Original Article

Correlation between circulating tumor cells and different molecular biological characteristics in breast cancer patients

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Background: This study sought to detect the number of circulating tumor cells (CTCs) in breast cancer patients, and examine the relationship between CTCs and molecular biological characteristics.

Methods: From June 2016 to June 2018, 150 female patients with invasive breast cancer detected by CTCs at the Department of Breast Surgery, Fudan University Cancer Hospital were enrolled in this study. The patients had an average age of 52.6±7.8 (range, 35–77) years. Routine pathological and immunohistochemical examinations were performed on tissues obtained during surgery. In this study, CTCs were detected using the immunomagnetic bead negative enrichment technique (i.e., the Cyttel technique). The measurement data are expressed by $\bar{x} \pm s$, and were compared by *t*-tests. A univariate analysis of variance was used to compare differences between groups. The count data are expressed as the absolute value, and the test χ^2 or Fisher's exact test were used to compare differences.

Results: There were 109 cases of positive CTC (≥3 CTCs/4 mL) (72.7%), and 41 cases of negative CTC (<3 CTCs/4 mL) (27.3%). There were no significant differences in terms of age and menopausal status between the two groups (P>0.05). There was no significant difference in the positive rate of CTC in T1, T2, and T3 and above patients (P>0.05). There was no significant difference in the CTC positive rate between ER positive and negative patients, PR positive and negative patients, and Ki-67 ≥14% and <14% patients (P>0.05). However, there was a statistical difference in the positive rate of CTC between human epidermal growth factor receptor 2 (HER-2) positive and negative patients (P<0.05). There was no significant difference in the CTC positive rate among patients with Luminal A type, B type, HER-2 overexpression type, and triple negative breast cancer (TNBC) (P>0.05). There was no significant difference in the positive rate of CTC among patients with invasive ductal carcinoma, invasive lobular carcinoma, and other types of invasive carcinoma (P>0.05).

Conclusions: It can be concluded that there is a relationship between CTC and HER-2 expression, which has certain predictive value for patients with positive HER-2 expression, thus predicting poor prognosis.

Keywords: Breast cancer; circulating tumor cells (CTCs); molecular typing; human epidermal growth factor receptor 2 (HER-2)

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Introduction

The incidence and death toll of breast cancer continue to increase every year, posing a serious threat to the physical and mental health of Chinese women (1). As a systemic disease, breast cancer often appears in the metastasis of tumor cells in the early stage of the disease, and distant metastasis is the main lethal factor for breast cancer patients (2,3). The abscission of breast tumor cells is the initial process of tumor tissue metastasis. These free cells spread to the whole body through blood or lymphatic channels, forming metastasis. The distant metastasis of tumor tissues is related to a variety of factors. Cells with metastatic potential are transferred to the blood, lymph nodes or distant organs of the human body. In fact, their number is very low, and it is often difficult to detect them using conventional detection methods. Circulating tumor cells (CTCs) are tumor cells that shed from the primary site or enter the peripheral blood circulation through clinical procedures. CTC is an essential factor for solid tumor metastasis. The detection of CTC plays an important role in the metastasis and prognosis of solid tumors, and has attracted great attention worldwide (4). This retrospective study sought to examine the relationship between CTCs and clinicopathological data.

We present the following article in accordance with the MDAR reporting checklist (available at https://gs.amegroups.com/article/view/10.21037/gs-22-43/rc).

Methods

Patients

From June 2016 to June 2018, 150 female patients with invasive breast cancer detected by CTCs at the Department of Breast Surgery, Fudan University Cancer Hospital were enrolled in this study. The patients had an average age of 52.6±7.8 (range, 35–77) years. Of the 150 patients, 68 were premenopausal, and 82 were postmenopausal. In relation to the pathological type, 105 patients had infiltrating ductal carcinoma, 15 patients had infiltrating lobular carcinoma, and 31 patients had other types of carcinomas.

Patients were excluded from the study if they met any of the following exclusion criteria: (I) had inflammatory breast cancer or lactation breast cancer; (II) had previously undergone chemotherapy; (III) had distant metastasis; and/or (IV) had other types of malignant tumors.

The study was approved by the Ethical Committee of Fudan University Cancer Hospital (No. 2016-02-0123-08). All patients signed the informed consent form. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Research methods

All the included patients underwent preoperative comprehensive evaluations, including a color doppler ultrasound, molybdenum target test, magnetic resonance imaging, and computed tomography examination. Surgery was performed after the exclusion of surgery-related contraindications. Routine pathological and immunohistochemical examinations were performed on the tissues obtained during surgery, and tumor-node-metastasis (TNM) staging was determined using the criteria set out in the 7th edition of the *Cancer Staging Manual* of the American Joint Committee on Cancer and the International Union for Cancer Control [2020]. Estrogen receptor/progesterone receptor (ER/PR) expression was determined according to the immunohistochemical guidelines of ER/PR for breast cancer published by the American Society of Clinical Oncology and the American College of Pathologists. Human epidermal growth factor receptor 2 (HER-2) expression was evaluated using the HerecepTest scoring system. The Ki-67 index was defined as 14% positive and <14% negative.

CTC detection method

In this study, CTCs were detected using the immunomagnetic bead negative enrichment technique (i.e., the Cyttel technique). Before surgery, 5 mL of venous blood was taken from each patient's upper limb on the healthy side, and 4 mL of the specimen was fully mixed with 3 working fluids, and placed into a 50-mL centrifuge tube. The first working solution was centrifuged to remove hemoglobin and heme, and the second working solution was added to cleave the red blood cells, remove the lysis matrix of the red blood cells, and obtain the white blood cells and rare cells. One hundred and fifty μL of the magnetic particles were absorbed, and the suspension was placed into an EP tube and washed 3 times before being added to the above samples to completely combine the magnetic beads and the white blood cells. Three mL of the third working solution and the previously treated sample were added to the new centrifuge tube to remove the white blood cells and magnetic beads. After centrifugation, the above 2 layers of solution were absorbed and transferred to a new centrifugal tube. The first working

Table 1 Comparison of positive and negative CTC (%) in patients with T1, T2, T3, and more severe patients

T stage	N	CTC positive (n=109), n (%)	CTC negative (n=41), n (%)	χ^2	P
T1	37	23 (21.1)	14 (34.1)	2.730	0.255
T2	75	57 (52.3)	18 (43.9)	–	–
T3 and above	38	29 (26.6)	9 (22.0)	–	–

CTC, circulating tumor cell.

solution was added and centrifuged, and the supernatant was discarded, leaving 300 μL. The obtained liquid was put in a 2-mL centrifugal tube, the residual magnetic beads were removed, the supernatant was discarded until 60–70 μL remained after centrifugation, and an equal volume of fixative solution was added, mixed, and smeared. The CTC standard was that the nucleus size was not uniform; nuclear diameter >18 μm; nuclear atypia; three-dimensional levels of chromatin; high nuclear/cytoplasmic ratio. CTC is defined if the above four items are met. According to Wu *et al.*, 3 is the threshold of positive CTC; that is, CTCs/4 mL ≥3 is positive, while CTCs/4 mL <3 is negative (5).

Statistical analysis

SPSS 17.0 software was used for the statistical analysis, and a P value <0.05 indicated a statistically significant difference. The measurement data are expressed as ($\bar{x}$ ± s), and were compared by *t*-tests. An analysis of variance was used to compare differences between groups. The count data are expressed as the absolute value, and the χ^2 test or Fisher's exact test were used to compare differences.

Results

CTC detection results

There were 109 cases of positive CTC (≥3 CTCs/4 mL) (72.7%), and 41 cases of negative CTC (<3 CTCs/4 mL) (27.3%). There were no significant differences in terms of age and menopausal status between the two patient groups (P>0.05).

The relationship between CTC and TNM staging

There was no significant difference in the positive rate of CTC in T1, T2, and T3 and above patients (P>0.05; *Table 1*).

Relationship between CTC and immunohistochemical results

As *Table 2* shows, there was no statistical difference in the positive rate of CTC between ER positive and negative patients, PR positive and negative patients, and Ki-67 ≥14% and <14% patients (P>0.05). However, there was a statistical difference in the positive rate of CTC between HER-2 positive and negative patients (P<0.05).

Relationship between CTC and molecular typing of breast cancer

There was no significant difference in the positive rate of CTC in patients with Luminal A type, B type, HER-2 overexpression type, and triple negative breast cancer (TNBC) (P>0.05; *Table 3*).

Relationship between CTC and clinicopathological features

There was no significant difference in the positive rate of CTC among patients with invasive ductal carcinoma, invasive lobular carcinoma, and other types of invasive cancer (P>0.05; *Table 4*).

Discussion

Breast cancer is a systemic disease. Distant metastasis is the primary cause of death of breast cancer patients. Indeed, more than 90% of deaths among breast cancer patients are caused by metastasis and recurrence (6). Local recurrence or distant metastasis still occurs in 20–30% of patients without lymph node metastasis 5 to 10 years after treatment (7). Thus, the distant diffusion of breast cancer tumor cells appears to occur in the early stage of the disease. With the continuous development of various technologies, the detection of CTCs can help in the early detection of distant metastasis of breast cancer patients, and can be used to predict the prognosis of breast cancer patients in clinical

Table 2 Relationship between CTC and immunohistochemical results

Gene	N	CTC positive (n=109), n (%)	CTC negative (n=41), n (%)	χ^2	P
ER				1.135	0.287
+	98	65 (59.6)	23 (56.1)		
–	52	44 (40.4)	18 (43.9)		
PR				1.645	0.200
+	82	58 (53.2)	17 (41.5)		
–	68	51 (46.8)	24 (58.5)		
HER-2				8.499	0.002
–	78	48 (44.0)	29 (70.7)		
+	72	61 (56.0)	12 (29.3)		
Ki-67				2.063	0.151
≥14%	117	82 (75.2)	26 (63.4)		
<14%	33	27 (24.8)	15 (36.6)		

CTC, circulating tumor cell; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2.

Table 3 Comparison of positive and negative CTC (%) in patients with Luminal A type, B type, HER-2 overexpression type, and TNBC

Pathological type	N	CTC positive (n=109), n (%)	CTC negative (n=41), n (%)	χ^2	P
Luminal A	28	17 (15.6)	11 (26.8)	7.567	0.056
Luminal B	51	33 (30.3)	18 (43.9)	–	–
HER-2 overexpression	55	46 (42.2)	9 (21.9)	–	–
TNBC	16	13 (11.9)	3 (7.3)	–	–

CTC, circulating tumor cell; HER-2, human epidermal growth factor receptor 2; TNBC, triple negative breast cancer.

Table 4 Comparison of positive and negative CTC (%) in patients with invasive ductal carcinoma, invasive lobular carcinoma, and other types of invasive carcinoma

Pathological type	N	CTC positive (n=109), n (%)	CTC negative (n=41), n (%)	χ^2	P
Invasive ductal carcinoma	102	79 (72.5)	23 (56.1)	1.081	0.582
Invasive lobular carcinoma	17	11 (10.1)	6 (14.6)	–	–
Other types of invasive carcinoma	31	19 (17.4)	12 (26.3)	–	–

CTC, circulating tumor cell.

practice. At present, CTCs has shown good research prospects, which is of great significance in determining recurrence and metastasis in breast cancer patients and improving the overall survival rate. This study investigated the relationship between CTCs and clinicopathological data, such as TNM staging, immunohistochemical indexes, and the molecular typing of breast cancer, to understand the effect of CTCs in the prognosis of breast cancer patients. CTCs identification must be specific and sensitive enough to separate tumor cells from the blood. With the recent development of nanotechnology. The immunomagnetic bead enrichment method adopted in this paper provides an advanced separation method for CTCs. The high specificity and sensitivity of CTCs are ensured because the monoclonal

antibody on the surface of the immunomagnetic beads only binds to the nuclear proteins containing specific antigens. The HER-2 immunomagnetic beads used in our study were obtained by coupling HER-2 antibody with magnetic microspheres. CTC used to capture breast cancer patients not only has good specificity and sensitivity, but also has rapid magnetic response, short enrichment time and high capture efficiency. The immune magnetic beads have stable properties, small particle size, good magnetic response and good dispersion.

TNM staging plays an important role in determining the prognosis of breast cancer patients. This study compared the relationship between T stage and CTC test results. As T stage progressed, the number of cases with positive and negative CTC results also changed. As *Table 1* shows, there was no significant difference in the positive rate of CTC among breast cancer patients with T1, T2, and T3 stages, which suggests that there is no correlation between T stage and CTCs. This conclusion is consistent with the results of Gong *et al.* (8,9); however, other studies have found that the positive rate of CTCs in the peripheral blood of breast cancer patients is related to TNM stage. The differences between the conclusions of these studies may be related to the different methods used for CTC testing or the number of patients included.

The immunohistochemical results of ER, PR, HER-2, and Ki-67 can provide valuable information for the adjuvant treatment of breast cancer, as they serve as indicators of the conventional prognostic stages of breast cancer. As *Table 2* shows, there were statistical differences in the positive and negative rates of CTC between patients with positive and negative HER-2 ($P<0.05$), which suggests that there is a correlation between CTCs and HER-2 expression. However, no statistically significant differences were observed between CTCs and the sub-groups of ER, PR, Ki-67, and other immunohistochemical indicators. In general, we believe that breast cancer patients with hormone receptor negative and HER-2 overexpression have a poor prognosis. In this study, the positive rate of CTC in patients with positive HER-2 was significantly higher than that of patients with negative HER-2, but similar findings were not found for the other three immunohistochemical indicators, which is consistent with the conclusions of Zhou *et al.* (10).

In addition, as a highly heterogeneous malignant tumor, breast cancer patients with the deletion of ER and PR and positive HER-2 expression have a higher degree of malignancy and poor prognosis. Accordingly, the probability of CTCs entering the circulation should be increased in these patients. At present, some studies have compared the relationship between the molecular typing of breast cancer and CTCs; for example, Jiang *et al.* (11) concluded that CTC detection results can be used as independent predictors of TNBC and HER-2 overexpression in breast cancer patients. The results of this study showed that the cases of HER-2 overexpression and CTC positive cases of TNBC were higher than those of Luminal A and B breast cancer patients, but the difference was not statistically significant ($P=0.600$). This may be due to the relatively small number of patients enrolled in this study. Thus, studies with larger sample sizes need to be conducted to gather further evidence.

In this study, we compared the relationship between CTCs and TNM staging, immunohistochemical results, molecular typing, and other indicators in breast cancer. Based on the existing results, it appears that there is a relationship between CTCs and HER-2 expression, which has a certain predictive value for patients with positive HER-2 expression. HER-2 pathological type of breast cancer often predicts poor prognosis, so the results of this paper indicate that TCT is of certain significance in guiding the treatment and judging the prognosis of breast cancer.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://gs.amegroups.com/article/view/10.21037/gs-22-43/rc

Data Sharing Statement: Available at https://gs.amegroups.com/article/view/10.21037/gs-22-43/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://gs.amegroups.com/article/view/10.21037/gs-22-43/coif). The authors

have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was approved by the Ethical Committee of Fudan University Cancer Hospital (No. 2016-02-0123-08). All patients signed the informed consent form. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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