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Clinical Implications of HER-2 and P53 in Taxane-Based and Anthracycline-Based Neoadjuvant Chemotherapy in Breast Cancer

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OBJECTIVE To evaluate the predictive value of human epidermal growth factor receptor-2 (HER-2) and P53 in taxane-based and anthracycline-based neoadjuvant chemotherapy (NAC) in breast cancer.

METHODS Sixty-two patients with breast cancer were included in this study. Twenty-two patients were treated with taxane-based (taxane group) and 40 with anthracycline-based (anthracycline group). ER, PR, c-erbB2 and P53 were detected by immunohistochemistry staining before NAC, and Fluorescence In Situ Hybridization(FISH) was used to detect the HER-2 gene amplification for the cases with the expression of c-erbB2 protein as (++) or (+++). The efficacy of the regimens was evaluated after NAC.

RESULTS In the anthracycline group, objective response (OR) was observed in 30 out of 40 patients (75%), whereas no response (NR) was observed in 10 patients (25%). In the taxane group, OR was observed in 15 patients out of 22 patients (68.2%), whereas NR was observed in 7 patients (31.8%). HER-2-negative status was correlated with a high OR in both taxane-based and anthracycline-based NAC (P = 0.023 and P = 0.029), whereas P53-negative status was correlated with high OR rate in anthracycline-based NAC (P = 0.041). The significant difference of the CR rates was observed between the patients took < 4 cycles and ≥ 4 cycles NAC (4.65% *vs.* 21.05%, $P < 0.05$).

CONCLUSION The patients with HER-2 gene non-amplication may be sensitive to both taxane-based and anthracycline-based chemotherapy; the patients without P53 overexpression may suitable to select anthracycline-based chemotherapy; and proper increased NAC cycles may increase CR rates.

KEY WORDS: breast neoplasms, neoadjuvant chemotherapy, HER-2, P53, taxane.

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Introduction

Breast carcinoma has been thought to be a systemic disease in recent years. Neoadjuvant chemotherapy (NAC) is commonly used as a systemic treatment of locally advanced breast cancer[1]. NAC is usually given to downstage tumors and promotes higher conservative breast surgery rates[2] and could be used to indicate suitable further therapy by evaluating NAC efficacy. Research in the expression of tumor biological markers in NAC treatment has been a hot topic in recent studies of cancer, but there is no agreement on the relationship between ER, PR, HER-2, P53 and NAC efficacy, so we carried out this retrospective study in order to further discuss the relationship between them and the NAC efficacy.

Patients and Methods

Patients

Sixty-two cases with locally advanced breast carcinoma (invasive carcinoma) were enrolled in the study and they were allocated to two groups: taxane group (22 cases) and anthracycline group (40 cases). All of cases were collected from the First Hospital of China Medical University during January 2006 to June 2008, and histologically diagnosed as breast carcinoma by core needle biopsy of the breast. Staging of tumor was defined referring to the International Tumor Node Metastasis classification. With a range of age from 25 to 70 years old (median 50.49 years), 28 cases were ≤ 50 years, 34 cases were > 50 years. Thirty cases were pre-menopause, 32 cases were post-menopause. None were found to have systemic metastasis.

Treatment

In the taxane group, 4 cases received TP regimen (docetaxel and cisplatin), 5 cases received TX regimen (docetaxel and capecitabine), 8 cases received TE regimen (docetaxel and epirubicin), 5 cases received T regimen(single-agent taxane), and the dosage of paclitaxel was 175 mg/m^2 or the dosage of docetaxel was 75 mg/m^2; In the anthracycline group, 3 cases received CE regimen, 37 cases received CEF regimen with the dosage of epirubicin 60 mg/m^2, 70 mg/m^2 or 80 mg/m^2, and the dosage was dependent on the patients' physical status. Chemotherapy was prescribed for the patients every 3 weeks. In fact, 36 cases received 2 cycles, 7 cases received 3 cycles and 19 cases received 4 or more cycles.

Assessment of clinical tumor response to NAC

The assessment of tumor clinical response to NAC was carried out at the end of NAC treatment according to the product of primary tumor diameters and the clinical lymph node status. It was classified as a clinical complete remission (CR), partial remission (PR), stable disease (SD) or progressive disease (PD) according to standard International Union Against Cancer criteria. CR was defined as the disappearance of all clinical evidence of the tumor including the axillary site; partial remission was defined as a reduction of 50% or more in the diameters of the products of measured lesions, without the appearance of new lesions. SD was defined as a decrease of less than 50% in the diameters of the products of measured lesions or an increase of less than 25%, without the appearance of new lesions. Any measured increase greater than 25% or appearance of new lesions was defined as PD. CR plus PR was defined as objective response rate (OR). SD plus PD was defined as no response (NR). Before and after NAC, all cases were subject to the same measurement to evaluate the efficacy. 54 cases take breast ultrasound examination, 8 cases take mammography examination.

Immunohistochemistry staining for ER, PR, P53 and c-erbB2

Serial sections 4-μm thick was taken from paraffin-embedded tissue for ER, PR, P53 and c-erbB2 immunostaining. Immunohistochemical study was performed following the manufacturer's instructions. Briefly, this procedure included the deparaffinization and rehydration steps, followed by an epitope retrieval step. The slide was then subjected to a series of alternating washes in tris (hydroxymethyl) aminomethane hydrochloride buffer and incubation steps with, first, a peroxidase-blocking reagent and then with monoclonal antibody for ER (MAB-0062; Maixin_Bio), monoclonal antibody for PR (MAB-0502; Maixin_Bio), monoclonal antibody for P53 (MAB-0142; Maixin_Bio), and monoclonal antibody for c-erbB2 (MAB-0198; Maixin_Bio), followed by a visualization reagent for 30 minutes each, and finally incubated with a 3, 3′-diaminobenzidine chromogen solution. After a final wash, the slide was counterstained with hematoxylin. For ER, PR and P53 nuclear staining of invasive tumor cells was scored as positive. The threshold for ER, PR and P53 was 10%. Scoring of c-erbB2 results was done as suggested for the HercepTest (DAKO) using the following categories: (-), negative result or membrane staining in < 30% tumor cells; (+), weak and incomplete membrane staining in > 30% tumor cells; (++), weak or moderate, complete membrane staining in > 30% tumor cells; (+++), strong, complete membrane staining in > 30% tumor cells. In this study, the patients with c-erbB2 (-) and (+) were thought to be HER-2 gene non-amplified; the patient c-erbB2 (++) and (+++) tissues were chosen for HER-2 gene amplification testing by Fluorescence In Situ Hybridization (FISH).

FISH detect HER-2 gene amplification

FISH was performed on formalin-fixed, paraffin-embedded tissue specimens from patients using the PathVysion kit (Vysis, Downers Grove, IL, USA). This kit includes probes to the HER-2 gene locus at 17q11.2-12 (labeled with Spectrum Orange) and to the centromeric region of chromosome 17 (CEP17; labeled with Spectrum Green). Briefly, unstained 4 μm thick paraffin sections were cut from blocks and placed on positively charged slides. The slides were placed in an oven at 94°C for approximately 5 h, deparaffinized in xylene, and dehydrated in a series of ethanol washes. After pretreatment in 0.2 N hydrochloric acid and sodium thiocyanate solutions, digestion in a protease solution for 16 minutes, and fixation in 10% neutral buffered formalin, the slides were subjected to denaturation and hybridization with 10 μl of the PathVysion probe/buffer mixture. Fluorescence microscopy integrated with a cooled CCD camera system and Smart Capture software (CytoVision Chromophour System; Applied Imaging Ltd., Carlsbad, CA, USA) for chromosome arrangement was used to investigate and analyze the FISH results.

FISH analysis was performed according to the manufacturer's instructions. Count 30 cells, if Ratio value < 1.8, the case was thought to be HER-2 gene non-amplified; if Ratio value > 2.2, the case was thought to be HER-2 gene amplified; if Ratio value was 1.8~2.2, count 100 cells or redo FISH experiment.

Statistical analysis

SPSS version 13.0 statistical package was used for statistical analysis. The McNemar test and Fisher's exact test were used to investigate the relationship between the pre-neoadjuvant protein expression status and the clinical tumor response. $P \leq 0.05$ was considered to be statistically significant.

Results

Clinical tumor response after neoadjuvant chemotherapy

All patients' clinical tumor responses were assessed after NAC according to the International Union Against Cancer criteria. Table 1 shows the statistical results. In the taxane group, OR was observed in 15 out of 22 cases (71.9%), whereas NR was observed in 7 cases (28.1%). In the anthracycline group, OR was observed in 30 cases (69.4%) out of 40 cases, whereas NR was observed in 10 cases (30.6%). No significant deviation was observed between these two groups' clinical tumor response after NAC.

Correlation between pre-neoadjuvant proteins expression status and clinical tumor response

The relationship between pre-neoadjuvant proteins expression status and the clinical tumor response was showed in Tables 2 and 3. In the taxane group, twelve cases (85.7%) showed OR and two (14.3%) showed NR out of fourteen cases with HER-2 gene non-amplificaton. There was a significant correlation between the non-amplification of HER-2 gene and high OR rate ($P = 0.023$). In the anthracycline group, 24 cases (80.8%) showed OR and 4 (19.2%) showed NR out of 28 cases with P53 negative; 26 cases (85.7%) showed OR and 5 (14.3%) showed NR out of the cases with HER-2 gene non-amplification. There were significant correlations between the P53 protein negative, HER-2 gene non-amplification and high clinical OR rates ($P = 0.041$, $P = 0.029$). From the above results, we concluded that HER-2 gene non-amplification correlates with high response rates regardless of receiving taxane or anthracycline.

The relationship of efficacy of NAC and NAC cycles was showed in table 4. The patients who took < 4 cycles and ≥ 4 cycles achieved CR rates are 4.65% and 21.05%, respectively, and the difference is of statistical significance ($P < 0.05$).

Table 1. Clinical tumor response after NAC.

	Taxane-based NAC	Anthracycline-based NAC	*P**
OR	15 (68.2%)	30 (75.0%)	
NR	7 (31.8%)	10 (25.0%)	1.000

*: McNemar

Table 2. Relationship between biomarker expression status before taxane-based NAC and clinical tumor response.

Biomarkers	OR group ($n = 15$)	NR group ($n = 7$)	*P**
P53+	4 (57.1%)	3 (42.9%)	
P53 –	11 (73.3%)	4 (26.7%)	0.630
ER+	8 (66.7%)	4 (33.3%)	
ER–	7 (70.0%)	3 (30.0%)	1.000
PR+	7 (63.6%)	4 (36.4%)	
PR–	8 (72.7%)	3 (27.3%)	1.000
HER-2+	3(37.5%)	5 (62.5%)	
HER-2–	12 (85.7%)	2 (14.3%)	0.023

*: Fisher test

Table 3. Relationship between biomarker protein expression status before anthracycline-based NAC and clinical tumor response.

Biomarkers	OR group ($n = 30$)	NR group ($n = 10$)	*P**
P53+	6 (50.0%)	6 (50.0%)	
P53–	24 (85.7%)	4 (14.3%)	0.041
ER+	11 (73.3%)	4 (26.7%)	
ER–	19(76.0%)	6 (24.0%)	1.000
PR+	13 (72.2%)	5 (27.8%)	
PR–	17 (77.3%)	5 (22.7%)	0.731
HER-2+	4 (43.8%)	5 (56.2%)	
HER-2–	26 (86.4%)	5 (13.6%)	0.029

*: Fisher test

Table 4. Relationship of efficacy of NAC and NAC cycles (cases).

Cycles	*n*	CR (pCR + cCR)	PR	SD	PD
< 4	43	2	28	12	1
≥ 4	19	4	11	3	1
Total	62	6	39	15	2

Discussion

The predicative value of the efficacy of NAC for the patients' prognosis has been paid more and more attention, and in NAC setting, pathological complete response (pCR) is a robust prognostic marker and is used as a surrogate clinical endpoint, therefore, clinicopathologic response to NAC might be used as predictive marker for the patients' prognosis. It has always been emphasized to find out the predictive markers of pCR in NAC.

Taxanes have emerged as fundamental drugs in the treatment of breast cancer since their initial licensing in Europe. The NSABP B-27 study showed that taxanes may have activity against anthracycline-resistant breast cancer[3], and docetaxel was the only drug to have shown superiority over single-agent anthracycline therapy as well as combination regimens in the metastatic setting[4]. In our study, no significant deviation was observed between the response rate to anthracycline-based and taxane-based NAC, but our results may be due to our small number of cases.

Many studies have investigated the correlation between HER-2 expression and clinical tumor response for anthracycline-based NAC in breast cancer. The results of Petit group's study indicated that HER-2 gene amplification is a negative predictor of response to anthracycline-based regimens[5,6]. Kariya et al.[7] studied 30 invasive breast cancer patients with two to five courses of CAF (cyclophosphamide 600 mg/m^2, pirarubicin 20~40 mg, 5-fluorouracil 600 mg/m^2), and it also indicated that HER-2-negative status was the only significant predictive factor of response. However, some studies have indicated that HER2-overexpressing tumors had a higher clinical response rate than non-HER-2-overepxressing tumors (91% *vs*. 50%, respectively)[8]. In our study, the results indicated that the HER-2 gene non-amplification is a predictive factor of clinical response in both taxane-based and anthracycline-based NAC, it was partly in concordance with the results of Petit group's study. Our earlier study found that, only 68% of the cases of c-erbB2 protein (+++) expression were HER-2 gene amplified in our patients, therefore, we use FISH to detect the HER-2 gene amplification in the cases with the expression of c-erbB2 protein as (++) or (+++), the results would be more accurate than immunohistochemistry staining, therefore, the results of our study were convincing and we would enlarge the sample in our future work.

In this study, it was found that the P53 protein is an useful predictive biomarker, P53 negative correlated with high response rates in the anthracycline-based group, so the patients with P53 protein overexpression may not suitable to select anthracycline-based regimens. Our results were in concordance with the research from which has been reported that there is a strong correlation between P53 gene mutation and anthracycline chemoresistance[9,10]. Other studies showed increased response rates for TP53 mutated tumors, but these works are not contradictory, rather, they address different tumor subtypes and/or different drug regimens [11,12]. Johnson and Fan found that human breast cancer BCap37 cells transfected with antisense P53 or p21$^{WAF1/CIP1}$ exhibited a significant increase in their sensitivity to paclitaxel[13]. Recently, Bao et al.[14] found that patients with an overexpression of serum P53 Abs showed low sensitivity to anthracycline (40% OR rate in P53 Ab-positive patients) but high sensitivity to taxane (72.7% OR rate in P53 Ab-positive patients), although this variance was not significant. Despite this trend for a better response to taxanes for TP53-mutated tumors[15], it is not yet possible to identify, before the initiation of therapy, the patients for whom docetaxel will be effective[16]. Our study has not show statistical significance in the taxane group, it may be correlated with the different drugs that the patients have used, the regimens including docetaxel and paclitaxel, or because of the small sample or the method adopted for detecting P53 protein.

There is no agreement on how many NAC cycles patients should receive. Generally speaking, patients should be given 3 to 4 cycles of NAC if the efficacy of NAC is evident, and proper increased NAC cycles may increase pCR. Six cycles of pre-operative epidoxorubicin/docetaxel versus three cycles of pre-operative epidoxorubicin/docetaxel significantly increases the pCR rates for breast cancer patients [17]. In our study, the lower CR rates may be correlated with fewer NAC cycles.

There are still many studies investigating the prognostic factors in breast cancer, such as the expression of PCNA, Bcl-2 and ki-67, but at present, not a single index can be used to predict all the chemotherapy regimens' efficacy. To achieve more reliable results, further examination of a greater number of patients and more regimens is necessary. If we could find an excellent prediction method for NAC efficacy, we believe it would be a step forward toward tailored, more precise therapies for individual patients.

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