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Original Research Article

Effect of exemestane endocrine therapy for hormone-receptor-positive breast cancer patients with varying levels of ctDNA

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Abstract

Purpose: *To determine the effectiveness of exemestane in the treatment of hormone receptor (HR)-positive breast cancer patients with different levels of circulating tumor DNA (ctDNA).*
Methods: *HR-positive breast cancer patients admitted at the Oncology Department of Harbin Medical University Cancer Hospital, Harbin, China from January 2017 to January 2019 were selected as subjects. They were given endocrine therapy with exemestane, and subjected to plasma ctDNA screening. The expression levels of ctDNA, levels of factors that influence ctDNA expression, ctDNA expression levels before and after treatment, clinical efficacy of treatment, long-term prognosis, and factors that influence prognosis of the disease, were determined.*
Results: *There were 57 ctDNA-positive patients and 23 ctDNA-negative patients, accounting for 71.25 % positive ctDNA expression. Post-treatment, 32 patients were ctDNA-positive, while 48 patients were ctDNA-negative. There were significant differences in age distribution, cases of lymph node metastasis, and levels of CEA, CA125 and CA153 between ctDNA-negative and ctDNA-positive groups ($p < 0.05$). The ORR of ctDNA-negative group was significantly higher than that of ctDNA-positive group ($\chi^2 = 6.841$; $p = 0.009$). Decrease (Δ) in the levels of CEA, CA125 and CA153 in the ctDNA-negative group was significantly higher than those in control group ($p < 0.001$). The overall survival time of ctDNA-negative group was significantly better than that of ctDNA-positive group.*
Conclusion: *ctDNA mutations in HR-positive breast cancer patients correlated with clinical outcomes of neoadjuvant treatment with exemestane. The treatment resulted in marked reduction in number of ctDNA-positive patients.*

Keywords: *Hormone receptor positive breast cancer, Exemestane, Circulating tumor DNA, Clinical outcomes*

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INTRODUCTION

Breast cancer occurs when breast epithelial cells proliferate out of control under the influence of a variety of carcinogenic factors [1]. Early breast cancer manifestations include breast lumps, nipple discharge and axillary lymphadenopathy. In the advanced stage, cancer cells undergo

metastasis to distant sites, resulting in multiple organ lesions which seriously affect the life of the patient [2]. Breast cancer ranks first among female malignant tumors. However, due to advances in medical research, breast cancer has become one of the solid tumors with excellent outcomes [3]. It has been estimated that 67 % of breast cancer patients express estrogen receptor and/or progesterone receptor, which is suitable for endocrine therapy. This is known as HR-positive breast cancer [4].

Exemestane is a second-generation aromatase inhibitor used for treatment of metastatic breast cancer, and as an adjuvant therapy for early breast cancer. The drug is widely applied in treatment of HR-positive breast cancer [5]. Circulatory tumor DNA (ctDNA) refers to DNA fragments released by tumor cells into the blood circulation. Thus, these DNA fragments reflect the tumor burden, and carry a large amount of information about the biological behavior of the tumor. Moreover, due to their merits of non-invasiveness and repeatability, ctDNA are regarded as *liquid biopsy* [6]. A lot of research interest on breast cancer is currently focused on assay of ctDNA which is critical in the diagnosis and clinical evaluation of prognosis of breast cancer [7]. However, not much is known on changes in expressions of ctDNA before and after treatment of breast cancer. Moreover, there is paucity of information on the effect of differences in ctDNA expression levels on the prognosis of breast cancer. In this study, exemestane was applied as adjuvant endocrine therapy on 80 patients with HR-positive breast cancer. The expression levels of ctDNA were assayed, and clinical prognosis of patients with different expression levels of ctDNA were evaluated.

METHODS

Subjects

From January 2017 to January 2019, 80 patients with HR-positive breast cancer treated with exemestane neoadjuvant therapy and ctDNA test, were selected from the Oncology Department of Harbin Medical University Cancer Hospital. This study was approved by the ethics committee of Harbin Medical University Cancer Hospital (approval no. 2017-34 (334), and followed international guidelines for human studies.

Inclusion criteria

Patients in the following categories were included in the study: (1) first-visit patients aged 18-80 years; (2) those confirmed as HR-positive breast cancer via histological or cytological examination, and whose TNM clinical stage was II or III [8]; (3) patients with at least one measurable primary lesion (RECIST v1.1 standard); (4) patients with ECOG scores of 0 - 2 points [9]; (5) those who received no other anti-tumor treatment before enrollment; and (6) patients who voluntarily participated in the clinical trial and signed a written informed consent.

Exclusion criteria

Patients in the following categories were excluded: (1) those without measurable lesions such as pleural or pericardial exudates or ascites; (2) patients who had inflammatory breast cancer or other malignant tumors; (3) those with a history of allergies to the drug components of the treatment regimen used; (4) patients who had a history of immunodeficiency, including positive HIV test result, or other acquired or congenital immunodeficiency diseases, or a history of organ transplantation; (5) those with impaired blood coagulation, and dysfunctions in important organs; and (6) patients with a history of mental disorders, and those who were unable to cooperate during the study, or who did not complete the follow-up.

Neoadjuvant endocrine therapy

All patients were treated orally with exemestane, a third-generation aromatase inhibitor, at a dose of 25 mg/day. During the treatment, attention was paid to the occurrence of osteoporosis. Appropriate supplementation with calcium, vitamin D and bisphosphonate supplements were carried out. All patients received neoadjuvant endocrine therapy for 6 consecutive months. Patients with worsening disease conditions were in addition, given chemotherapy or radiotherapy or other therapies. The other patients did not receive additional treatments during the treatment period.

Assay of ctDNA

All patients were screened for ctDNA on the first day of treatment, and at 6 months after treatment. Fasting venous blood was taken from the patients. The specific assay procedure was as described earlier [10]. Plasma was obtained after centrifugation of blood, and ctDNA was extracted from the plasma using DNA extraction kits, strictly in accordance with the kit instructions. The kit was used to construct a library of the interrupted DNA. The library was amplified via polymerase chain reaction (PCR), followed by sequencing to obtain the whole

genome length. Thereafter, blast comparison, analysis of CNVs, and calculation of ctDNA content and comparison with percentage of ctDNA in healthy people as a reference, were carried out. The classification of ctDNA was set as negative (for ctDNA content < 0.1%), or positive (for ctDNA content ≥ 0.1%).

Measurement of indicators

ctDNA expression

The ctDNA-positive expressions before treatment, and 6 months after treatment, were

Tumor indices

Before treatment, and 6 months after treatment, fasting venous blood was taken from each patient, and serum concentrations of CEA, CA125, and CA153 were determined using enzyme-linked immunosorbent assay (ELISA).

Clinical treatment effectiveness

After 6 months of treatment, the patients' conditions were followed up and recorded as indexes of treatment effectiveness. According to the *Response Evaluation Criteria in Solid Tumors (RECIST)* [11], clinical efficacy was divided into complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). The objective response rate (ORR) was calculated as in Eq 1. ORR (%) = {(Nc + Np)/Nc+Np+Ns+Np1}100%, where Nc = no. of complete response, Np = no. of partial response, Ns = no. of stable disease, and Np1= no. of progressive disease.

Long-term curative effect

The patients were followed up from the beginning of treatment, up to 24 months post-treatment, or to time of death. The follow-up was done at 4-month intervals, and survival curves of the patients were plotted.

Statistical analysis

All statistical analysis were performed with SPSS, while GraphPad prism 8.0 software was used to plot graphs. Enumeration data are expressed as percentage (%). The chi-square test or Fisher's exact test was employed to compare differences between groups. Measured data are expressed as mean ± standard deviation (SD), and the comparison between groups was conducted using independent sample *t*-test. Intra-group comparison was done with paired *t*-test. Kaplan-Meier survival analysis (Log-rank test) was used to analyze data on survival. Significant difference was assumed at $p \leq 0.05$.

RESULTS

Basic information on patients

As shown in Table 1, a total of 80 patients were enrolled, with an average age of 61.28 ±13.57 years. Among these, 56 patients were aged ≥ 60 years, while 24 patients were aged < 60 years. Tumor diameters of 18 patients were < 2 cm; 48 patients had tumor diameters of 2 – 5 cm, while 14 patients had tumor diameters > 5 cm. With respect to lymph node metastases, 44 patients were in N1 stage, 30 patients were in N2 stage, while 6 patients were in N3 stage. The mean values of plasma baseline tumor indexes were 32.61 ± 4.22 ng/mL (CEA), 67.26 ± 7.70 U/mL (CA125) and 61.29 ± 12.11 U/mL (CA153).

Table 1: General profile of the enrolled patients

Parameter	Overall (n=80)
Age	61.28±13.57
≥ 60 years	56 (70.0%)
< 60 years	24 (30.0%)
Tumor size	
< 2 cm	18 (22.5%)
2~5 cm	48 (60.0%)
> 5 cm	14 (17.5%)
Lymph node metastasis	
N1	44 (55.0%)
N2	30 (37.5%)
N3	6 (7.5%)
Baseline serological indicators	
CEA (ng/mL)	32.61±4.22
CA125 (U/mL)	67.26±7.70
CA153 (U/mL)	61.29±12.11

ctDNA-positive expression

As shown in Table 2, before treatment, 57 patients were ctDNA-positive, while 23 patients were ctDNA-negative, resulting in 71.25 % positive ctDNA expression. However, after treatment, 25 patients were converted from ctDNA-positive to ctDNA-negative, while 3 pre-treatment ctDNA-negative patients became ctDNA-positive. In all, there were 32 post-treatment cases of ctDNA-positive, and 48 cases of ctDNA-negative, accounting for 60 % ctDNA-positive expression. Thus, after treatment, there was marked decrease in ctDNA-positive expression ($\chi^2 = 15.83$, $p < 0.001$).

Table 2: ctDNA expression levels before and after treatment

Variable		After treatment		
		Positive	***Negative***	***Total***
Baseline	Positive	20	37	57
	Negative	12	11	23
	Total	32	48	80

Single-factor analysis of different expression levels of baseline ctDNA

All patients were divided into ctDNA-positive (n = 57) and ctDNA-negative (n = 23) groups, based on the ctDNA expression level (Table 3). A comparison of the baseline data of the two groups of patients showed that age, lymph node metastasis, and concentrations of CEA, CA125, and CA153 differed significantly between them ($p < 0.05$). However, tumor diameter was comparable between the two groups of patients ($p > 0.05$).

Clinical efficacy of treatment in patients with different baseline ctDNA levels

Table 4 shows that 14, 18, 18, and 7 of the 57 ctDNA-positive patients manifested CR, PR, SD, and PD, respectively, resulting in ORR value of 56.14 % (32/56). In the crDNA-negative patients (n=23), the corresponding number of patients with CR, PR, SD, and PD were 8, 12, 2, and 1, respectively, resulting in ORR value of 86.96 % (20/23). The ORR of the ctDNA-negative group was significantly higher than that of the ctDNA-positive group (χ^2=6.841, p = 0.009).

Change (Δ%) in serum tumor indices in patients

Figure 1 shows that after treatment, CEA in the ctDNA-positive group was decreased by 31.83±9.67 %, CA125 was decreased by 36.68±11.85 %, while CA153 was reduced by 42.63±8.54 %. In the ctDNA-negative group, CEA was decreased by 45.28 ± 12.45 %, CA125 was reduced by 62.28 ± 14.86 %, while CA153 was decreased by 71.84 ± 18.24 %. The levels of CEA, CA125, and CA153 in the ctDNA-negative group were markedly higher than those in the control group ($p < 0.001$).

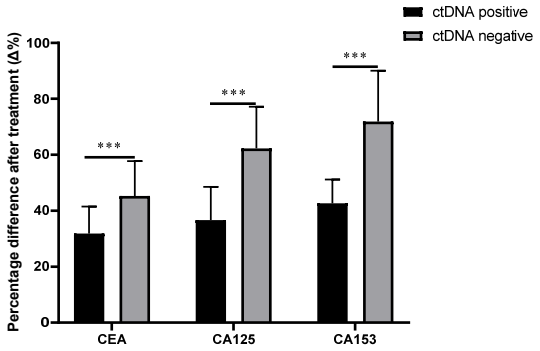

Figure 1: Percentage differences in serological indicators after treatment. ***$P < 0.001$

Table 3: Univariate analysis of ctDNA expression levels at baseline

Parameter	ctDNA-positive (n=57)	ctDNA-negative (n=23)	t/χ^2	*P*-value
Age	64.85±15.84	58.29±11.55	1.800	0.076
≥60 years	45	12	5.735	0.017
＜60 years	12	11		
Tumor size			2.860	0.239
＜2 cm	10	8		
2-5 cm	36	12		
＞5 cm	11	3		
Lymph node metastasis			7.084	0.029
N1	26	18		
N2	26	4		
N3	5	1		
Baseline serological indicators				
CEA (ng/ml)	34.91±8.28	29.87±6.13	2.638	0.010
CA125 (U/ml)	70.82±18.26	62.89±14.30	2.337	0.022
CA153 (U/ml)	65.94±15.65	58.98±14.29	2.553	0.013

Table 4: Clinical efficacy in patients with different baseline ctDNA levels

Variable	CR	PR	SD	PD	ORR
ctDNA-positive (n=57)	14	18	18	7	32
ctDNA-negative (n=23)	8	12	2	1	20
χ^2					6.841
P-value					0.009

Overall survival of patients with different ctDNA levels

The overall survival time of ctDNA-positive/all group was 18.737 (17.095/20.379) months, while the overall survival time of the ctDNA negative/all group was 20.696 (18.302/23.089) months, and HR was 2.230 (1.154/4.311) (χ^2 = 4.447, p =0.035). The overall survival time of the ctDNA all/positive group was 17.500 (14.915/20.085) months, while the overall survival time of the ctDNA all/negative group was 20.500 (19.050/21.950)] months, and the HR was 1.717 (0.871/3.387)] (χ^2 = 2.436, p = 0.119). These results are shown in Figure 2 and Table 5.

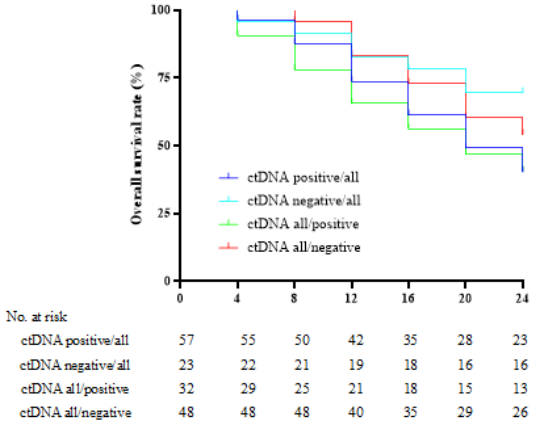

Figure 2: Overall survival times of patients with different ctDNA levels

The overall survival of patients in the ctDNA positive/positive, ctDNA positive/negative, ctDNA negative/positive, and ctDNA negative/negative groups were 17.750 (15.426/20.074)], 20.000 (17.731/22.269), 10.667 (3.752/17.581), and 22.200 (20.322/24.078) months, respectively (χ^2 = 14.93, p < 0.001). Pairwise comparison revealed that the overall survival of the ctDNA negative/negative group was considerably better than that of the ctDNA positive/positive group (χ^2=15.69, p < 0.001). These results are presented in Figure 3 and Table 5.

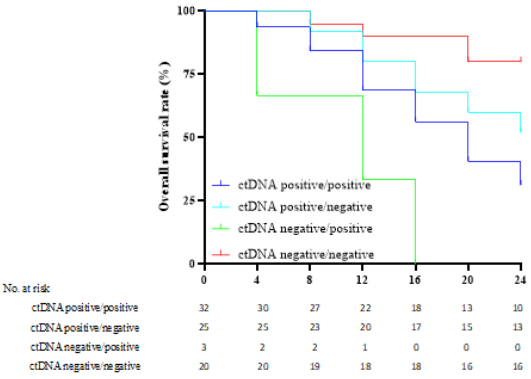

Figure 3: Overall survival times associated with different ctDNA sub-levels

DISCUSSION

In the current study, there were decreases in ctDNA-positive expression of HR-positive breast cancer patients after neoadjuvant endocrine therapy with exemestane. The ORR of ctDNA-negative patients was markedly superior to that of the ctDNA-positive patients. At present, the diagnosis of breast cancer involves the use of mammography and breast ultrasound. However, these strategies are associated with risk of missed diagnosis and misdiagnosis, and a lack of effective means to assess the prognosis of patients [12].

Circulating tumor DNAs (ctDNAs) which are derived from somatic mutations in tumor cells, have identical epigenetic characteristics as tumor tissues. They provide broad prospects in the diagnosis and prognosis evaluation of breast cancer, and their use overcomes the problem of heterogeneity in tumor detection [13,14]. However, due to the high requirements for sample preservation in ctDNA testing, no consensus has been achieved with respect to the testing procedure and quantitative testing, as well as baseline ctDNA level for breast cancer diagnosis [15]. It is known that changes in ctDNA levels are closely related to tumor progression, and they are useful for predicting treatment response.

Table 5: Overall survival time of different ctDNA levels

Variable	Overall survival time	HR	χ^2	*P*-value
ctDNA-positive/all	18.737 (17.095, 20.379)	2.230 (1.154,4.311)	4.447	0.035
ctDNA-negative/all	20.696 (18.302, 23.089)	-		
ctDNA all/positive	17.500 (14.915, 20.085)	1.717 (0.871,3.387)	2.436	0.119
ctDNA all/negative	20.500 (19.050, 21.950)	-		
ctDNA positive/positive	17.750 (15.426, 20.074)	1.667 (0.856, 3.287)	2.456	0.117
ctDNA positive/negative	20.000 (17.731, 22.269)	-		
ctDNA negative/positive	10.667 (3.752, 17.581)	10.550 (0.538,207.0)	15.69	<0.001
ctDNA negative/negative	22.200 (20.322, 24.078)	-		

Studies have compared the potential of using tumor serum markers, imaging examinations, and ctDNA expression levels in assessing breast cancer progression. The results indicate that ctDNA levels reflect the status of disease and clinical progress in its treatment [16].

The expression levels of ctDNA are dynamic. It has been reported that the pre-treatment ctDNA levels of patients in neoadjuvant chemotherapy-insensitive group were markedly higher than those of patients who benefited from treatment [17]. This finding suggests that the level of ctDNA before treatment may serve as predictor of the degree of benefit from neoadjuvant chemotherapy, which is consistent with the results of the present study [17].

Drug resistance is a serious problem in endocrine therapy for HR-positive breast cancer patients. Indeed, approximately 30 % of HR-positive patients have primary drug resistance. There is a close correlation between mutations in the estrogen receptor alpha gene (ESR1) in ctDNA and drug resistance [18]. Previous work on breast cancer patients revealed that the extent of ESR1 mutation in ctDNA was more than 50 %, but it was only 30 % in metastases, indicating that ctDNA measurement can be used to predict the outcome of endocrine therapy [19]. The results of this study showed that there were disparities in the age distribution, lymph node metastasis, and concentrations of CEA, CA125 and CA153 in patients with different levels of ctDNA expression. Moreover, the tumor serological indicators in ctDNA-negative patients were more significantly decreased after treatment, which further confirmed that ctDNA is associated with tumor burden.

The present study compared the overall survival of patients with different expression levels of ctDNA, and found that the overall survival of ctDNA-negative/all patients was better than that of ctDNA-positive/all patients, and the overall survival of ctDNA-negative/negative patients was superior to that of ctDNA-positive/positive patients. These results suggest that the baseline ctDNA expression level can be used as an indicator for prognostic evaluation, while the ctDNA expression level after treatment is of no significant prognostic value. In addition, patients who were ctDNA-negative before and after treatment had optimum long-term prognosis. In this study, 3 ctDNA-negative patients became ctDNA-positive after treatment, possibly due to poor treatment efficacy and tumor progression. On the other hand, it also shows that ctDNA has a short half-life, and is vulnerable to inflammation, trauma, and chemotherapy drugs [20]. Consequently, the sensitivity and specificity of ctDNA results need to be further strengthened.

CONCLUSION

This study has demonstrated that ctDNA is correlated with the outcome of exemestane neoadjuvant therapy in HR-positive breast cancer patients. The therapy resulted in marked decreases in ctDNA-positive expression.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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