Predictive value of serum anti-p53 antibodies, carcino-embryonic antigen, carbohydrate antigen 15-3, estrogen receptor, progesterone receptor and human epidermal growth factor receptor-2 in taxane-based and anthracycline-based neoadjuvant chemotherapy in locally advanced breast cancer patients

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Breast carcinoma is the most common malignancy in Chinese women. The purpose of this study is to evaluate the predictive value of serum anti-p53 antibodies (p53 Abs), carcino-embryonic antigen (CEA), carbohydrate antigen (CA) 15-3, estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER)-2 in taxane-based and anthracycline-based neoadjuvant chemotherapy (NAC). Sixty-eight patients with locally advanced breast carcinoma were included. Thirty-two were treated with taxane (the taxane group) and 36 with anthracycline (the anthracycline group). The standard dosage of docetaxel was 100 mg/m^2 (day 1) and those of cyclophosphamide, adriamycin and 5-flurouracil were 500 mg/m^2 (day 1–8), 40 mg/m^2 (day 1) and 500 mg/m^2 (day 1–8), respectively. The p53 Abs were detected by enzyme-linked immunosorbent assay; CEA and CA15-3 were detected by Elecsys 2010 Disc System; ER, PR and HER-2 were detected by immunohistochemistry staining. The biomarkers p53 Abs, CEA and CA15-3 were detected in serum samples, and the immunohistochemistry staining for ER, PR and HER-2 was performed in tumor samples before and after NAC. The expression of p53 Abs was significantly reduced by taxane ($P=0.006$). The serum CEA and CA15-3 levels were significantly affected by both taxane ($P=0.004$ and $P=0.008$) and anthracycline ($P=0.002$ and $P=0.000$) drugs. HER-2-negative status (pre-neoadjuvant) was correlated with a high objective response rate (OR) in both taxane-based and anthracycline-based chemotherapy ($P=0.022$ and $P=0.025$), whereas p53 Ab-negative status (pre-neoadjuvant) was correlated with high OR rate in anthracycline-based chemotherapy ($P=0.039$). This study shows that the serum p53 Ab level is easily changed by taxane. CEA and CA15-3 levels are easily changed by taxane and anthracycline. The p53 Ab-negative patients may predict a high clinical OR rate in anthracycline-based NAC. HER-2-negative may predict a high OR in both taxane-based and anthracycline-based NAC. *Anti-Cancer Drugs* 19:317–323 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Breast carcinoma is the most common malignancy in women. The incidence of breast cancer has been increasing in recent years. Neoadjuvant chemotherapy (NAC) is commonly used as a systemic treatment of locally advanced breast cancer [1]. This treatment is usually given to downstage tumors and promotes higher conservative breast surgery rates [2].

Taxane and anthracycline are the two drugs that are being commonly used in NAC treatment in China. Taxane has been shown to bind to polymerized microtubules, resulting in a shift in the equilibrium between tubulin dimers and microtubules [3]. The most important actions of anthracycline are its interaction with the nuclear enzyme topoisomerase α and its blockage of DNA transcription [4]. It was proved that the addition of paclitaxel or docetaxel, either in combination with anthracycline or as a separate regimen administered before or after anthracycline-based therapy, increased clinical and pathologic response rates and improved disease-free survival [5].

As tumors with the same histological type, grade and clinical staging behave differently, clinical and pathological criteria could neither predict the response to NAC nor

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accurately define tumor biology [6]. It is supposed, however, that morphologically similar tumors have distinct gene expression patterns [7] and these distinct patterns could be directly or indirectly reflected in changes in biomarker expression.

It is commonly believed that tumor markers are associated with clinical response. Recent studies found that serum marker expression may also be associated with therapy response during NAC [8]. In this study, we focused on three serum biomarkers: serum anti-p53 antibodies (Abs), carcino-embryonic antigen (CEA) and carbohydrate antigen (CA)15-3, and three tumor biomarkers: estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER)-2. We detected ER, PR and HER-2 expression by immunohistochemistry staining, CEA and CA15-3 expression by Elecsys 2010 Disc System (Roche Diagnostics AG, Basel, Switzerland) and the serum p53 Abs by enzyme-linked immunosorbent assay before and after NAC in locally advanced breast cancer patients. We monitored the six changes in biomarker expression before and after NAC. Meanwhile, the values of biomarkers for predicting clinical response to tumor were evaluated.

Methods

Patients

Sixty-eight locally advanced breast carcinoma (invasive ductual carcinoma) patients were enrolled in this study and they were allocated randomly to two groups: taxane group (32 patients) and anthracycline group (36 patients). All of them were from the Jilin Provincial Tumor Hospital from April 2005 to April 2006 and histologically diagnosed as breast carcinoma by ultrasound-guided needle biopsy of the breast. For each patient, age, menstrual status, clinical stage and lymph node status were recorded. Staging of tumor was defined according to the International Tumor Node Metastasis classification proposed by the American Joint Committee on Cancer. Before NAC, each patient underwent a staging consisting of radiographs of chest, spine and pelvis; a liver ultrasound examination; and a bone scan. Serum samples were obtained, 1 week before and 1 week after NAC and were stored at $-80^{\circ}C$ until used. Patient recruitment and sample collection were performed under the guidelines of protocols approved by the institutional review board. Informed consent was obtained from all patients.

Treatment

Patients were administered four standard courses of neoadjuvant monotherapy docetaxel and CAF (cyclophosphamide, adriamycin, 5-fluorouracil). One course lasted for 3 weeks. The standard dose of docetaxel was 100 mg/m^2 (day 1) and those for cyclophosphamide, adriamycin and 5-fluorouracil were 500 mg/m^2 (day 1–8), 40 mg/m^2 (day 1) and 500 mg/m^2 (day 1–8), respectively. Chemotherapy was prescribed for the patients every 3 weeks, as long as leucopenia did not occur.

Assessment of clinical tumor response to neoadjuvant chemotherapy

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 axillary clinical status. It was classified as a clinical complete remission (CR), partial remission, 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 sum of the products of measured lesions or an estimated decrease in tumor size of at least 50%, without the appearance of new lesions. SD was defined as a decrease of less than 50% in the sum of the products of measured lesions, or an estimated decrease of less than 50% in lesion size or an increase of less than 25%, without the appearance of new lesions. Any measured or estimated increase greater than 25% or appearance of new lesions was defined as PD. CR plus Partial remission was defined as objective response rate (OR). SD plus PD was defined as no response (NR).

Establishment of enzyme-linked immunosorbent assay procedure for detection of serum p53 antibodies

The wild-type human p53 protein with 6-His tag at N-termini was overproduced in *Escherichia coli* and purified.

Polystyrene 96-well microtiter plates (Nunc, Roskilde, Denmark) were coated overnight at 4°C with 50 µl wild-type human p53 protein at a concentration of 5 µg/ml dissolved in 0.05 mol/l carbonate buffer, pH 9.6. Plates were subsequently washed three times with PBST [PBS containing 0.05% (v/v) Tween 20] and then twice with PBS. Excess binding sites were blocked using 100 µl of the blocking buffer (5% powdered nonfat milk dissolved in PBS, pH 7.5). Wells were washed and 50 µl of serum diluted to 1/200 in the blocking buffer were added and incubated for 1 h at 37°C. Plates were washed, incubated for 45 min at 37°C with 50 µl of peroxidase-conjugated goat anti-human IgG antibodies (Santa Cruz, Biotechnology, Santa Cruz, California USA) diluted 1/10 000, and washed again. The peroxidase activity retained in the wells was assayed by the addition of 100 µl of tetramethylbenzidine (TMB; AMRESCO, Solon, Ohio, USA) solution. The reaction was stopped by adding 50 µl 2 N H_2SO_4 per well and the absorbance in each well was measured at 450/620 nm in a microtiter plate reader (Multiskan Ascent; Labsystems, Helsinki, Finland). All samples were measured in duplicate and the mean of the duplicate values was taken as the final read-out.

We detected sera from 400 healthy volunteers using the optimal conditions of the enzyme-linked immunosorbent assay format to determine the cut-off values. With a specificity of 95%, a serum has been chosen as the control serum. All results were expressed as p53 index: p53 index = OD450/620 nm absorbance of a sample/OD450/620 nm absorbance of the control serum. Serum samples with the p53 index of more than 1.7 were considered positive.

Detection of serum carcino-embryonic antigen and carbohydrate antigen15-3

The serum CEA and CA15-3 were detected with the specific CEA and CA15-3 Elecsys 2010 reagents (Roche Diagnostics AG) for Elecsys 2010 Disc System (Roche Diagnostics AG). For CEA, the serum concentrations that exceeded 3.4 ng/ml were scored as positive. For CA15-3, the serum volume that exceeded 25 U/ml was scored as positive.

Immunohistochemistry staining for estrogen receptor, progesterone receptor and human epidermal growth factor receptor-2

Serial sections 4-μm thick were taken from paraffin-embedded tissue for ER, PR and HER-2 immunostaining. After being dried at 60°C overnight, slides were dewaxed by xylene and rehydrated in graded ethanols. Endogenous peroxidase activity was blocked by treatment with 3% solution of hydrogen peroxide in methanol for 20 min, and was then rinsed in PBS. For epitope retrieval, the slides were treated in 10 mmol/l citrate buffer (pH 6.0) using a microwave oven at 650 W for 2 × 6 min, and were then blocked in normal goat serum (1 : 10) for 10 min. The slides were incubated with the following antibodies for 30 min: monoclonal antibody for ER (clone 1D5 + 6F11; NeoMarker, Fremont, California, USA) at dilution 1 : 100, monoclonal antibody for PR (clone hPRa2 + hPRa3; NeoMarker) at 4 μg/ml, and monoclonal antibody for HER-2 (clone e2-4001 + 3B5; NeoMarker) at 0.5 μg/ml, followed by a rinse in PBS. Subsequently, the slides were incubated for 20 min with biotinylated goat anti-mouse IgG (H + L) (NeoMarker), followed by incubation for 20 min with 1 : 200 streptavidin–biotin–peroxidase complex. Peroxidase was detected with 3,3-diaminobenzidine as chromogen. The slides were then counterstained with hematoxylin, mounted and coverslipped. The sections known to be positively stained were included in each run, receiving either a primary antibody as a positive control, or irrelevant mouse IgG as a negative control.

For ER and PR nuclear staining of invasive tumor cells was scored as positive. For HER-2 membranous staining of invasive tumor cells was scored as positive and according to the Herceptest scoring system, the patients with HER-2 (++) and HER-2 (+++) were enrolled. The threshold for ER, PR and HER-2 was 10%.

Statistical analysis and study endpoints

SPSS (Chicago, Illinois, USA) version 13.0 statistical program was used for statistical analysis. The paired *t*-test, McNemar test and Fisher's exact test were used to assay: first, the protein expression change level after NAC; second, the relationship between the pre-neoadjuvant protein expression status and the clinical tumor response.

Results

Clinical and pathological characteristics of breast cancer patients

The patients' clinical and pathological characteristics are listed in Table 1. Their ages were between 22 and 71 years, with a median of 52.3 years. The patients were in 30 cases aged ≤ 50 years (44.1%) and in 38 cases aged > 50 years (55.9%); 28 cases (41.2%) were observed in premenopausal status and 40 cases (58.8%) were observed in postmenopausal status. Of the 68 patients, 11 (16.2%) were at clinical stage α and 57 (83.8%) at clinical stage β; 54 patients (79.4%) had cancer metastasis to their axillary lymph nodes.

Biomarker expression status of the 68 patients before and after NAC

Before NAC, positive protein accumulation was observed in 21(30.9%) patients for serum p53 Abs, 30 patients (44.1%) for CEA, 27 patients (39.7%) for CA15-3, 37 patients (54.4%) for ER, 39 patients (57.4%) for PR and 29 patients (42.6%) for HER-2. After NAC, positive cases were observed in nine patients (13.2%) for serum p53 Abs, 23 patients (33.8%) for CEA, 17 patients (25.0%) for CA15-3, 34 patients (50.0%) for ER, 30 patients (44.1%) for PR and 19 patients (27.9%) for HER-2.

Biomarker expression variations in different anticancer drug schemes

The biomarker expression changes after taxane-based and anthracycline-based NAC are summarized in Tables 2 and 3, respectively.

For the patients treated with taxane, before NAC, positive serum protein expressions were observed in 11

Table 1 Patient characteristics

Characteristics	*n* = 68 (range)	%
Age		
Median (years)	52.3 (22–71)	
≤ 50	30	44.1
>50	38	55.9
Menstrual status		
Pre	28	41.2
Post	40	58.8
Clinical stage		
II	11	16.2
III	57	83.8
Lymph node		
Positive	54	79.4
Negative	14	20.6

cases (34.4%) for p53 Abs, 13 cases (40.6%) for CEA and 12 cases (37.5%) for CA15-3; after NAC, those expressions were observed in three cases (9.4%) for p53 Abs, 15 cases (46.9%) for CEA and 11 cases (34.4%) for CA15-3. The serum levels of p53 Abs, CEA and CA15-3 were significantly changed ($P = 0.006$, $P = 0.004$ and $P = 0.008$). For the patients treated with anthracycline, before therapy, there were 17 patients (47.2%) with CEA-positive status and 15 cases (41.7%) with CA15-3-positive status; after therapy, there were eight patients (22.2%) with CEA-positive status and six patients (16.7%) with CA15-3-positive status. The serum protein levels were significantly changed ($P = 0.002$ and $P = 0.000$).

Patients' clinical response to neoadjuvant chemotherapy

All patients' clinical tumor response was assessed after four cycles of treatment using the International Union Against Cancer criteria. Table 4 shows the statistical results. In the taxane group, OR was observed in 23 patients out of 32 patients (71.9%), whereas NR was observed in nine patients (28.1%). In the anthracycline group, OR was observed in 25 patients (69.4%) out of 36 patients, whereas NR was observed in 11 patients (30.6%). No significant deviation was observed between these two groups' clinical tumor response to NAC.

Table 2 Variations in biomarker expression after taxane-based NAC in 32 patients

Biomarkers	Pre-NAC [*n* (%)]	Post-NAC [*n* (%)]	*P* value	Test
p53 Abs+	11 (34.4)	3 (9.4)		
p53 Abs−	21 (65.6)	29 (90.6)	0.006	Paired *t*
CEA+	13 (40.6)	15 (46.9)		
CEA−	19 (59.4)	17 (53.1)	0.004	Paired *t*
CA15-3+	12 (37.5)	11 (34.4)		
CA15-3−	20 (62.5)	21 (65.6)	0.008	Paired *t*
ER+	15 (46.9)	18 (56.3)		
ER−	17 (53.1)	14 (43.7)	NS	McNemar
PR+	16 (50.0)	10 (31.3)		
PR−	16 (50.0)	22 (68.7)	NS	McNemar
HER-2+	14 (43.8)	9 (28.1)		
HER-2−	18 (56.2)	23 (71.9)	NS	McNemar

Abs, antibodies; CEA, carcino-embryonic antigen; CA15-3, carbohydrate antigen 15-3; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor-2; NAC, neoadjuvant chemotherapy; NS, not significant.

Table 3 Variations in biomarker protein expression after anthracycline-based NAC in 36 patients

Biomarkers	Pre-NAC [*n* (%)]	Post-NAC [*n* (%)]	*P* value	Test
p53 Abs+	10 (27.8)	6 (16.7)		
p53 Abs−	26 (72.2)	30 (83.3)	NS	Paired *t*
CEA+	17 (47.2)	8 (22.2)		
CEA−	19 (52.8)	28 (77.8)	0.002	Paired *t*
CA15-3+	15 (41.7)	6 (16.7)		
CA15-3−	21 (58.3)	30 (83.3)	0.000	Paired *t*
ER+	22 (61.1)	16 (44.4)		
ER−	14 (38.9)	20 (55.6)	NS	McNemar
PR+	23 (63.9)	20 (55.6)		
PR−	13 (36.1)	16 (44.4)	NS	McNemar
HER-2+	15 (41.7)	10 (27.8)		
HER-2−	21 (58.3)	26 (72.2)	NS	McNemar

Abs, antibodies; CEA, carcino-embryonic antigen; CA15-3, carbohydrate antigen 15-3; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor-2; NAC, neoadjuvant chemotherapy; NS, not significant.

Table 4 Patients' clinical tumor response to NAC

	Taxane-based NAC [*n* (%)]	Anthracycline-based NAC [*n* (%)]	*P* value	Test
OR	23 (71.9)	25 (69.4)		
NR	9 (28.1)	11 (30.6)	NS	Fisher

OR, objective response rate; NR, no response; NAC, neoadjuvant chemotherapy; NS, not significant.

Table 5 Relationship between biomarker expression status before taxane-based NAC and clinical tumor response in 32 breast carcinoma patients [*n* (%)]

Factors	OR group ($n=23$)	NR group ($n=9$)	*P* value	Test
p53 Abs+	8 (72.7)	3 (27.3)		
p53 Abs−	15 (71.4)	6 (28.6)	NS	Fisher
CEA+	8 (61.5)	5 (38.5)		
CEA−	15 (78.9)	4 (21.1)	NS	Fisher
CA15-3+	7 (58.3)	5 (41.7)		
CA15-3−	16 (80.0)	4 (20.0)	NS	Fisher
ER+	11 (73.3)	4 (26.7)		
ER−	12 (70.6)	5 (29.4)	NS	Fisher
PR+	12 (75.0)	4 (25.0)		
PR−	11 (68.8)	5 (31.2)	NS	Fisher
HER-2+	7 (50.0)	7 (50.0)		
HER-2−	16 (88.9)	2 (11.1)	0.022	Fisher

Abs, antibodies; CEA, carcino-embryonic antigen; CA15-3, carbohydrate antigen 15-3; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor-2; OR, objective response rate; NR, no response; NAC, neoadjuvant chemotherapy; NS, not significant.

Table 6 Relationship between biomarker protein expression status before anthracycline-based NAC and clinical tumor response in 36 breast carcinoma patients [*n* (%)]

Factors	OR group ($n=25$)	NR group ($n=11$)	*P* value	Test
p53 Abs+	4 (40.0)	6 (60.0)		
p53 Abs−	21 (80.8)	5 (19.2)	0.039	Fisher
CEA+	10 (58.8)	7 (41.2)		
CEA−	15 (78.9)	4 (21.1)	NS	Fisher
CA15-3+	9 (60.0)	6 (40.0)		
CA15-3−	16 (76.2)	5 (23.8)	NS	Fisher
ER+	16 (72.7)	6 (27.3)		
ER−	9 (64.3)	5 (35.7)	NS	Fisher
PR+	15 (65.2)	8 (34.8)		
PR−	10 (76.9)	3 (23.1)	NS	Fisher
HER-2+	7 (46.7)	8 (53.3)		
HER-2−	18 (85.7)	3 (14.3)	0.025	Fisher

Abs, antibodies; CEA, carcino-embryonic antigen; CA15-3, carbohydrate antigen15-3; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor-2; OR, objective response rate; NR, no response; NAC, neoadjuvant chemotherapy; NS, not significant.

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

Tables 5 and 6 show the relationship between pre-neoadjuvant proteins expression status and the clinical tumor response. In the taxane group, 16 HER-2-negative

patients (88.9%) showed OR and two HER-2-negative patients (11.1%) showed NR. There was a significant correlation between the low expression of HER-2 and high OR rate ($P = 0.022$). In the anthracycline group, 21 serum p53Abs-negative patients (80.8%) showed an OR and five p53 Abs-negative patients (19.2%) showed NR. There was a significant correlation between the low expression of serum p53 Abs and high OR rate ($P = 0.039$). Eighteen HER-2-positive patients (85.7%) showed OR and three HER-2-negative patients (14.3%) showed NR. There was a significant correlation between the low expression of HER-2 and high clinical OR rate ($P = 0.025$).

Discussion

NAC can reduce advanced breast tumor volume, lead to less aggressive surgery and provide favorable local conditions for surgery in inoperable tumors [1,2].

Taxanes have emerged as fundamental drugs in the treatment of breast cancer since their initial licensing in Europe [9]. The NSABP B-27 study showed that taxanes may have activity for those anthracycline-resistant diseases [10]. After these kinds of drugs were used in clinical trials, their effect was compared with anthracycline. Crown *et al.* [9] concluded that docetaxel was the only drug to have shown superiority over single-agent anthracycline therapy as well as combination regimens in the metastatic setting. In our study, single-agent taxane (docetaxel) and combination anthracycline (CAF) schemes were performed. The clinical OR after chemotherapy was identified in 23 patients (71.9%) and 25 patients (69.4%), respectively. No significant deviation was observed between these two drugs' therapeutic effect. However, the different mechanisms of these two drugs could be partly identified through protein expression change. It is apparent that these two drugs could bring about distinct protein expression in serum, and they will be supplementary in the treatment of breast cancer.

Changes in protein expression after NAC have been reported by several researchers. Such observation suggests that chemotherapy can alter the tumor gene expression and that event is an important point in drug sensitivity [11]. Tiezzi *et al.* [6] studied HER-2, p53 and p21 expression status of 60 patients before and after neoadjuvant treatment with docetaxel ($75\,mg/m^2$) in combination with epirubicin ($50\,mg/m^2$). After two to five cycles (median = three cycles, one cycle contained 3 weeks) of treatment, they found that p53 and p21 levels were significantly decreased, but HER-2 level was steady before and after NAC. In the current study, three tumor markers (ER, PR and HER-2) and three serum markers (the serum p53 Abs, CEA and CA15-3) before and after chemotherapy were studied. It was found that the protein level of the serum p53 Abs was significantly decreased only by taxane ($P = 0.006$). Moreover, the serum CEA and CA15-3 expressions were significantly affected by both taxane ($P = 0.004$ and $P = 0.008$) and anthracycline ($P = 0.002$ and $P = 0.000$). Three tumor markers, ER, PR and HER-2, were, however, detected as having no significant change in either taxane-based or anthracycline-based NAC.

CEA and CA15-3 were regarded as two important serum biomarkers. In this study, the levels of CEA and CA15-3 were significantly changed during NAC treatments, but they showed no ability in predicting tumor response during either taxane-based or anthracycline-based NAC. Many studies have revealed that the expressions of CEA and CA15-3 were not stable and one third of patients showed a spike phenomenon although they experienced clinical benefit: an initial increase and subsequent decrease [8]. A spike phenomenon was not found in the study because of short observation time and lesser detection times. It is clear, however, that these two factors are more sensitive to certain doses of taxane and anthracycline than the other four factors. It is supposed that the change regularities of serum CEA and CA15-3 were diverse in different drug schemes, different treatment phases and different patients.

In this study, it was found that the serum p53 Ab is a useful predictive serum biomarker. The serum p53 Abs are supposed to be the immune production of tumor p53 protein, which is released into the extracellular environment and is absorbed into the circulation [12]. Gao *et al.* [12] found that there was a strong association between the presence of serum p53 Abs and tumor p53 protein ($P < 0.0001$). In the meantime, others have reported that there is a strong correlation between p53 gene mutation and anthracycline chemoresistance [13]. Some of these mutations, however, could not cause the enhanced staining for p53 protein and this might explain why immunohistochemical studies have always been inconclusive [13]. To date, few researchers have studied the correlation between serum p53 Ab expression and chemoresistance. According to the study, 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 (Fisher's exact test). Moreover, a high OR rate was significantly associated with low serum p53 Ab concentration in the anthracycline-based treatment ($P = 0.039$). It is possible that the relationship between serum p53 Abs and drug sensitivity could indirectly reflect the correlation between tumor p53 protein and drug sensitivity, and detection of the serum p53 Abs may complement the discrepancy of tumor p53 immunohistochemistry staining.

Many studies have investigated the correlation between HER-2 expression and clinical tumor response for anthracycline-based NAC in breast cancer. Petit *et al.* [14] studied 64 patients with 5-fluorouracil, epirubicin and cyclophosphamide at two epirubicin dose levels, 50 or 100 mg/m^2. Finally, they concluded that low-dose anthracycline and HER-2 overexpression predicted a poor response rate. Low-dose or high-dose anthracycline and HER-2 normal expression predicted an intermediate OR. High-dose anthracycline and HER-2 overexpression predicted a high OR. Kariya *et al.* [15] 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). Their study indicated that HER-2-negtive status was the only significant predictive factor of response. Some studies, however, have shown that there is no correlation between tumor response and HER-2 expression. Zhang *et al.* [16] found no correlation between the expression of HER-2 and clinical tumor response. In this study, with a taxane dosage of 100 mg/m^2, and cyclophosphamide, adriamycin and 5-fluorouracil doses of 500/40/500 mg/m^2, low HER-2 expression is a predictive factor of clinical response in both taxane-based and anthracycline-based NAC. Our experiment result was partly coincident with that of Kariya *et al.* [15].

Currently, the focus has been on three tumor serum biomarkers and three tumor biomarkers during the NAC treatments. In conclusion, the serum p53 Ab level is easily changed by a certain dose of taxane, whereas CEA and CA15-3 levels are easily changed by both taxane and anthracycline. Breast cancer patients that are serum p53 Ab-negative may lead to a high predicted OR rate in anthracycline-based NAC, whereas HER-2-positive patients may lead to a low predicted OR in both taxane-based and anthracycline-based NAC.

Research in the expression of tumor biological markers in NAC treatment has been a hot topic in recent studies of cancer. It has been clearly demonstrated that the *p*53 gene for tumor or p53-mediated apoptosis pathway could be modified by some kind of anticancer drug [13,17,18]. It is assumed that this modification will directly affect the expression of p53 protein as well as the serum p53 Abs. There is little evidence to show the correlations between the expression changes of CEA and CA15-3 and their gene modifications. It is assumed that changes in these two proteins for serum take place concurrently with the variation in volume and size of the tumors, and that is why their expressions are easily changed during chemotherapy.

Of all the six biomarkers, the expression of serum p53 Abs is distinct under the influence of different anticancer drugs and it may be helpful for drug selection before NAC is executed. The expressions of CEA and CA15-3 are labile when tumor cells are stimulated by drugs. Therefore, CEA and CA15-3 can potentially be used to monitor the tumor status of the patients. ER and PR are frequently used as two typical biomarkers in the endocrine therapy of the tumor. The experiments showed that ER and PR levels were not changed in NAC, and they offered no predictive value of tumor response. HER-2 expression was shown to have significant correlation with the clinical response to the tumor. It is marked that in predicting tumor prognosis, HER-2 is more useful than the other five tumor biological markers.

To achieve more reliable results, further examination of a greater number of patients is necessary.

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