfDNA can also be used as an emerging tool for breast cancer research. By analyzing cfDNA, it is possible to explore the pathogenesis, drug resistance, and metastasis of breast cancer, and provide more ideas and basis for the prevention, diagnosis, and treatment.

The cfDNA released by TNBC cells may have some unique characteristics, such as high concentration and high mutation rate. If a mutation in a specific gene is detected in a patient's cfDNA, targeted drug therapy targeting that mutation can be selected. The results of this study show that the cfDNA content in the peripheral blood of TNBC patients has no obvious correlation with tumor stage and clinicopathological features, but there is a correlation with relatively good clinical prognosis. This study suggests that cfDNA content is an important indicator of poor prognosis in TNBC patients, and cfDNA content can be considered as one of the reference indicators when formulating clinical treatment plans for TNBC patients. This study shows that the cfDNA content in the peripheral blood of TNBC patients is correlated with clinicopathological features (tumor size, lymph node metastasis, TNBC pathological type) and hormone receptor expression. Moreover, the content of cfDNA in the TNBC group was higher than that in the non-TNBC group ($P < 0.05$). The results of this study show that cfDNA content can be used as a diagnostic marker of TNBC, which can be used to guide the early diagnosis and treatment of TNBC and provide more treatment opportunities for TNBC patients.

However, the number of cases included in this study is small, and there may be problems such as a small sample size and short follow-up time, which lead to the fact that the research results cannot fully

represent the actual situation of TNBC patients. Therefore, it is necessary to expand the sample size and conduct multi-center follow-ups of patients to further verify the correlation between cfDNA content and clinicopathological features.

In summary, cfDNA offers significant clinical benefits in the detection and management of breast cancer. It is possible to obtain valuable information on the progression and treatment effects of breast cancer by investigating the cfDNA level and its gene variation. This non-invasive detection method has a wide range of applications including early screening, auxiliary diagnosis, curative effect evaluation, and monitoring of recurrence in breast cancer.

Disclosure statement

The authors declare no conflict of interest.

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