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Original Article

Evaluation of biomarker changes after administration of various neoadjuvant chemotherapies in breast cancer

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Abstract: To assess the changes in estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and Ki-67 expression in breast cancer patients after various neoadjuvant chemotherapies. Data from 138 locally advanced breast cancer patients with histological diagnoses were reviewed. Seventy patients (group 1) were given 4 cycles of 500 mg/m^2 cyclophosphamide and 50 mg/m^2 pirarubicin every 21 days. Sixty-eight patients (group 2) were given 4 cycles of 500 mg/m^2 cyclophosphamide and 75 mg/m^2 docetaxel every 21 days. The biomarker changes of the operated tumor tissues were compared with the initial core biopsies. ER, PR, HER2 and Ki-67 expression changed by 28.6%, 22.9%, 17.1% and 54.3%, respectively, after neoadjuvant chemotherapy in group 1 and 16.2%, 22.1%, 13.2% and 70.6%, respectively, after neoadjuvant chemotherapy in group 2. There were significant differences between the groups regarding ER and Ki-67 status changes, and these changes can be used to inform treatment strategies.

Keywords: Breast cancer, neoadjuvant chemotherapy, ER, PR, HER2, Ki-67

Introduction

The introduction of neoadjuvant chemotherapy in locally advanced breast cancer offered us advantages like initiation of early systemic therapy, delivery of drugs through intact vasculature, down-staging of tumors, which makes inoperable tumors operable and renders tumors suitable for breast conserving surgery [1, 2]. Biomarkers have been applied in the detection, screening, diagnosis, and monitoring of cancer treatment. Increasing evidence indicates that tumor biomarker levels can change following neoadjuvant chemotherapy [3-7].

Understanding the relationship between changes in tumor biomarker levels following different neoadjuvant chemotherapies could help assess the effectiveness of these chemotherapies. But previous studies reported are not in complete accord results regarding the impact of neoadjuvant chemotherapy on tumor biomarker status [6-11]. In addition, there was almost no study on the effects of different regimens of neoadjuvant chemotherapy on changes in biomarker of breast cancer. In this study, we assessed the changes in the ER, PR, HER2 and Ki-67 biomarkers in locally advanced breast cancer patients after various neoadjuvant chemotherapies.

Materials and methods

Patients

From December 2012 to June 2014, 144 patients with stage II or III breast cancer who were scheduled for surgery were analyzed in hospital. Patients with early-stage or metastatic breast cancer were excluded from the study. The diagnosis of breast cancer was made via clinical, radiological and histological assessment in all patients. This retrospective study was approved by the institutional ethics committee without the patient informed consent. All

Table 1. Clinical characteristics of the group 1 patients and their responses to neoadjuvant therapy

Characteristics	SD/PD	Overall clinical response		χ^2	*P*	PCR	χ^2	*P*
		PR	CR					
Number of patients	15 (21.40%)	46 (65.70%)	9 (12.90%)			4 (5.71%)		
Age				1.289	0.525		0.449*	0.503
> 50	7 (10.00%)	29 (41.43%)	5 (7.14%)			3 (4.29%)		
≤ 50	8 (11.43%)	17 (24.29%)	4 (5.71%)			1 (1.43%)		
Tumor size				5.581*	0.216		6.992*	0.018
T2	3 (4.29%)	18 (25.71%)	5 (7.14%)			1 (1.43%)		
T3	6 (8.57%)	21 (30.00%)	3 (4.29%)			2 (2.86%)		
T4	6 (8.57%)	7 (10.00%)	1 (1.43%)			1 (1.43%)		
Axillary lymph node				4.162*	0.114		0.047*	0.809
Negative	8 (11.43%)	11 (15.71%)	2 (2.86%)			1 (1.43%)		
Positive	7 (10.00%)	35 (50.00%)	7 (10.00%)			3 (4.29%)		
ER				7.739*	0.019		0.639*	0.424
Negative	3 (4.29%)	19 (27.14%)	7 (10.00%)			3 (4.29%)		
Positive	12 (17.14%)	27 (38.57%)	2 (2.86%)			1 (1.43%)		
PR				6.691	0.034		3.225*	0.082
Negative	4 (5.71%)	28 (40.00%)	6 (8.57%)			4 (5.71%)		
Positive	11 (15.71%)	16 (22.86%)	3 (4.29%)			0 (0)		
HER2				0.979*	0.64		0.078*	0.7857
Negative	11 (15.71%)	32 (45.71%)	5 (7.14%)			3 (4.29%)		
Positive	4 (5.71%)	14 (20.00%)	4 (5.71%)			1 (1.43%)		
Ki-67				0.278*	0.87		0.146*	0.707
Negative	6 (8.57%)	15 (21.43%)	3 (4.29%)			1 (1.43%)		
Positive	9 (12.86%)	31 (44.29%)	6 (8.57%)			3 (4.29%)		

*: Fisher's exact test probability value, hereafter the same.

of the patients were female. The patients' mean age was 46 years (range, 28-69 years). The study included patients with stages IIB (n = 45), IIIA (n = 62), and IIIB (n = 37) breast cancer assessed clinically and radiologically according to the American Joint Committee on Cancer (AJCC) staging guidelines. The histologic classification of the tumors was as follows: 127 patients had infiltrative ductal carcinoma, 9 patients had infiltrative lobular carcinoma, 3 patients had medullary carcinoma, 2 patients had metaplastic carcinoma, 2 patients had mucinous adenocarcinoma, and 1 patient had signet ring cell carcinoma. Written informed consent was obtained from all patients prior to enrollment. The patients were again asked for their permission to participate following neoadjuvant chemotherapy and surgery.

Neoadjuvant chemotherapy

Due to insufficient clinical data and non-representative biopsies, only 138 cases were considered for analysis. The 70 patients in group 1 were given 4 cycles of 500 mg/m^2 cyclophosphamide and 50 mg/m^2 pirarubicin every 21 days. The 68 patients in group 2 were given 4 cycles of 500 mg/m^2 cyclophosphamide and 75 mg/m^2 docetaxel every 21 days. Surgery was performed within 2 weeks after the last chemotherapy cycle. The changes in ER, PR, HER2 and Ki-67 status in the operated tumor tissue were compared with the material obtained via the initial core biopsies. The ER, PR and Ki-67 assessments were performed using standard immunohistochemical techniques. Nuclear expression in > 1% of the tumor cells was considered positive for ER and PR. The Ki-67 proliferation index was defined as the percent of Ki-67-positive cells (among 1,000 cancer cells). The ER, PR and Ki-67 expression levels were compared between the pre-therapy tumor core biopsies and post-neoadjuvant chemotherapy surgical tumor biopsies. The evaluation of HER2 status was performed according to the ASCO/CAP guidelines

Table 2. Clinical characteristics of the group 2 patients and their responses to neoadjuvant therapy

Characteristics	SD/PD	Overall clinical response		χ^2	P	PCR	χ^2	*P*
		PR	CR					
Number of patients	20 (29.40%)	41 (60.30%)	7 (10.30%)			5 (7.40%)		
Age				0.309*	0.857		0.136*	0.897
> 50	9 (13.24%)	21 (30.88%)	3 (4.41%)			2 (2.94%)		
≤ 50	11 (16.18%)	20 (29.41%)	4 (5.88%)			3 (4.41%)		
Tumor size				0.315*	0.989		0.337*	0.845
T2	7 (10.29%)	13 (19.12%)	2 (2.94%)			1 (1.40%)		
T3	7 (10.29%)	14 (20.59%)	3 (4.41%)			2 (2.94%)		
T4	6 (8.82%)	14 (20.59%)	2 (2.94%)			2 (2.94%)		
Axillary lymph node				0.304*	0.809		0.176*	0.773
Negative	10 (14.71%)	19 (27.94%)	4 (5.88%)			3 (4.41%)		
Positive	10 (14.71%)	22 (32.35%)	3 (4.41%)			2 (2.94%)		
ER				11.849*	0.004		0.672*	0.412
Negative	9 (13.24%)	35 (51.47%)	6 (8.82%)			3 (4.41%)		
Positive	11 (16.18%)	6 (8.82%)	1 (1.40%)			2 (2.94%)		
PR				7.299*	0.026		0.045*	0.832
Negative	8 (11.76%)	31 (45.59%)	5 (7.35%)			3 (4.41%)		
Positive	12 (17.65%)	10 (14.71%)	2 (2.94%)			2 (2.94%)		
HER2				1.162	0.559		1.680*	0.165
Negative	12 (17.65%)	19 (27.94%)	3 (4.41%)			4 (5.88%)		
Positive	8 (11.76%)	22 (32.35%)	4 (5.88%)			1 (1.40%)		
Ki-67				8.103*	0.013		3.965*	0.048
Negative	13 (19.12%)	11 (15.71%)	3 (4.41%)			4 (5.88%)		
Positive	7 (10.29%)	30 (44.12%)	4 (5.88%)			1 (1.40%)		

via immunohistochemistry using a system with 4 grades (0-3+). Cases with grade 2+ were further evaluated using fluorescence in situ hybridization. All specimens were reviewed by two pathologists independently.

Clinical response categories

The assessment of the clinical response was based on the change in tumor size, which was obtained from pretreatment clinical and radiological measurements. The clinical measurement was the product of the two greatest palpable perpendicular dimensions of the tumor.

The clinical response was categorized into the following four groups: 1. A complete response (CR) was defined as the complete resolution of the entire tumor, as determined via physical examination and imaging studies; 2. A partial response (PRP) was defined as an incomplete reduction (> 50%) in tumor size; 3. Stable disease (SD) was defined as a reduction of < 50% or an increase of < 25% in tumor size; 4. Progressive disease (PD) was defined as a tumor size increase of > 25%.

Statistics

Normally distributed continuous variables are presented as the mean ± standard deviation, and continuous variables with a skewed distribution are presented as the median and range. Categorical variables are shown as percentages. The Statistical Package for Social Science (SPSS) version 16.0 (SPSS Inc, USA) was used to compare groups. The chi square and Fisher's exact tests were used to assess differences. Statistical significance was set at < 0.05.

Results

Relationship between the clinical characteristics of the patients and response to neoadjuvant therapy

A 78.6% overall neoadjuvant therapy response rate was observed in group 1 (55/70). CR was

Table 3. Relationship between biomarker levels prior to and after neoadjuvant chemotherapy and treatment efficacy in group 1

Changes in biomarkers	Number of patients	SD/PD	Overall clinical response		χ^2	*P*
			PR	CR		
ER					18.166*	0.002
-/+	8	1	5	2		
+/+	39	4	30	5		
-/-	11	8	2	1		
+/-	12	2	9	1		
PR					14.916*	0.008
-/+	5	2	1	2		
+/+	15	7	6	2		
-/-	39	5	29	5		
+/-	11	1	10	0		
HER2					15.218*	0.008
-/+	6	3	2	1		
+/+	27	8	15	4		
-/-	31	2	27	2		
+/-	6	2	2	2		
Ki-67					15.415*	0.007
-/+	11	1	6	4		
+/+	6	2	3	1		
-/-	26	3	23	0		
+/-	27	9	14	4		

observed in 12.9% (9/70) of the patients, and 65.7% (46/70) had a PRP. SD was noted after neoadjuvant therapy in 21.4% (15/70) of the patients. The pathological CR rate was 5.71% (n = 4; **Table 1**). Patients with negative ER expression and patients with negative PR expression had high CR rates to neoadjuvant therapy in group 1 ($P < 0.05$). Changes in age, mean tumor diameter, axillary lymph node status, HER2 expression, and Ki-67 expression were not statistically significant ($P > 0.05$, **Table 1**).

The overall clinical response rate obtained with neoadjuvant therapy was 70.6% (48/68) in group 2. A CR was observed in 10.3% (7/68) of the patients, and 60.3% (41/68) had a PR. SD was observed in 29.4% (20/68) of the patients after neoadjuvant therapy. The pathological CR rate was 7.4% (n = 5; **Table 2**). In group 2, patients with negative ER expression, patients with negative PR expression and patients with high Ki-67 expression levels had a high response rate to neoadjuvant therapy ($P < 0.05$). Differences in age, mean tumor diameter, axillary lymph node status, and HER2 expression were not statistically significant ($P > 0.05$, **Table 2**).

Relationship between biomarker levels prior to and after neoadjuvant chemotherapy and the efficacy of neoadjuvant chemotherapy

Qualitative changes in the biomarkers prior to and after therapy are shown in detail in **Tables 3** and **4**. In group 1, 28.6% (20/70) of the patients showed ER status changes, 22.9% (16/70) of the patients showed PR status changes, 17.1% (12/70) of the patients showed HER2 status changes, and 54.3% (38/70) of the patients showed Ki-67 status changes. The change in hormonal status before and after treatment was significantly associated with treatment efficacy ($P < 0.05$, **Table 3**).

In group 2, 16.2% (11/68) of the patients showed ER status changes, 22.1% (15/68) of the patients showed PR status changes, 13.2% (9/68) of the patients showed HER2 status changes, and 70.6% (48/68) of the patients showed Ki-67 status changes. The change in hormonal status before and after treatment was significantly associated with treatment efficacy ($P < 0.05$, **Table 4**).

Relationship between biomarker levels prior to and after chemotherapy and differences in neoadjuvant treatment

In this study, 22.5% (31/138) of the patients showed ER status changes. These patients comprised 28.6% (20/70) of the total patients in group 1 and 16.2% (11/68) of the patients in group 2. There were significant between-group differences regarding ER status changes. The treatment more easily induced ER status changes in group 1 compared with group 2. Furthermore, 62.3% (86/138) of the patients showed Ki-67 status changes. These patients made up 54.3% (38/70) of group 1 and 70.6% (48/68) of group 2. Ki-67 status changes between the two groups were statistically significant. The treatment more easily induced Ki-67 status changes in group 2 compared with group 1. There were no statistically significant

Table 4. Relationship between biomarker levels prior to and after neoadjuvant chemotherapy and the efficacy in group 2

Changes in biomarkers	Number of patients	SD/PD	Overall clinical response		χ^2	*P*
			PR	CR		
ER					15.342*	0.018
-/+	10	4	5	1		
+/+	22	12	7	3		
-/-	35	4	28	3		
+/-	1	0	1	0		
PR					15.746*	0.015
-/+	13	3	9	1		
+/+	15	10	3	2		
-/-	38	6	28	4		
+/-	2	1	1	0		
HER2					13.160*	0.041
-/+	3	2	1	0		
+/+	24	10	9	5		
-/-	35	7	27	1		
+/-	6	1	4	1		
Ki-67					14.607*	0.011
-/+	16	3	13	0		
+/+	11	8	2	1		
-/-	9	1	7	1		
+/-	32	8	19	5		

changes of PR or HER2 status between the two groups ($P > 0.05$, **Table 5**).

Discussion

Neoadjuvant therapy involves treatment prior to primary therapy and has become a valuable strategy in the multidisciplinary treatment of breast cancer. Neoadjuvant therapy offers several advantages compared with traditional postoperative regimens. Invasive breast cancer patients have a significant risk of harboring occult micrometastatic disease in distant organs. Neoadjuvant chemotherapy enables the earlier administration of chemotherapeutic agents to treat these micrometastases, and an observed response to chemotherapy in the primary breast disease site indicates that the regimen has effective antitumor activity. Additionally, for women who experience significant tumor regression, neoadjuvant chemotherapy facilitates a more conservative surgical procedure [12]. Clinical trials for neoadjuvant therapy offer a rapid and cost-effective means to evaluate the effectiveness of novel systemic therapeutic agents compared with conventional adjuvant therapy trials. The latter format is extremely labor-intensive and requires thousands of patients who must be followed-up over many years.

There is controversy regarding the effectiveness of neoadjuvant chemotherapy in promoting overall survival in breast cancer; however, many of its advantages have been accepted and integrated into clinical treatments. To date, several clinical trials comparing neoadjuvant chemotherapy with postoperative therapy have demonstrated equivalent survival in the two treatment modalities. Neoadjuvant therapy is intended to reduce the size or metastatic ability of the cancer prior to radical treatment intervention, thereby making procedures easier and more likely to succeed and eliminating the need for extensive treatment technique that would be required for larger or more metastatically active tumors. Neoadjuvant therapy also acts on micrometastatic disease. The downstaging is a surrogate marker for efficacy against undetected dissemination and results in improved survival compared with a strategy involving surgery alone. The use of such a therapy can effectively reduce the difficulty and morbidity associated with more extensive procedures. Some physicians provide this therapy in hopes that a response will inform the best course of action.

Neoadjuvant chemotherapeutic agents are known to induce intracellular changes that lead to cell death. The changes in the molecular properties of the cancer cells may affect tumor behavior, tumor biomarkers, tumor grade, properties of the tumor cells and tumor proliferation rates. In this study, we examined and compared the changes in ER, PR, HER2 and Ki-67 levels in breast cancer patients receiving different neoadjuvant chemotherapies. In the evaluation of breast cancer, ER, PR, HER2 and Ki-67 can be prognostic, predictive, or both. Several studies have studied hormone receptor changes with neoadjuvant chemotherapy in tumor cells.

Taucher et al. [13] found that patients showed a significant increase in ER-negative ($P = 0.02$) and PR-negative ($P = 0.0005$) measurements following preoperative chemotherapy. Preoperative cytotoxic chemotherapy induced significant variations in the steroid receptor expression of breast cancer cells. Powles et al. [14] found that the ER expression was reduced after neoadjuvant chemotherapy, whereas PR expression was increased in the group that experienced effective treatment. Patei et al. [15] suggested that ER and/or PR expression did not change prior to or after neoadjuvant chemotherapy. Some studies have suggested that neoadjuvant chemotherapy did not alter the proliferative status of the HER2/neu gene [16]. Vande ven reported that ER, PR and HER2 inconsistent rates after neoadjuvant chemotherapy ranged from 3 to 17%, 6 to 52% and 8 to 30%, respectively [17]. Experimental results have previously been contradictory due to differences in neoadjuvant chemotherapy strategies and application cycles. In our study, biomarker levels in breast cancer changed following various chemotherapy schemes. In group 1, 28.6% (20/70) of the patients showed ER status changes, 22.9% (16/70) of the patients showed PR status changes, 17.1% (12/70) of the patients showed HER2 status changes, and 54.3% (38/70) of the patients showed Ki-67 status changes. In group 2, 16.2% (11/68) of the patients showed ER status changes, 22.1% (15/68) of the patients showed PR status changes, 13.2% (9/68) of the patients showed HER2 status changes, and 70.6% (48/68) of the patients showed Ki-67 status changes. There were statistically significant between-group changes regarding ER status. The treatment more easily induced ER status changes in group 1 compared with group 2. The Ki-67 status changes between the two groups were statistically significant (the treatment more easily induced Ki-67 status changes in group 2 compared with group 1). There were no statistically significant between-group status changes in PR or HER2 ($P > 0.05$).

Predicting the efficacy of neoadjuvant chemotherapy has become one of the primary focuses of breast cancer treatment. In our study, approximately 15/70 (21.4%) of the patients in group 1 were not sensitive to neoadjuvant chemotherapy, and approximately 20/68 (29.4%) of those in group 2 were not sensitive to neoadjuvant chemotherapy. If efficacy predictors were detected prior to neoadjuvant therapy, tumor progression or the prolonged use of toxic reactions could potentially be avoided for patients who are not sensitive to chemotherapy. A study by Zambetti M showed that patients who were ER-negative prior to neoadjuvant chemotherapy were sensitive to neoadjuvant chemotherapy and that this item could be used to predict neoadjuvant chemotherapy sensitivity [18]. Estevez et al. [19] applied neoadjuvant docetaxel to stage II and III breast cancer patients and showed that HER2, ER, and Ki-67 status had no correlation with the response to chemotherapy. Learn et al. [20] reported that combined docetaxel and anthracycline as the neoadjuvant chemotherapy scheme could improve the response rate to the HER2-negative breast cancer; however, ER and PR status were unrelated to chemotherapy response. Zhou et al. [21] combined docetaxel and anthracycline in a neoadjuvant chemotherapy scheme; HER2 over-expression, as well as ER-negative and PR-negative status, was used as indicators for response to neoadjuvant chemotherapy. Ki-67 and p53 expression had no correlation with chemotherapy response. Mieog et al. [22] used the FEC scheme for neoadjuvant chemotherapy with four cycles; Ki-67 overexpression was an independent predictor of anthracycline-containing neoadjuvant chemotherapy drug efficacy with a higher Ki-67 expression reflecting increased tumor proliferation. They also reported that the tumor response to anthracycline was better when combined with taxol [23]. A Ki-67 positivity rate ≥ 20% prior to neoadjuvant chemotherapy and hormone receptor-negative tumor status was a good indicator of complete remission [24]. A retrospective analysis by Nishimura et al. [25] was based on 144 cases of locally advanced breast cancer; they reported that Ki-67 levels prior to neoadjuvant chemotherapy were strong indicators predicting the efficacy of neoadjuvant chemotherapy, with higher Ki-67 expression often indicating a high level of complete remission. Additionally, disease-free survival was significantly lengthened if Ki-67 expression levels were significantly reduced after neoadjuvant chemotherapy. Matsubara [26] reported that the percentage of cells with positive Ki-67 changes prior to and after neoadjuvant chemotherapy were independent prognostic factors (based on a 56-month follow up) as well as Ki-67 testing of core biopsies and surgical specimens prior to and after neoadjuvant chemotherapy in 385 cases. In

our study, the patients in group 1 with negative expressions of ER and PR had high response rates to neoadjuvant therapy ($P < 0.05$); however, the changes in age, mean tumor diameter, axillary lymph node status, HER2 expression, and Ki-67 expression were not statistically significant ($P > 0.05$). The patients with negative ER expression, patients with negative expression of PR and patients with high expression of Ki-67 had high response rates to neoadjuvant therapy in group 2 ($P < 0.05$); however, the changes in age, mean tumor diameter, axillary lymph node status, and HER2 expression were not statistically significant ($P > 0.05$, **Table 2**). In both the AC and TC programs, neoadjuvant chemotherapy efficacy was associated with ER, PR, Ki-67, and HER2 changes prior to and after chemotherapy ($P < 0.05$).

Our study has some limitations. Immunohistochemical methods used in the evaluation of biomarkers and hormone receptors may have affected the results due to improper tissue sampling, an insufficient number of tissue specimens or sampling from an area that did not represent the heterogeneity within the tumor. Direct effects of the chemotherapy itself on immunohistochemical staining and factors related to the observing pathologist are other important factors. Only 138 patients were included in this study; more patients should be recruited to more accurately assess the effects of chemotherapeutic agents on cancer cells.

In conclusion, biomarker levels of ER, PR, HER2 and Ki-67 were differentially changed following various neoadjuvant treatments. There were significant differences between groups regarding ER and Ki-67 status changes; besides, our results showed that neoadjuvant chemotherapy was more effective in patients who were ER- and PR-negative prior to treatment. These changes may affect treatment decisions, as changes in cellular proliferation and hormone receptors have been shown to be associated with tumor response. We investigated whether pretreatment features and molecular markers and changes in these factors can predict the treatment response and survival in patients with primary operable breast cancer who receive neoadjuvant therapy. Studies on different cancer types may increase our understanding of the effects of chemotherapeutic agents on tumor biology, thereby informing decisions regarding adjuvant therapy and disease follow up.

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Disclosure of conflict of interest

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