RESEARCH ARTICLE

Predictive Factors Determining Neoadjuvant Chemotherapy Outcomes in Breast Cancer - a Single Center Experience

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Abstract

From January 1, 2008 to March 31, 2010, 101 patients with stage II-III breast cancer were enrolled in this study and subjected to an anthracycline-based neoadjuvant chemotherapy regimen with or without docetaxel. Surgery was performed after 2-6 cycles of chemotherapy, and the clinical response was determined by pathological and histochemical assessments. The clinical response rate, as indicated by complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD), were 6.9, 52.5, 36.6, and 4.0%, respectively. A multivariable correlation analysis indicated that the overall clinical response rate correlated with the number of metastatic lymph nodes, number of chemotherapy cycles, and vessel invasion status. Importantly, the CR rate was only associated with the number of chemotherapy cycles. Nonparametric tests failed to detect a correlation between HER2 or Topo IIα status and clinical response to neoadjuvant chemotherapy in these patients. When they were stratified by HER2 or HR status, for HER2-positive patients the CR rate was associated with vessel invasion and Topo IIα status. Based on our findings, we propose that HR, HER-2 and Topo IIα are not putative predictive biomarkers of chemotherapy outcome for breast cancer patients. Topo IIα expression level was only inversely correlated with CR rate among HR-positive patients. Importantly, the achievement of CR was largely related to the number of chemotherapy cycles.

Keywords: Neoadjuvant chemotherapy - invasive breast cancer - hormone receptor - HER2 - topoisomerase IIα

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Introduction

Neoadjuvant chemotherapy, which was originally established by Haagnesen and Stout in the 1970s, has been increasingly used in the management of patients with large, operable, and locally advanced breast cancers. This treatment is administered with the goal of downstaging and avoiding a mastectomy. Moreover, as an in vivo measurement of chemosensitivity, it enables systemic treatment of occult micrometastatic disease (Aigner et al., 2011; Moreno-Aspitia, 2012). A large number of studies have since explored the clinical significance of neoadjuvant chemotherapy in breast cancer treatment, including two well-known large-scale clinical trials, namely NSAPB B18 and B27 (Wolmark et al., 2001; Rastogi et al., 2008).

However, most randomized trials comparing pre-operative and post-operative adjuvant chemotherapy in early breast cancer have shown that pre-operative chemotherapy is not beneficial for patients in terms of local control and overall survival (OS) compared to post-operative adjuvant chemotherapy. Some studies have indicated that the patients who benefited the most from neoadjuvant chemotherapy were those who achieved a pathological complete response (pCR) with no residual microscopic tumor present (Wolmark et al., 2001; Rastogi et al., 2008). The current understanding of neoadjuvant chemotherapy suggests that it may improve the rate of successful mastectomies and breast-conservation compared to the pre-operative approach. However, despite a consensus within the field about the benefits of neoadjuvant chemotherapy, its use remains controversial, as resistance to therapy could affect the appropriate timing for surgery (Kurosumi, 2006). Therefore, the ability to identify those most likely to benefit from neoadjuvant chemotherapy is critically needed.

Indeed, increasing clinical and experimental studies have focused on identifying predictors that indicate positive responses to breast cancer treatments. Established biomarkers, such as the estrogen receptor (ER) and progesterone receptor (PR), which already play a significant role in the selection of patients for endocrine therapy, have also been implicated in impacting the response to chemotherapy. The role of the ER and Human Epidermal Growth Factor Receptor 2 (HER2) as negative and positive indicators for chemotherapy, respectively,

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has also been explored. ER status has been considered predictive of pCR in patients receiving neoadjuvant chemotherapy for operable and locally advanced breast cancer. Topoisomerase IIα (Topo IIα), which is a direct molecular target of anthracyclines, has recently been associated with the clinical response to chemotherapy in breast cancer. In spite of the tremendous amount of evidence, the impact of ER, PR, HER2, and Topo IIα expression on the efficacy of neoadjuvant chemotherapy remains controversial (Järvinen et al., 1998; Di Leo et al., 2001; Harris et al., 2001; Coon et al., 2002; Lips et al., 2012). For example, some findings suggest that HER2 overexpression or gene amplification together with Topo IIα positive or negative status could collectively predict the response to anthracycline-based chemotherapy; however, this finding has been questioned by other studies (Di Leo et al., 2002; Järvinen et al., 2003; Arpino et al., 2005; Fritz et al., 2005; Harris et al., 2009; Munro et al., 2010). Moreover, an increasing number of factors, such as Ki67, oncoprotein K-RAS, and tumor suppressor p53, have been proposed to affect neoadjuvant chemotherapy in breast cancer, but the overall consensus remains inconclusive (Bonnefoi et al., 2011; Dowsett et al., 2011; Generali et al., 2011; Oshima et al., 2011; Petrarca et al., 2011). However, a few studies conducted at major breast cancer centers in China have indicated that the HER2 and Topo IIα status together may have a predictive role in breast cancer therapy (Zhu et al., 2008; Li et al., 2011).

In our previous study, we attempted to analyze the impact of relevant clinical and pathological factors, including those mentioned above, on the clinical response of breast cancer patients to neoadjuvant chemotherapy, but failed to identify significant factors for indicating a response to chemotherapy. In this study, we conducted a prospective study on breast cancer patients treated in our hospital from the local region of East China, of which 80% were diagnosed with invasive breast ductal carcinoma. Treatment consisted of an anthracycline-based regimen plus docetaxel, which has been widely used as breast cancer neoadjuvant chemotherapy (Zhu et al., 2008; Wu et al., 2011). The findings presented here provide useful information for the current treatment selection criteria and may potentially provide a substantial benefit for the patients.

Materials and Methods

Patient Eligibility

One hundred and one breast cancer patients from East China (excluding the Shanghai area) who met the following eligibility criteria were invited to participate in this study: a) diagnosis with stage II and III breast cancer with a pathological diagnosis of invasive ductal carcinoma, b) no history of allergic reaction to paclitaxel or other taxanes, c) no active infection or other significant illness that could affect tolerance of treatment for at least six cycles of chemotherapy following surgery, and d) adequate hematologic, renal, and hepatic function. Specific criteria for exclusion from the study included a previous history of malignancy, previous cytotoxic, radiation, or endocrine therapy, or inability to provide informed consent. The study protocol was approved by the ethics committee and the academic committee of Zhejiang Cancer Hospital (Hangzhou, China). All patients provided informed written consent before commencing the study.

Study Design

Patients who participated in the study received a pathological diagnosis and histochemical examination of ER, PR, P53, HER2, and Topo IIα expression levels prior to receiving an anthracycline-based neoadjuvant chemotherapy regimen composed of epirubicin (75 mg/m^2) or epirubicin (60 mg/m^2) plus paclitaxel (75 mg/m^2) every 3 wks. After 2-6 cycles of treatment, the clinical response was assessed according to RECIST criteria (Duffaud, 2000).

Statistics

Data were analyzed using SPSS (SPSS, Inc, Chicago, IL). Age, disease staging, tumor size prior to treatment, chemotherapy cycles, histological grades, and axillary lymph node metastasis were considered parametric factors for analyzing clinical response to neoadjuvant chemotherapy. Nonparametric factors included menopause status, vessel invasion, lymph nodes metastasis, and chemotherapy regimen as well as ER, PR, p53, HER2, and Topo IIα expression status. The clinical response was stratified by complete response (CR) or non-CR. A multivariable correlation analysis was applied to test the impact factors of neoadjuvant chemotherapy, followed by HR and HER2 stratification. The predictive factors for neoadjuvant chemotherapy were validated using a logistic regression analysis. A p value less than 0.05 was considered statistically significant.

Patient Characteristics

All patients enrolled in the study were female with a median age of 48 y (range, 28-66 y). The onset of menopause occurred in 35.6% patients (36/101). The duration of time since the initial detection of a breast lump ranged from 1 mo to 20 y, with an average of 18.01 ± 3.97 mo. Of the patients, 56.4% (57/101) had a tumor on the left breast, and the remaining patients (43.6%) had a mass on the right breast. From the cohort, 101 lumps were detected in total, with 14.9% (15/101) in the upper inner quadrant, 1% (1/101) in the inner lower quadrant, 10.9% (11/101) in the lower outer quadrant, 34.7% (35/101) in the upper outer quadrant, 8.9% (9/101) in the upper quadrant, 1% (1/101) in the lower quadrant, 5% (5/101) in the outer quadrant, and 24.8% (24/101) in the center.

Treatment Regimen

An anthracycline-based neoadjuvant chemotherapy regimen plus paclitaxel was used to treat 87 patients, among whom 26 were subjected to the docetaxel-epirubicin-cyclophosphamide (TEC) regimen every 3 wks, which was composed of paclitaxel (75 mg/m^2), epirubicin (60 mg/m^2), and cyclophosphamide (400 mg/m^2). The remaining 61 patients were treated with the docetaxel-epirubicin (TE) regimen (paclitaxel, 75 mg/m^2, plus epirubicin 60 mg/m^2) every 3 wks. The cyclophosphamide-epirubicin-fluorouracil (CEF) regimen

Table 1. Patient Characteristics

	Group	Number	%
Age (y)	≤ 35	14	13.9
	> 35	87	86.1
Phase (mo)	≤ 18	81	80.2
	> 18	18	17.8
Histological grade	1	10	9.9
	2	59	58.4
	3	32	31.7
Vessel invasion	N	45	44.6
	P	56	55.4
Lymph node metastases	N	24	23.8
	P	77	76.2
	1-5	45	
	≥ 6	32	
Estrogen Receptor	N	41	40.6
	P	60	59.4
Progesterone Receptor	N	61	60.4
	P	40	39.6
HER2	N	70	69.3
	P	31	30.7
TOPO2α	N	37	36.6
	P	64	63.4
P53	N	41	40.6
	P	60	59.4
Chemotherapy reaction	CR	7	6.9
	PR	53	52.5
	SD	37	36.6
	PD	4	4

consisted of cyclophosphamide (500 mg/m^2), epirubicin (75 mg/m^2), and fluorouracil (500 mg/m^2) and was used to treat 14 patients (13.9%; 14/87) every 3 wks. Neoadjuvant chemotherapy lasted for 4-6 cycles, with an average of 3.24 ± 0.11 cycles, among which 32.7% (33/101) of patients were treated for 2 cycles, 22.8% (23/108) of patients received 3 cycles, 35.6% (36/101) of patients received 4 cycles, 5.9% (6/101) of patients received 5 cycles, and 3% (3/101) of patients received 6 cycles. After completion of the chemotherapy regimen, the final clinical response of primary breast cancer was assessed and appropriate surgery, including radical mastectomy (6/101), modified radical mastectomy (87/101), and breast-conserving lumpectomy (8/101) was performed to remove the primary tumor and sample (or clear) the axillary lymph nodes. The pathologic response of primary breast cancer, the presence of axillary lymph node metastases, and response to primary chemotherapy were assessed. All patients received chemotherapy following the same regimen after surgery.

Clinical and pathological features

All patients were diagnosed with invasive breast ductal carcinoma. The mean tumor size upon physical examination pre- and post-neoadjuvant chemotherapy was 4.66 ± 0.21 cm and 3.04 ± 0.21 cm based on the largest single diameter. The number of axillary lymph nodes dissection ranged from 5 to 42 (mean 19.15 ± 0.69), and 0-24 (mean 4.50 ± 0.51) of the nodes were determined to be metastatic. All other clinical and pathological characteristics as well as clinical responses are shown in Table 1.

Table 2. Correlation Between Listed Parameters and Patient Response

Factors	Statistics	RR	CR
Total patients N =101			
Metastasized LN number	Correlation	-0.316**	-0.158
	Sig. (2-tailed)	0.001	0.114
Neoadjuvant chemo cycles	Correlation	0.223*	0.269**
	Sig. (2-tailed)	0.025	0.007
Vessel invasion	Correlation	-0.214*	-0.148
	Sig. (2-tailed)	0.032	0.141
HER2(-) N = 70			
Metastasized LN number	Correlation	-0.321**	-0.123
	Sig. (2-tailed)	0.007	0.311
Neoadjuvant chemo cycles	Correlation	0.243*	0.181
	Sig. (2-tailed)	0.043	0.133
Tumor size	Correlation	0.015	0.241*
	Sig. (2-tailed)	0.899	0.045
HER2(+) N = 31			
Age	Correlation	0.028	0.388*
	Sig. (2-tailed)	0.883	0.031
Histological grade	Correlation	-0.359*	-0.302
	Sig. (2-tailed)	0.047	0.099
Neoadjuvant chemo cycles	Correlation	0.187	0.410*
	Sig. (2-tailed)	0.313	0.022
Menopausal	Correlation	-0.265	-0.474**
	Sig. (2-tailed)	0.15	0.007
Vessel invasion	Correlation	-0.360*	-0.412*
	Sig. (2-tailed)	0.047	0.021
HR(-) N = 40			
Metastasized LN number	Correlation	-0.321*	-0.146
	Sig. (2-tailed)	0.044	0.37
Neoadjuvant chemo cycles	Correlation	0.101	0.477**
	Sig. (2-tailed)	0.536	0.002
HR(+) N = 61			
Metastasized LN number	Correlation	-0.330**	-0.158
	Sig. (2-tailed)	0.009	0.224
Neoadjuvant chemo cycles	Correlation	0.310*	0.075
	Sig. (2-tailed)	0.015	0.564
Vessel invasion	Correlation	-0.304*	-0.273*
	Sig. (2-tailed)	0.017	0.033
TOPO2α	Correlation	-0.117	-0.292*
	Sig. (2-tailed)	0.370	0.022

Results

Clinical response analysis

Clinical response was assessed in 101 patients who received chemotherapy as described. Sixty patients responded to the therapy, including seven CRs and 53 partial responses (PRs). The overall clinical response rate (CR and PR) in this group of patients was 59.4% (Table 1) and the CR rate was 6.9%. Thirty-seven patients achieved stable disease (SD) and four patients had progressive disease (PD).

A multivariable correlation analysis indicated that the overall clinical response rate significantly correlated with the number of metastatic lymph nodes (p=0.001), chemotherapy cycle number (p=0.025), and vessel invasion status (p=0.032). Patients with less than six metastatic lymph nodes showed a higher response rate (Fisher's exact test, χ^2=5.454, p=0.023). In contrast, the CR rate was only associated with the number of chemotherapy cycles (Table 2; p=0.007).

A logistic regression analysis showed that lymph node status, number of chemotherapy cycles, and Topo IIα status represented predictive factors for a clinical response to neoadjuvant chemotherapy in these patients.

Table 3. Logistic Regression Analysis

		B	S.E.	Wald	df	Sig.	Exp(B)	95.0% CI for EXP(B)	
								Lower	Upper
RR	METN	-0.146	0.068	4.6	1	0.032	0.864	0.757	0.988
	PRECYCLE	0.623	0.265	5.549	1	0.018	1.865	1.11	3.134
	TOPO2α	-1.254	0.562	4.978	1	0.026	0.285	0.095	0.859

Specifically, patients with fewer metastatic lymph nodes who received a higher number of chemotherapy cycles and had negative expression of Topo IIα achieved a better clinical response. However, these factors were not able to predict the CR rate (Table 3).

Stratification by HER2 status

When patients were stratified by HER2 status, HER2-negative patients with fewer metastatic lymph nodes (Pearson correlation, $p = 0.007$), no more than six metastatic lymph nodes ($\chi^2 = 3.984$; $p = 0.046$), and a higher number of chemotherapy cycles (Pearson correlation, $p = 0.043$) exhibited an improved clinical response (Table 2). However, the CR rate in these patients appeared to only be related to tumor size (Pearson correlation; $p = 0.045$). In contrast, patients with HER2 expression benefited from both no vessel invasion (Pearson correlation, $p = 0.047$) and better pathological differentiation (Pearson correlation, $p = 0.047$). Instead, the CR rate of HER2-positive patients was associated with multiple factors, including menopause status (Pearson correlation, $p = 0.007$), vessel invasion (Pearson correlation, $p = 0.021$), number of chemotherapy cycles (Pearson correlation, $p = 0.022$), and age (Pearson correlation, $p = 0.031$).

Stratification by HR status

HR-negative patients (ER- and PR-) with fewer metastatic lymph nodes (Pearson correlation, p = 0.044) responded better to chemotherapy, whereas a higher number of chemotherapy cycles had a positive effect on the CR rate in these patients (Table 2). Among HR-positive patients (ER+ and/or PR+), the clinical response was improved in patients with fewer metastatic lymph nodes (Pearson correlation, $p = 0.009$), no vessel invasion (Pearson correlation, $p = 0.017$), and those who received a higher number of chemotherapy cycles (Pearson correlation, $p = 0.015$). The CR rate in the same patient population was associated with invasive status of vessels (Spearman correlation, $p = 0.033$) and Topo IIα status (Spearman correlation, $p = 0.022$). However, the response difference between patients with < 6 and ≥ 6 metastatic lymph nodes, regardless of HR status, was not significant ($p > 0.05$).

Correlation between Topo IIα and HR status

The correlation between Topo IIα and HR status was also examined. We found that Topo IIα expression in HER2-positive patients was significantly lower than in HER2-negative patients (15/30 vs. 49/70, respectively; $\chi^2 = 4.323$, $p = 0.046$). In contrast, we failed to detect any correlation between Topo IIα and HR status ($\chi^2 = 0.075$, $p > 0.05$). Moreover, nonparametric analysis did not demonstrate a correlation between either Topo IIα or HER2 status with clinical response to chemotherapy ($p > 0.05$). Therefore, among the HR-positive patients, patients negative for Topo IIα expression had a significantly higher CR rate compared to patients positive for Topo IIα expression (Fisher's exact test, $\chi^2 = 5.213$, $p = 0.049$).

Discussion

The patient enrollment in this study determined several features of this study. First, we observed low disparities in the cancer types of breast cancer incidence within this region, which indeed reflects the characteristics of breast cancer occurrence in East China. In this geographic area, invasive breast ductal carcinoma constitutes 75% of all breast cancer cases, and neoadjuvant chemotherapy is generally applied to patients in stage II and III of the disease, which ensured equality of the subjects enrolled and strengthened the credibility of the study. Specifically, most patients (86.5%) participating in the study were over the age of 35 years, with a median age of 48 years, among which 64.6% had not experienced menopause. This type of age distribution and menopause status indeed fits the characteristics of breast cancer incidence in a broader context of Chinese females (Leong et al., 2010). Another obvious feature was that the majority of patients were diagnosed with relatively advanced stage breast cancer, with an average duration of 18.01 ± 3.97 months between the detection of a breast lump and the time of enrollment, which was largely due to the fact that most patients were from rural areas of China. The most common TE/TEC and CEF neoadjuvant chemotherapy regimens used in practice were applied in this study. To avoid the incredibility due to the small sample size, we chose not to include trastuzumab in the regimen for HER2-positive patients. The study protocol followed the NCCN/cNCCN instructions to avoid interference to the outcome (Jiang et al., 2009; Ng et al., 2009). The overall pathological features of the participating patients, including histologic grading, vessel invasion status, and lymph nodes metastasis as well as a positive ratio of HER2, p53, Topo IIα, ER, PR, and HR were consistent with previous reports. Taken together, these findings and procedures ensure the credibility of this study.

In our study, the tumor size and clinical response were determined by measuring the resected tumor samples. The response rate (59.4%) and percent of patients who achieved CR (6.9%) were a little lower than those reported in previous studies (Rastogi et al., 2008; Zhu et al., 2008). We speculate that this difference was most likely due to a lower tolerable dosage of the patients from East China. Given the side effects, the applied dosage of chemotherapy in our study was approximately 85-100% of the recommended dosage. This may have accounted

for the compromised therapeutic effect observed in our study. In agreement with this hypothesis, we indeed did not observe any difference between the two regimens. According to our data from the multiparametric analysis, the clinical response was associated with multiple factors, including the number of metastatic lymph nodes, number of chemotherapy cycles, and vessel invasion. On the other hand, the CR rate was mainly dependent on the number of chemotherapy cycles, and higher CR rates were achieved in patients receiving more cycles. This observation suggests that patients may benefit from a longer neoadjuvant chemotherapy treatment regimen. However, while this idea sounds provocative, caution should be taken before addressing this possibility. The anxiety of patients receiving longer neoadjuvant chemotherapy prior to surgery could cause both physical and psychological distress for the patient. In addition, the potential of developing resistance to post-surgical adjuvant chemotherapy is a serious concern (Aigner et al., 2011; Kong et al., 2011). Notably, recent studies have reported that neoadjuvant chemotherapy did not significantly improve the prognosis of breast cancer patients (Kurosumi, 2006), and therefore clarification of this discrepancy requires further analysis of patients' survival. Considering that 91.1% of the patients in our study were treated for 2-4 weeks, we postulate that neoadjuvant chemotherapy lasting for this duration will potentially be able to induce a CR. In addition, four weeks of chemotherapy will likely benefit patients for the purpose of breast conservation, but more than four cycles of treatment is not recommended.

The multivariable correlation analysis in this study failed to show any correlation between biomarkers often used in breast cancer, including HER2 and HR, with the overall clinical response rate to chemotherapy, and therefore we further stratified the data by HER2 or HR epxression, respectively. We found that pathological features, including age, tumor size, pathological grades, vessel invasion, lymph nodes metastasis, and HER2 status were all associated with the clinical response to chemotherapy regardless of HER2 or HR status stratification. Importantly, it has been shown that these factors are indeed risk factors for breast cancer recurrence (Gnant et al., 2011). These findings suggest that regardless of differences in local treatment, such as radiation and surgery, the prognosis of breast cancer is largely dependent on the response of the patients to systemic treatment. In this regard, factors affecting chemotherapy response would determine prognosis (Kurosumi, 2004; Arpino et al., 2005; Untch et al., 2011).

Based on our data, the percentage of patients with Topo IIα-positive expression was 63.4%, among which HER2-positive patients had lower Topo IIα expression levels compared to HER2-negative patients. This observation is inconsistent with previous findings by Glynn et al. (Glynn et al., 2011), who found no correlation between Topo IIα and HER2 expression, except for the finding that most HER2-negative patients had higher Topo IIα expression. The discrepancy between our findings and the current understanding that HER2/Topo IIα status is associated with the response to anthracyclines (Järvinen et al., 2000; Knoop et al., 2005; Du et al., 2011) was most likely due to the small sample size, lower dose of anthracyclines used in our regimens, or differences in the patient population.

In summary, our findings indicate that in contrast to previous studies, age and pathological features of breast cancer patients are key predictive factors for response to neoadjuvant chemotherapy, and the CR rate is closely associated with the number of chemotherapy cycles that the patient receives. In contrast, HR, HER2, and Topo IIα expression had no effect on the clinical response to chemotherapy.

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