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Predictive Value of Circulating Tumor Cells for Evaluating Short- and Long-Term Efficacy of Chemotherapy for Breast Cancer

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Background: The present study investigated the role of circulating tumor cells (CTCs) counts in predicting the short- and long-term efficacy of chemotherapy for breast cancer (BC).

Material/Methods: Peripheral venous blood was extracted from 187 BC patients. CTCs were measured by flow cytometry. Spearman's correlation analysis was performed to examine the correlation between the efficacy of chemotherapy and CTC counts. A receiver operating characteristic (ROC) curve was plotted to estimate the predictive value of CTC counts. The Kaplan-Meier method was employed to calculate disease-free survival (DFS) and overall survival (OS). Cox regression analysis was used to determine risk factors for prognosis of BC.

Results: Complete response (CR) + partial response (PR) was achieved by 65.8% of BC patients. After chemotherapy, CTC counts were decreased in both the CR + PR and SD + PD groups. Spearman's correlation analysis indicated that CTC counts before chemotherapy were positively correlated with clinical response to chemotherapy (r=0.45, P<0.05). For predicting clinical response to chemotherapy, CTC counts yielded an area under the curve (AUC) of 0.958, with sensitivity reaching 96.9% and specificity reaching 85.4%. The Kaplan-Meier method and Cox regression analysis indicated that tumor node metastasis (TNM) staging, lymph node metastasis (LNM), ki-67, endocrine therapy, and CTC counts were risk factors for prognosis of BC.

Conclusions: These findings indicate that BC patients with CTCs ≥8 exhibited poor response to chemotherapy and poor OS. CTC counts can serve as an indicator in predicting short- and long-term efficacy of chemotherapy for BC.

MeSH Keywords: Breast Neoplasms, Male • Chemotherapy, Cancer, Regional Perfusion • Neoplastic Cells, Circulating • Prognosis • Self Efficacy

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Background

Breast cancer (BC) is one of the most common malignancies threatening female health, with high prevalence throughout the world [1]. In 2012, it was estimated that 272 700 BC cases were newly diagnosed among women and approximately 61 500 women died of BC in China [2]. The therapy for BC primarily consists of surgical intervention, radiotherapy, adjuvant chemotherapy after definitive surgery, hormone therapy, and molecular-targeted therapy [3]. In spite of increasing benefits from adjuvant or neoadjuvant treatments, there are still early BC patients at high risk of metastasis and relapse [4]. Clinically, the common methods used to detect BC include ultrasound, magnetic resonance imaging (MRI), mammography, and serum tumor markers such as carcinoembryonic antigen (CEA) and cancer antigen 135 (CA153) [5,6]. Recently, the use of molecular biomarkers has been reported to help BC patients receive optimal treatment, and established biomarkers, including estrogen receptor(ER), progesterone receptor (PR), epidermal growth factor receptor 2 (HER2), and Ki67, play important roles in the subcategorization of BC to predict prognosis and decide the specific treatment plans for BC patients [7,8]. Also, genetic and epigenetic association studies of microRNAs and its host gene may provide a theoretical basis to reveal the relationship between them and may help explore the mechanism of BC [9]. Nonetheless, only a small number of tumor cells are considered to be involved in BC development and progression. More improvements are required and recent advancements in molecular diagnostics should translate into more personalized treatment for better clinical outcomes.

Circulating tumor cells (CTCs) are reported to circulate in the peripheral blood of patients diagnosed with breast, prostate, colorectal, and lung cancers [10–15]. They are rare in healthy individuals or in patients who have nonmalignant disease [16]. Immunomagnetic platforms are used for the reproducible measurement of CTCs at low frequencies and in small blood volumes [17]. It has been reported that detection of CTCs in peripheral blood of BC patients has great value in the early diagnosis, prognosis, and treatment of BC [18,19], and the study of its molecular level is beneficial to the development of new molecular-targeted drugs and the discovery of mechanism underlying tumor metastasis [20,21]. A previous study revealed a strong relationship between CTCs and the progression of radiographic disease in patients undergoing chemotherapy or endocrine therapy for metastatic BC (MBC) [17]. Furthermore, Cristofanilli et al. reported that the enumeration of CTCs prior to treatment may be an independent predictor of poor progression-free survival (PFS) and overall survival (OS) in patients with MBC [22]. Similarly, Giordano et al. found that higher CTCs counts at any time indicated the poor prognosis of advanced BC, and the dynamics of CTCs can effectively predict prognosis in patients with advanced BC [23].

A variety of detection methods for CTCs, such as immunocytochemistry (ICC) and reverse transcription polymerase chain reaction (RT-PCR), can improve the sensitivity and specificity of CTCs to some extent [24–27]. The present study, by detecting CTCs with flow cytometry before and after chemotherapy, investigated the role of CTC counts in predicting short- and long-term efficacy of chemotherapy for BC.

Material and Methods

Study subjects

A total of 187 female BC patients, admitted into our hospital between January 2013 and October 2013, were randomly selected for this study. These patients, who were pathologically diagnosed as having BC by fine-needle aspiration biopsy, were aged 26–70 years, with a median age of 51 years. Histopathological types were: 139 cases of invasive ductal carcinoma (IDC), 23 cases of invasive lobular carcinoma (ILC), 10 cases of squamous cell carcinoma (SCC), and 15 cases of other types. Tumor node metastasis (TNM) staging before chemotherapy was: 17 cases in stage IIa, 76 cases in IIb, 62 cases in IIIa, and 32 cases in IIIb. Among these BC patients, there were 133 undergoing endocrine therapy. Inclusion criteria were: female; an expected survival of more than 6 months; a complete catalogue of clinical data; pathologically confirmed BC by physical examination, color Doppler ultrasound, mammography screening, X-ray, magnetic resonance imaging (MRI), and core-needle breast biopsy; no history of other primary malignancy; and normal indicators of routine laboratory blood and urine testing, blood coagulation, liver, and kidney functions. Exclusion criteria were: initial diagnosis during pregnancy and lactation; evidence of active infection, coagulation disorders, acute and chronic inflammatory and autoimmune diseases; associated with cardiac dysfunction, liver and kidney diseases, or failing to undergone chemotherapy or to visit the doctor on time due to personal reasons; failing to complete chemotherapy regimens or experiencing a delay (more than 2 weeks) in initiation of chemotherapy due to severe toxicity and side effects; being given related therapies except adjuvant chemotherapy and endocrine therapy at 3 months before blood extraction; incomplete record; and poor compliance. The protocol was approved by the Ethics Committee of our hospital. Written informed consent was obtained from each subject.

Treatment regimes

All patients underwent 3 cycles of 5-fluorouracil/epirubicin/cyclophosphamide (FEC). Continuous intravenous infusion of Fluorouracil (5-Fu) (initial dose of 500 mg/m^2, d 1 and 8) for 4 h; intravenous infusion of epirubicin (EPI) (initial dose of 50 mg/m^2, d 1); intravenous infusion of Cytoxan (CTX) (initial

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dose of 500 mg/m^2, d 1 and 8); and 1 cycle lasted for 3–4 weeks. After 2 weeks of chemotherapy, the operation was performed, and 2 weeks after that the planned chemotherapy performed. Estrogen receptor (ER)- or progesterone receptor (PR)-positive patients underwent endocrine therapy using tamoxifen following 3 cycles of chemotherapy.

Specimen collection and immunohistochemistry (IHC)

Before chemotherapy (T0), 1 day before the third cycle of chemotherapy (T1), and 1 week after the third cycle of chemotherapy (T2), fasting venous blood (7.5 ml) extracted from each subject in the morning was put into tubes containing ethylene diamine tetraacetic acid EDTA. Tumor tissues collected from BC patients were fixed by 10% formalin. These paraffin-embedded slices were subject to IHC for detection of expressions of ER, PR, human epidermal growth factor receptor-2 (HER-2), and Ki-67. All results were independently confirmed by 2 or more pathologists, as was the further identification of molecular classification in BC.

Extraction of CTCs and antibody labeling

Venous blood-containing tubes were diluted by equal amounts of phosphate-buffered saline (PBS) and buffy coats were aspirated by Ficoll-Hypaque gradient centrifugation (500 g, 25 min). With the addition of 5 ml PBS, the supernatant was removed by centrifugation (2200 rpm, 6 min). After the addition of 0.5 ml PBS and 1 ml fixation, the tubes with marks were stored at 4°C for 30 min. Subsequently, the tubes were subject to the first centrifugation (2200 rpm, 3 min) and the supernatant was aspirated. After resuspension in 4 ml PBS, the second centrifugation (2200 rpm, 3 min) was performed and the supernatant was also aspirated. Following the addition of 1–2 ml cell-penetrating agents, the tubes were subject to the third centrifugation (2200 rpm, 3 min), with the supernatant aspirated. Then, the forth centrifugation (2200 rpm, 3 min) was performed for removal of the supernatant after resuspension in 4 ml of 2% serum PBS. Blood samples were resuspended with 70 ul 2% serum PBS, and then were added to l0 ul FcR. Ten minutes later, we added 2.5 ul Epcam, 20 ul CD45, and 5 ul CK18 antibody to the tubes, and stored them in a refrigerator for 20 min. Again, we added 4 ml of 2% serum PBS to the tubes and centrifuged them at 2200 rpm for 3 min. After the removal of the supernatant, the samples were resuspended with 500 ul 2% serum PBS and finally put into Eppendorf (EP) tubes for flow cytometry detection.

Response evaluation criteria in solid tumors (RECIST)

Clinical response to chemotherapy was evaluated by measurement of tumor size using MRI. According to the revised RECIST guideline (version 1.1) [28], clinical response to chemotherapy was classified into complete response (CR, disappearance of all target lesions), partial response (PR, at least a 30% decrease in the sum of the largest diameter (LD) of target lesions), stable disease (SD, neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for progressive disease [PD]), and PD (at least a 20% increase in the sum of the LD of target lesions). The BC patients with CR and PR were included into a CR + PR group and those with SD and PD into a SD + PD group. Response rate was defined as the rates of CR + PR.

Follow-up

The follow-up was made by phone call and outpatient office visits and ended on October 31, 2016. The follow-up started on the day operation was performed and ended with death, missing visit, or deadline. Among 187 patients, 7 were lost to follow-up, giving a follow-up rate of 96.3%. OS was defined as the time from BC diagnosis (surgery) to either death (regardless of cause of death) or the last known date alive. Disease-free survival (DFS) was defined as the time from surgery to events such as relapse or metastasis, dearth from any cause, or the last known date alive.

Statistical analysis

Data were analyzed using the statistical package for the social sciences (SPSS) version 19.0 (SPSS Inc., Chicago, IL). Continuous data are expressed as mean ± standard deviation; one-way analysis of variance (ANOVA) was applied for comparisons of 3 or more independent groups, and the t-test was used for comparisons of 2 groups. Categorical data are expressed as ratio and percentage and the chi-square test was performed for comparison in a group. Spearman's correlation analysis was used to examine the correlation between the efficacy of chemotherapy and CTCs. A receiver operating characteristic (ROC) curve was plotted to estimate the predictive value of CTC counts for clinical response to chemotherapy. The Kaplan-Meier method was used to calculate DFS and OS of BC patients undergoing chemotherapy. The log-rank test is employed for examining treatment differences in survival between 2 treatment groups. Cox regression analysis was used to determine risk factors for prognosis of BC. A significance level of $P<0.05$ was set as a statistically significant difference.

Results

CTC count at T0, T1, and T2 and its correlation with clinical response to chemotherapy

Among 187 patients, there were 22 with CR (11.8%), 101 with PR (54.0%), 56 with SD (29.9%), and 8 with PD (4.3%). CR + PR was achieved by 123 cases, 65.8% of BC patients. According to

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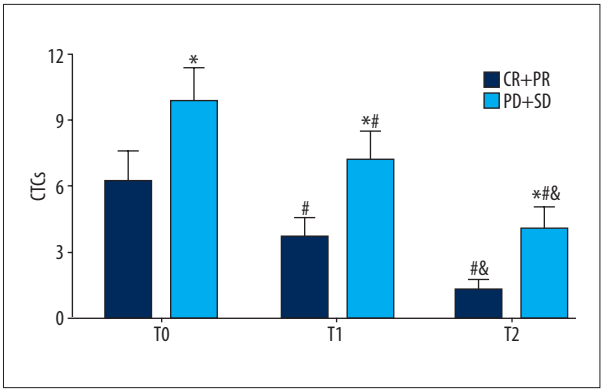

Figure 1. Comparison of CTC counts at T0, T1, and T2 between the CR + PR group and the SD + PD group. T0, before chemotherapy; T1, 1 day before the third cycle of chemotherapy; T2, 1 week after the third cycle of chemotherapy; * $P<0.05$ compared with the CR + PR group; # $P<0.05$ compared with the T0; & $P<0.05$ compared with the T2. CTC – circulating tumor cell; CR – complete response; PR – partial response; SD – stable disease; PD – progressive disease.

clinical response to chemotherapy, BC patients with CR and PR were assigned into the CR + PR group and those with SD and PD into the SD + PD group. As shown in Figure 1, CTC counts were lower in the CR + PR group than in the SD + PD group at T0, T1, and T2 ($P<0.05$). After chemotherapy, CTC counts were decreased in both the CR + PR and SD + PD groups ($P<0.05$). Spearman's correlation analysis indicated that CTC counts at T0 were positively correlated with clinical response to chemotherapy ($r=0.66$, $P<0.05$).

The prediction of CTC count at T0 for clinical response to chemotherapy in BC

As shown in Figure 2, for predicting clinical response to chemotherapy, with cutoff of 8 for CTCs, CTC counts yielded an area under the curve (AUC) of 0.958, with the sensitivity reaching 96.9% and the specificity reaching 85.4% (95%CI: 0.927-0.990). These data suggested that CTC counts exhibited good performance for predicting clinical response to chemotherapy.

Association between the CTC count at T0 and clinicopathological characteristics of BC patients

CTCs-negative patients were defined as those with CTCs <8 at T0 and CTCs-positive patients as those with CTCs ≥8 at T0. As shown in Table 1, CTC counts were associated with tumor node metastasis (TNM) staging, tumor size, lymph node metastasis (LNM), ki-67, molecular classification, and endocrine therapy ($P < 0.05$), but were not related with age, menstruation, family medical history, histopathological types, tumor thrombus, ER, PR, or HER-2 ($P>0.05$).

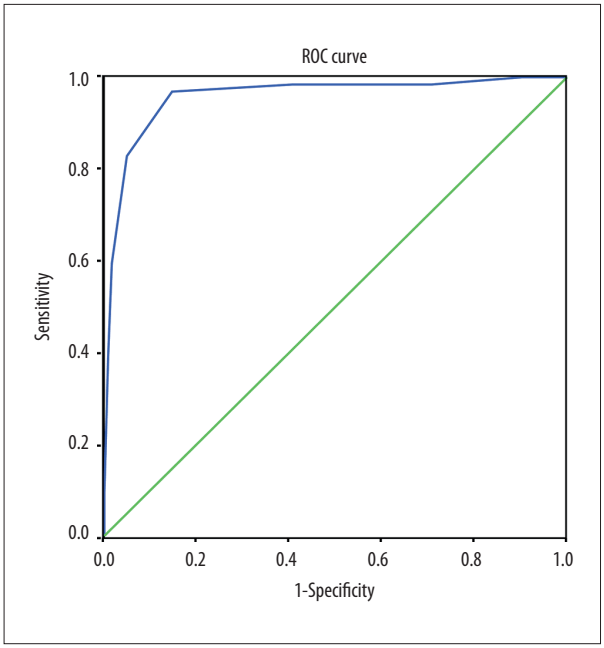

Figure 2. The predictive performance of CTC counts at T0 for clinical response to chemotherapy in BC, estimated by the ROC curve. CTC counts yielded an AUC of 0.898 for predicting clinical response to chemotherapy, with sensitivity reaching 82.6% and specificity reaching 87.1%. T0 – before chemotherapy; CTC – circulating tumor cell; BC – breast cancer; ROC – receiver operating characteristic; AUC – area under the curve.

The Kaplan-Meier method for determination of factors influencing DFS and OS of BC patients

The BC patients exhibited a 3-year OS rate of 78.1% (median OS: 28.68±0.52 months) and a DFS rate of 62.0% (median DFS: 25.66±1.03 months). The Kaplan-Meier results suggested that factors influencing DFS and OS of patients included TNM staging, tumor size, LNM, ki-67, molecular classification, endocrine therapy, and CTC counts ($P<0.05$). The DFS and OS of patients were not associated with age, menstruation, family medical history, histopathological types, tumor thrombus, ER, PR, or HER-2 ($P>0.05$) (Table 2).

Cox regression analysis for determination of factors influencing the prognosis of BC patients

With DFS and OS as dependent variables, and TNM staging, tumor size, LNM, Ki-67, molecular classification, endocrine therapy, and CTC counts as independent variable, the Cox regression analysis was performed, showing that TNM staging, LNM, Ki-67, and CTC counts were independent risk factors, but endocrine therapy was a protective factor for the prognosis of BC patients ($P<0.05$). However, tumor size and molecular classification were not associated with DFS and OS of BC patients ($P>0.05$) (Table 3).

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Table 1. Association between the CTC count at T0 and clinicopathological characteristics of BC patients.

Clinicopathological characteristics		Case	CTCs				χ^2	*P*
			Negative (n, %)		Positive (n, %)			
Age (years)	<50	90	49	(26.2)	41	(21.9)	0.55	0.46
	≥50	97	58	(31.0)	39	(20.9)		
Menstruation	Yes	108	66	(35.3)	42	(22.5)	1.58	0.209
	No	79	41	(21.9)	38	(20.3)		
Family medical history	No	165	95	(50.8)	70	(37.4)	0.07	0.787
	Yes	22	12	(6.4)	10	(5.3)		
TNM staging	IIa	17	12	(6.4)	5	(2.7)	16.42	0.001
	IIb	76	53	(28.3)	23	(12.3)		
	IIIa	62	23	(12.3)	39	(20.9)		
	IIIb	32	19	(10.2)	13	(7.0)		
Histological classification	IDC	139	83	(44.4)	56	(29.9)	1.49	0.685
	ILC	23	11	(5.9)	12	(6.4)		
	SCC	10	5	(2.7)	5	(2.7)		
	Others	15	8	(4.3)	7	(3.7)		
Tumor size	≤5 cm	147	92	(49.2)	55	(29.4)	8.08	0.005
	>5 cm	40	15	(8.0)	25	(13.4)		
Tumor thrombus	No	118	72	(38.5)	46	(24.6)	1.88	0.170
	Yes	69	35	(18.7)	34	(18.2)		
LNM	No	36	26	(13.9)	10	(5.3)	4.10	0.043
	Yes	151	81	(43.3)	70	(37.4)		
ER	Negative	77	46	(24.6)	31	(16.6)	0.34	0.560
	Positive	110	61	(32.6)	49	(26.2)		
PR	Negative	98	55	(29.4)	43	(23.0)	0.10	0.750
	Positive	89	52	(27.8)	37	(19.8)		
HER-2	Negative	64	35	(18.7)	29	(15.5)	0.25	0.614
	Positive	123	72	(38.5)	51	(27.3)		
Ki-67	Negative	52	37	(19.8)	15	(8.0)	5.71	0.017
	Positive	135	70	(37.4)	65	(34.8)		
Molecular classification	Luminal A	67	44	(23.5)	23	(12.3)	9.29	0.026
	Luminal B	60	38	(20.3)	22	(11.8)		
	HER-2	32	12	(6.4)	20	(10.7)		
	TNBC	28	13	(7.0)	15	(8.0)		
Endocrine therapy	Yes	133	70	(37.4)	63	(33.7)	3.96	0.047
	No	54	37	(19.8)	17	(9.1)	0.55	0.46

CTC – circulating tumor cell; T0 – before chemotherapy; BC – breast cancer; TNM – tumor node metastasis; IDC – invasive ductal carcinoma; ILC – invasive lobular carcinoma; SCC – squamous cell carcinoma; LNM – lymph node metastasis; ER – estrogen receptor; PR – progesterone receptor; HER-2 – human epidermal growth factor receptor-2; TNBC – triple negative breast cancer.

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Table 2. The Kaplan-Meier method for determination of factors influencing DFS and OS of BC patients.

Clinicopathological characteristics		Case	OS	*P*	DFS	*P*
Age (years)	<50	90	28.93±0.70	0.994	25.47±0.99	0.985
	≥50	97	28.44±0.76		24.66±1.03	
Menstruation	Yes	108	29.01±0.67	0.317	26.05±0.91	0.066
	No	79	28.22±0.81		23.68±1.14	
Family medical history	No	165	28.92±0.54	0.433	25.20±0.76	0.581
	Yes	22	26.86±1.77		23.86±2.25	
TNM staging	IIa	17	30.78±1.19	0.002	29.56±1.64	0.001
	IIb	76	30.17±0.60		27.13±1.00	
	IIIa	62	28.17±0.89		23.19±1.27	
	IIIb	32	24.96±1.78		21.22±1.98	
Histological classification	IDC	139	28.51±0.62	0.867	24.76±0.84	0.745
	ILC	23	29.70±0.89		26.39±1.72	
	SCC	10	27.00±3.17		22.70±3.78	
	Others	15	29.87±1.42		27.20±2.26	
Tumor size	≤5 cm	147	29.48±0.50	0.012	26.21±0.73	0.007
	>5 cm	40	25.73±1.50		20.78±1.85	
Tumor thrombus	No	118	29.10±0.61	0.274	25.77±0.88	0.135
	Yes	69	27.95±0.95		23.81±1.23	
LNM	No	36	30.81±0.77	0.031	28.64±1.15	0.028
	Yes	151	28.16±0.61		24.19±0.83	
ER	Negative	77	29.26±0.74	0.318	25.21±1.15	0.587
	Positive	110	28.27±0.71		24.94±0.92	
PR	Negative	98	28.93±0.70	0.615	25.28±0.98	0.558
	Positive	89	28.40±0.78		24.80±1.06	
HER-2	Negative	64	29.49±0.76	0.262	25.86±1.15	0.438
	Positive	123	28.25±0.68		24.63±0.91	
Ki-67	Negative	52	29.79±0.87	0.045	27.60±1.27	0.003
	Positive	135	28.25±0.63		24.07±0.85	
Molecular classification	Luminal A	67	29.63±0.75	0.038	27.22±1.08	<0.001
	Luminal B	60	29.05±0.90		26.47±1.22	
	HER-2	32	28.70±1.11		22.28±1.70	
	TNBC	28	25.57±1.76		19.96±1.99	
Endocrine therapy	Yes	133	30.52±0.65	0.021	28.04±1.06	0.012
	No	54	27.92±0.67		23.84±0.89	
CTCs	Negative	103	30.15±0.56	<0.001	27.54±0.82	<0.001
	Positive	84	26.70±0.91		21.71±1.17	

OS – overall survival; DFS – disease-free survival; T0 – before chemotherapy; BC – breast cancer; TNM – tumor node metastasis; IDC – invasive ductal carcinoma; ILC – invasive lobular carcinoma; SCC – squamous cell carcinoma; LNM – lymph node metastasis; ER – estrogen receptor; PR – progesterone receptor; HER-2 – human epidermal growth factor receptor-2; TNBC – triple negative breast cancer; CTCs – circulating tumor cells.

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Table 3. The cox regression analysis for determination of factors influencing the prognosis of BC patients.

Histological classification	OS			DFS		
	P	EXP	95%CI	*P*	EXP	95%CI
TNM staging	0.004	1.92	1.23–2.99	0.002	1.64	1.20–2.25
Tumor size	0.609	1.22	0.56–2.65	0.952	1.02	0.57–1.81
LNM	0.019	4.25	1.27–14.18	0.024	2.14	1.10–4.14
Ki-67	0.043	2.95	1.03–8.45	0.001	3.65	1.64–8.11
Molecular classification	0.564	0.91	0.65–1.26	0.272	1.14	0.90–1.45
Endocrine therapy	0.041	0.37	0.14–0.96	0.043	0.53	0.29–0.98
CTCs	0.010	2.62	1.26–5.47	0.036	1.73	1.04–2.88

OS – overall survival; DFS – disease-free survival; T0 – before chemotherapy; BC – breast cancer; TNM – tumor node metastasis; LNM – lymph node metastasis; CTCs – circulating tumor cells.

Discussion

CTCs in the peripheral blood are considered to be a predictor for the prognosis of BC, which is of great help in monitoring and treatment [29]. In consideration of this point, flow cytometry was performed to count CTCs in patients with BC before and after chemotherapy, and the predictive value of CTC count was evaluated using the ROC curve. The results indicated that CTC counts had a high predictive value for both short- and long-term efficacy of chemotherapy in patients with BC.

The results first indicated that patients in the CR + PR group had lower CTC counts than the SD + PD group before and after chemotherapy. Several methods have been used in practical measurement of CTC counts, such as positron emission tomography-CT (PET-CT), magnetic resonance imaging (MRI), and flow cytometry [30,31]. To obtain reliable results, flow cytometry was used in the present study to measure the number of CTCs. Generally speaking, more than 2 CTCs per 7.5 ml of blood are present in 60% of patients with MBC [32]. The measurement of CTCs before and after surgical treatment for esophageal squamous cancer (EAC) is of great help in predicting tumor recurrence [33]. Chemotherapy is reported to be an effective way to kill CTCs in the blood of cancer patients, which is superior to tumor surgery and radiation therapy [34]. Consistent with our finding that CTCs were decreased after chemotherapy, another study found that CTCs in patients with urothelial carcinoma are lower in CR + PR patients after chemotherapy [35]. In line with those findings, Spearman correlation analysis also showed that CTC counts before chemotherapy was positively correlated with the clinical response to chemotherapy. Additionally, CTC counts in the blood before and after surgical treatment are related to indicators of long-term efficacy of chemotherapy for patients with BC, including recurrence-free survival (RFS) and OS [36]. The presence of CTCs prior to treatment was significantly related to progression-free survival (PFS) and OS [37]. Also, the 3-year follow-up indicated the CTCs count was associated with disease-free survival and OS in patients with BC. The CTC count had an AUC of 0.958, as well as high sensitivity and specificity as evaluated with the ROC curve. Thus, the CTC count in the peripheral blood of patients with BC before chemotherapy has a predictive value for both short- and long-term efficacy of chemotherapy.

In the present study, we also analyzed the correlation between CTC counts and clinicopathological features of BC patients. As a preoperative staging factor in EAC, the CTC count is considered to be independent of tumor-related indicators, such as tumor stage and grade, histological subtype, and lymph node invasion [33]. However, in patients with BC, there was a correlation between CTC counts and TNM staging, tumor size, LNM, ki-67, molecular classification, and endocrine therapy. Expression of Ki-67 protein, as a qualitative marker for cell proliferation, especially the potential for cell division [38], may predict tumor progression. Ki-67 expression is also reported to be a predictor of poor prognosis in patients with aggressive BC [39]. Here, Ki-67 was indicated to be a risk factor for the prognosis of BC, and the CTC count was associated with Ki-67 expression, which may provide a possible explanation for the mechanism underlying the ability of CTCs to predict the efficacy of treatment in BC. Endocrine therapy has been recommended as an initial therapy in HR-positive MBC [40]. The present study indicated that endocrine therapy was an independent protective factor for the prognosis of BC. Hormone receptor-negative BC consists of the Her-2 subtype, which is characterized by enrichment of the HER-2 gene, and basal cell subtype with ER and PR, of which the triple-negative type accounts for the greatest percentage [41]. The results of this study also demonstrated that the CTC count was closely associated with the molecular classification of BC. However, the CTC count has no

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significant association with cancer-associated markers ER/PR or HER-2 status [42], which was similar to our results.

Conclusions

To conclude, BC patients with CTCs ≥8 exhibited a worse prognosis after chemotherapy, as well as a shorter overall survival. CTC count can serve as an indicator in predicting short- and long-term efficacy of chemotherapy for BC. However, the detailed mechanism should be further investigated to verify the molecular pathway of CTCs in the progression of BC and the role of CTCs in more cancers. Our next study protocol will evaluate the radiologic response to treatment in MBC patients and evaluation of the correlation with CTC counts.

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