RESEARCH ARTICLE

Serum Tumor Marker Levels might have Little Significance in Evaluating Neoadjuvant Treatment Response in Locally Advanced Breast Cancer

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Abstract

Background: To determine the potential value of serum tumor markers in predicting pCR (pathological complete response) during neoadjuvant chemotherapy. Materials and Methods: We retrospectively monitored the pro-, mid-, and post- neoadjuvant treatment serum tumor marker concentrations in patients with locally advanced breast cancer (stage II-III) who accepted pre-surgical chemotherapy or chemotherapy in combination with targeted therapy at Fudan University Shanghai Cancer Center between September 2011 and January 2014 and investigated the association of serum tumor marker levels with therapeutic effect. Core needle biopsy samples were assessed using immunohistochemistry (IHC) prior to neoadjuvant treatment to determine hormone receptor, human epidermal growth factor receptor 2(*HER2*), and proliferation index Ki67 values. In our study, therapeutic response was evaluated by pCR, defined as the disappearance of all invasive cancer cells from excised tissue (including primary lesion and axillary lymph nodes) after completion of chemotherapy. Analysis of variance of repeated measures and receiver operating characteristic (ROC) curves were employed for statistical analysis of the data. Results: A total of 348 patients were recruited in our study after excluding patients with incomplete clinical information. Of these, 106 patients were observed to have acquired pCR status after treatment completion, accounting for approximately 30.5% of study individuals. In addition, 147patients were determined to be Her-2 positive, among whom the pCR rate was 45.6% (69 patients). General linear model analysis (repeated measures analysis of variance) showed that the concentration of cancer antigen (CA) 15-3 increased after neoadjuvant chemotherapy in both pCR and non-pCR groups, and that there were significant differences between the two groups (*P*=0.008). The areas under the ROC curves (AUCs) of pre-, mid-, and post-treatment CA15-3 concentrations demonstrated low-level predictive value (AUC=0.594, 0.644, 0.621, respectively). No significant differences in carcinoembryonic antigen (CEA) or CA12-5 serum levels were observed between the pCR and non-pCR groups (*P*=0.196 and 0.693, respectively). No efficient AUC of CEA or CA12-5 concentrations were observed to predict patient response toward neoadjuvant treatment (both less than 0.7), nor were differences between the two groups observed at different time points. We then analyzed the Her-2 positive subset of our cohort. Significant differences in CEA concentrations were identified between the pCR and non-pCR groups (*P*=0.039), but not in CA15-3 or CA12-5 levels (*p*=0.092 and 0.89, respectively). None of the ROC curves showed underlying prognostic value, as the AUCs of these three markers were less than 0.7. The ROC-AUCs for the CA12-5 concentrations of inter-and post-neoadjuvant chemotherapy in the estrogen receptor negative *HER2* positive subgroup were 0.735 and 0.767, respectively. However, the specificity and sensitivity values were at odds with each other which meant that improving either the sensitivity or specificity would impair the efficiency of the other. Conclusions: Serum tumor markers CA15-3, CA12-5, and CEA might have little clinical significance in predicting neoadjuvant treatment response in locally advanced breast cancer.

Keywords: Serum tumor markers - neoadjuvant chemotherapy - pCR - prognostic function

Asian Pac J Cancer Prev, **16 (11)**, 4603-4608

Introduction

Serum tumor biomarkers including carcinoembryonic antigen (CEA), cancer antigen 15-3 (CA15-3), and cancer antigen 12-5 (CA12-5) can serve as indicators of recurrence or metastasis at breast cancer follow-up, although the American Society of Clinical Oncology (ASCO) guidelines do not currently recommend their use as a routine surveillance tool or for early diagnosis due to inconsistencies with sensitivity and specificity (Khatcheressian et al., 2013). Recent studies have demonstrated that elevated levels of preoperative CA 15-3

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and CEA might independently predict poor prognosis in breast cancer (Park et al., 2008; Lee et al., 2013; Zhang et al., 2013); these might therefore provide us with a promising method to predict outcomes. Generally, neoadjuvant chemotherapy (NAC) is administered for the treatment of locally advanced disease and is being increasingly used for early-stage breast cancer to downstage primary tumors in order to allow less radical surgeries and to increase the rate of operations permitting breast-conservation (Wolmark et al., 2001; Cho et al., 2013; Shin et al., 2013). A pathological complete response (pCR) is considered to be the main target of NAC and is mainly evaluated on the basis of pathological results after surgery (Cho et al., 2013). According to the findings of the CTNeoBC meta-analysis, patients with pCR had a correspondingly better chance of event free survival (EFS) and a potential for an improved overall survival (OS) rate when compared to patients with non-pCR (Cortazar et al., 2014). Many studies that have focusing on determining the predictive factors of neoadjuvant treatment response have reported that intrinsic subtypes, Ki67 values, primary tumor size, and axillary lymph node status were considered as factors relevant to determining the effect of preoperative adjuvant therapy (Denkert et al., 2013; Lips et al., 2013; Yoshioka et al., 2015). Patients with human epidermal growth factor receptor 2 (HER2) gene overexpression who had received chemo-regimens containing trastuzumab and those with basal-like breast cancer who were sensitive to chemotherapy often realized higher rates of pCR than did patients positive for hormone receptor expression but who were *HER2* negative (Houssami et al., 2012; Denkert et al., 2013; de Ronde et al., 2014). Other methods such as nomogramms have been proven not to be accurate for the prediction of neoadjuvant chemotherapy response (Takada et al., 2012). In contrast, positron emission tomography/computed tomography (PET/CT) carried out during the process of preoperative treatment was reported to be a latent approach to predict pCR with a reasonable sensitivity and specificity in spite of its high expense (Hatt et al., 2013; Coudert et al., 2014; Koolen et al., 2014). Furthermore, an earlier retrospective study concluded that elevated pre-chemotherapy CA 15-3 levels, large tumor size, and the presence of lympho-vascular invasion predicted a poor pathological response to chemotherapy in locally advanced breast cancer (Al-azawi et al., 2006). As the analysis of serum biomarkers is rapid, noninvasive, reproducible, and quantitative, their assessment is widely used in clinical practice for the surveillance of local or regional recurrence and distant metastasis. Accordingly, in this study, our objective was to determine the role of serum tumor markers in predicting the effect of NAC when applied to patients with locally advanced breast cancer.

Materials and Methods

Subjects

We enrolled patients with locally advanced breast cancer who were undergoing NAC including targeted regimens from September, 2011 to September, 2012 in the Department of Breast Surgery in Fudan University Shanghai Cancer Center Hospital. All patients were well informed in advance and provided signed informed consent prior to participation in this study. Study exclusion criteria included poor general conditions, failure to tolerate the side effects of the chemotherapeutic agent(s), malignant disease (other than breast cancer) in the past 5 years, and immunological disease. There were 348 patients recruited in our study whose blood specimens were collected before, during, and after all of the treatment cycles respectively, from which the concentrations of the serum tumor markers CA15-3, CA12-5, and CEA were evaluated. Immunohistochemistry of hormone receptors, *HER2*, and proliferation index Ki67 values were assessed prior to neoadjuvant treatment using the core needle biopsy tissue for guidance of establishing suitable regimens. The procedure of detecting ER, PR, and *HER2* is following the same method as previous study in our center (Liu et al., 2014). The effect of preoperative treatment was determined as being either pCR or non-pCR according to the pathological diagnoses of surgical specimens. pCR was defined as the absence of malignant tumor cells as visualized under the microscope from the resected lesions and axillary lymph nodes. In this study, we divided the patients into two groups: those with pCR and those with non-pCR, in an attempt to investigate the relevance of serum tumor marker concentrations to the therapeutic response obtained from the process of pre-operative treatment.

Statistical analysis

We defined the cut-off values of tumor markers as the 95th percentiles of values found in the serum of healthy individuals, as determined according to the Chinese population normal value range. According to this standard, the normal value range for CA15-3 was 0-25 units/L, 0-35 units/L for CA12-5, and 0-5.2 units/L for CEA based upon the inspection technique performed by the Department of Laboratory Medicine in our center. For pathologic determination, tumors with ≥ 1% nuclear-stained cells were considered positive for both the estrogen receptor (ER) and progesterone receptor (PR). Immunohistochemistry (IHC) analysis of *HER2* was considered positive in cases with an IHC 3+ score, and fluorescence in situ hybridization (FISH) was required to re-confirm the status of the status of *HER2* if the IHC score was 2+. We define FISH positive as *HER2* copy number over 6.0 or HER2/CEP17 ratio over 2.0. (Wolff et al., 2014).

SPSS (IBM SPSS Statistic, version 20.0; SPSS, Chicago, IL, USA) was utilized to analyze the statistics. A χ^2-test was performed to determine whether a significant difference existed between the numbers of samples in different subgroups (pCR and non-pCR). For repeated measurement data, the general linear model was chosen to observe the dynamic changes of different serum biomarkers and to compare whether the differences between the pCR and non-pCR groups were maintained across changing time points. ROC curves were generated to investigate whether the serum tumor markers exhibited any predictive value. A *P* value of less than 0.05 (two-sided) was considered to indicate a significant result.

Results

We enrolled 348 patients with locally advanced breast cancer in this study, who had received chemotherapy or targeted therapy prior to surgery to downstage their primary lesion to allow less radical surgery and to increase the rate of breast-conservation during treatment. The general characteristics of the enrolled population are shown in Table 1. During all treatment cycles, patients were monitored at pro-, inter-, and post-neoadjuvant treatment stages for serum tumor markers using assessment of CA15-3, CA12-5, and CEA levels in circulating blood and by other clinical and imaging methods. Among the 348 patients, 28 Ki67 and one *HER2* result were missing. The 348 patients were assigned to the following groups on the basis of biopsy IHC assessment prior to chemotherapy: basal-like (50 patients, 14.4%), hormone receptor positive and *HER2* negative (143 patients, 41.1%), and Her-2 positive group (147 patients, 42.2%). The median age of the study patients was 47.9 years (24-78); 59 were found to have elevated CA15-3 concentrations, 47 had elevated CA12-5 concentrations, and 41 exhibited elevated CEA concentrations before treatment. After completion of all treatment cycles, 106 patients were observed to have acquired pCR from the final pathological reports, accounting for 30.5% of study individuals. In the basal-like breast cancer group, 40% of patients achieved pCR; the *HER2* positive group seemed to have the highest pCR rate (45.6%), which is in accordance with the results from other studies, especially for those incorporating a trastuzumab regimen (Houssami et al., 2012).

Table 1. General Characteristics of Study Population

Characteristics		n	%
Age(n=348)	≤35	46	13.2
	>35	302	86.8
Tumor size(n=348)	T1	37	10.6
	T2	193	55.5
	≥T3	116	33.3
	Tx	2	0.57
Nodal status(n=346)	N0	58	16.8
	N1	230	66.5
	N2	15	4.3
	N3	43	12.4
TNM stage(n=344)	I	5	1.5
	II	190	55.2
	III	149	43.3
ER(n=348)	Negative	150	43.1
	Positive	198	56.9
PR (n=348)	Negative	165	47.4
	Positive	183	52.6
Her2 (n=347)	Negative	200	57.6
	Positive	147	42.4
CA 15-3 (n=310)	Normal(≤25)	251	81
	Elevated(>25)	59	19
CA 12-5 (n=311)	Normal(≤35)	264	84.9
	Elevated(>35)	47	15.1
CEA (n=311)	Normal(≤5.2)	270	86.8
	Elevated(>5.2)	41	13.2
NAC response(n=348)	pCR	106	30.5
	non-pCR	242	69.5

A great majority of the enrolled patients (86.5%) who were diagnosed as having locally advanced breast cancer (stages II and III, according to the American Joint Committee on Cancer (AJCC) Cancer Staging Manual, Version 7) (Edge and Compton, 2010). Concentrations of the targeted biomarkers were in the normal range among most of the patients (81.0, 84.9, and 86.8% for CA15-3, CA12-5, and CEA respectively). No differences existed in the CA12-5 and CEA values between the pCR and non-pCR groups except for the post-NAC stage in CA15-3 (P=0.07), which was consistent with the result obtained from repeated measures analysis of variance. The data mentioned above is shown in Table 2. We noted an apparent difference in the CEA concentrations when calculating the means of the three time points, whereby a slight increase at the inter-NAC stage in the non-pCR group followed a drastic decrease after NAC treatment.

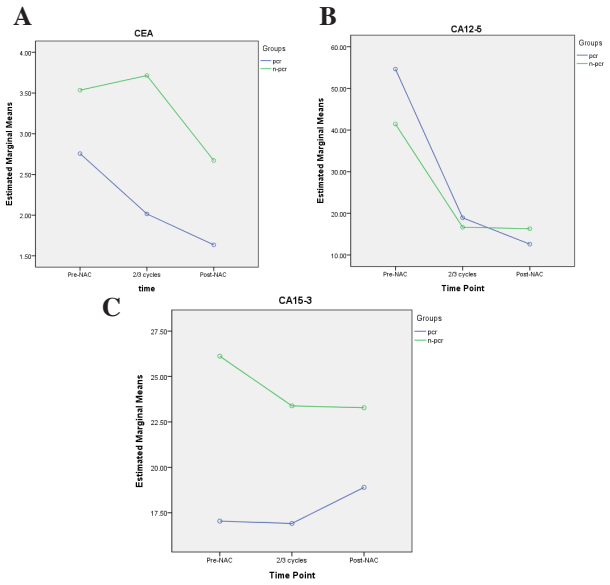

Figure 1. Results of Repeated Measures Analysis of Variance. (A) Compared to consecutive decrease in pCR group, n-pCR group has a slight increase after 2 or 3 cycles chemotherapy following a drastic decrease. **(B)** Both the pCR and n-pCR groups decrease during the process of treatment. **(C)** The entire trend of pCR group is increasing. The discrepancy of these two groups seemed to be significantly different (P=0.008)

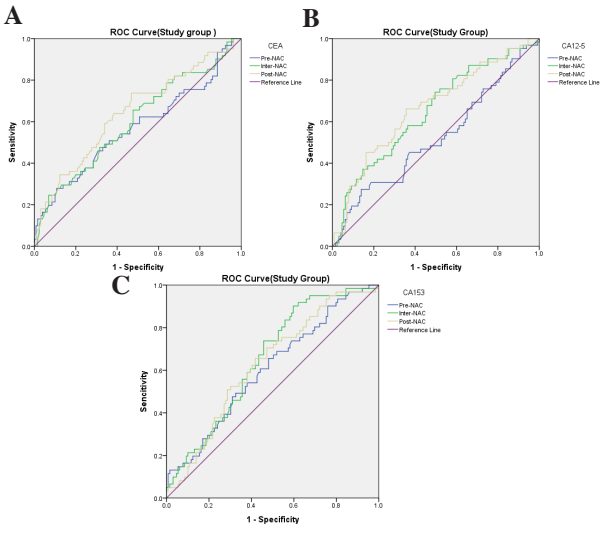

Figure 2. ROC of Study Group

Table 2. Mean Value of pCR and non-pCR Groups for Each Serum Tumor Marker. Only the Difference between two Groups in Post-NAC CA15-3 has Significance

	Pre-NAC			Inter-NAC			Post-NAC		
	Mean	SD	*P*	Mean	SD	*P*	Mean	SD	*P*
CA15-3	19.47	14.8	0.36	19.94	11.34	0.029	20.91	9.46	0.07
pCR	17.32	14.37		16.61	10.06		18.95	8.81	
n-pCR	20.43	14.92		21.5	11.59		21.82	9.63	
CA12-5	19.28	16.2	0.16	14.35	8.42	0.22	13.72	6.76	0.37
pCR	20.91	17.37		12.38	7.73		12.33	5.66	
n-pCR	18.52	15.61		15.26	8.59		14.37	7.14	
CEA	2.97	3.5	0.37	2.97	10.34	0.37	2.16	4.24	0.27
pCR	2.69	2.67		2.18	2.05		1.62	1.19	
n-pCR	3.09	3.81		3.33	12.4		2.39	5.03	

*Abbreviation NAC, neoadjuvant chemotherapy pCR, pathological complete response

Table 3. ROC-AUCs of Each Study Biomarkers at Different Time Points

Study Group	Biomarker	Time Point	AUC	95% CI		Sensitivity	Specificity
Study Population	CEA	Pre	0.57	0.48	0.66	27.9%	89.2%
		Inter	0.60	0.51	0.69	65.6%	52.3%
		Post	0.64	0.56	0.73	73.8%	53.1%
	CA125	Pre	0.52	0.43	0.61	27.4%	86.0%
		Inter	0.65	0.57	0.73	74.2%	50.4%
		Post	0.66	0.58	0.75	66.1%	64.3%
	CA153	Pre	0.60	0.52	0.69	65.6%	51.9%
		Inter	0.65	0.57	0.73	90.2%	40.3%
		Post	0.63	0.55	0.71	65.6%	58.1%
Her+ Subset	CEA	Pre	0.54	0.41	0.66	34.3%	71.1%
		Inter	0.59	0.47	0.71	91.4%	35.4%
		Post	0.56	0.43	0.68	77.1%	43.7%
	CA125	Pre	0.53	0.40	0.66	48.6%	63.3%
		Inter	0.64	0.52	0.76	77.1%	51.0%
		Post	0.62	0.50	0.74	91.4%	32.7%
	CA153	Pre	0.60	0.14	0.47	58.8%	61.2%
		Inter	0.58	0.22	0.46	94.1%	32.7%
		Post	0.54	0.52	0.42	94.1%	18.4%
ER- Her2+ Subset	CEA	Pre	0.65	0.50	0.80	73.3%	52.2%
		Inter	0.65	0.50	0.80	83.3%	47.8%
		Post	0.65	0.51	0.80	56.7%	73.9%
	CA125	Pre	0.65	0.50	0.80	53.3%	78.3%
		Inter	0.74	0.60	0.87	83.3%	56.5%
		Post	0.77	0.64	0.90	70.0%	82.6%
	CA153	Pre	0.57	0.42	0.73	63.3%	56.5%
		Inter	0.62	0.46	0.77	90.0%	39.1%
		Post	0.60	0.44	0.75	66.7%	56.5%

*In ER- Her2+ subgrop,CA125 seems to embrace the most valuable AUC,but the contradictions between sensitivity and specificity have limited its application

However, this response pattern did not appear in the pCR group wherein the mean concentrations of CEA demonstrated a consecutive decline as time went on (Figure 1). The seemingly apparent difference between pCR and non-pCR groups appeared to indicate a potential significance, but this was not supported by statistical analysis (*P*=0.196). In contrast, analysis of variance of repeated measures demonstrated that for the CEA and CA12-5 concentrations measured pre-, inter-, and post-NAC, the alternations were definitely associated with the time points (*P*=0.000 and 0.031 respectively), whereas when the variables were segregated by group (pCR or non-pCR), no relationships with the time points were observed (*P*=0.796 and 0.693, respectively). For the alterations of CA125, both pCR and non-pCR groups showed dramatic decreases after 2 or 3 cycles of chemotherapy, and the differences between pre-NAC and inter-NAC or post-NAC time points were obvious (*P*=0.31 and 0.18). Unfortunately, when comparing the differences between the two response groups, we had no positive outcomes (*P*=0.693). Neither timing nor grouping had effects on CA15-3 level variation. We noticed that in the pCR group, the CA15-3 concentration increased compared to the level measured before primary treatment after the completion of scheduled regimens, in contrast with the successive decline observed in the non-pCR group, and the discrepancy between these two groups seemed to be significant (*P*=0.008). However, whether patients with increased CA15-3 levels would have more chances to achieve pCR remains to be verified. In this study, logistic regression analysis suggested that the final outcome of the treatment and the difference of CA 15-3 (the difference

between the first, second, and third measurements) were irrelevant.

To investigate the prognostic value of serum tumor biomarkers, ROC curves were applied to the whole study group, the *HER2* positive subset, and the ER negative plus *HER2* positive subgroup. For the entire study group, use of the three serum biomarkers assessed in this study seemed to fail to predict the response of pre-operation systemic treatment accurately (Table 3 and Figure 2). The ROC-AUCs for the CEA concentrations of pre-, inter- and post-NAC treatment were only 0.568, 0.601, and 0.643, respectively. For CA12-5, the AUCs were 0.521, 0.648, and 0.664, respectively, and for CA15-3, the AUCs were 0.604, 0.652, and 0.627, respectively. Subgroup analyses stratified by *HER2* positive status also did not reveal any specific prognostic value of these three biomarkers (Table 3 and Figure 3). The ROC-AUC for predicting pCR was 0.650 for CEA at pre-NAC, 0.654 after 2/3 cycles of chemotherapy, and 0.654 before the final surgery in the ER negative plus *HER2* positive subset of patients. A similar situation was likely to be true for CA15-3 as well,

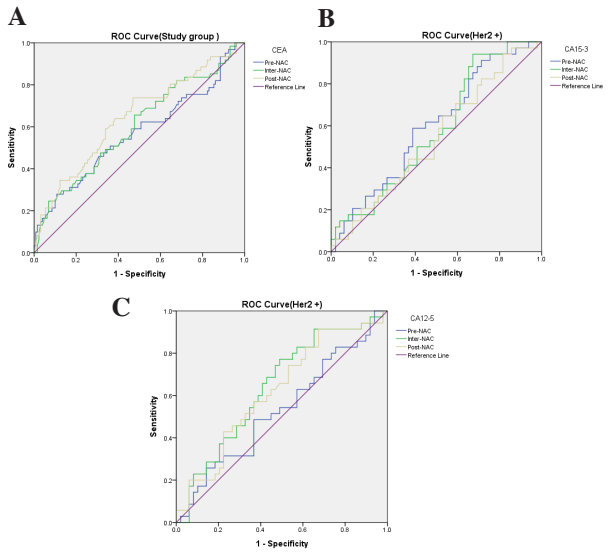

Figure 3. ROC of Her2+ Subset

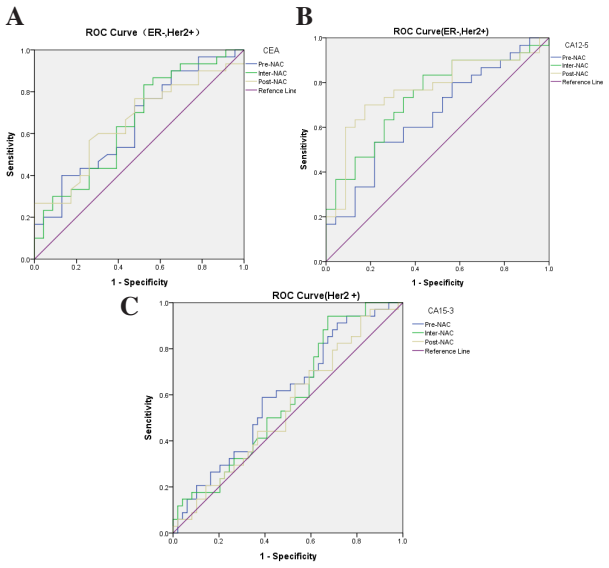

Figure 4. ROC of ER- Her2+ Subset

because the ROC-AUCs for this marker were under 0.7 (0.571, 0.615, and 0.595 respectively). It appeared likely, however, that CA12-5 could have a potential value in predicting favorable response in patients with ER negative, *HER2* positive breast cancer (Table 3 and Figure 4). The ROC-AUCs for the CA12-5 concentrations of pre-, inter-, and post-NAC were 0.625, 0.735, and 0.767, respectively. However, the specificity and sensitivity were at odds with each other which meant that improving either the sensitivity or the specificity would impair the efficiency of the other. We also analyzed the ROC-AUCs of the differences and the rates of change between two time points, only to find that no effective AUCs were feasible (data not provided). Subset analyses of basal-like breast cancer patients were not carried out because of the limited number of cases and to missing data.

Discussion

AS products produced by tumor cells or generated by host cells in response to tumorigenesis, serum tumor biomarkers such as CEA, CA15-3, and CA-125 have been widely investigated (Duffy, 2006; 2007). To our knowledge, serum CEA and CA15-3 are also associated with the tumor burden of breast cancer patients and might reflect the therapeutic responses in these patients (Yerushalmi et al., 2012); however, their roles in predicting early response during NAC remained ambiguous.

There have been a few studies which have suggested that assessments of elevated tumor marker concentrations during post-surgery follow-up prompted adverse outcomes (worse DFS and OS) in patients with non-metastatic breast cancer. American Society of Clinical Oncology listed the top 5 unnecessary uses of health care resources recommended, in which serum tumor biomarker assessment was included (Schnipper et al., 2012). Recent study have also reported that breast cancer tumor markers are frequently used among women with early-stage disease and are associated with an increase in both diagnostic procedures and in the total cost of care through analysis of Surveillance, Epidemiology, and End Results-Medicare (SEER) records (Ramsey et al., 2015).

This study was designed to investigate the clinical value of serum tumor biomarkers with the purpose of discovering their underlying relevance to the effects of preoperative treatment, which might provide a fast, noninvasive, reproducible, and quantitative evaluation method to screen for patients favorable to the treatment. The ROC-AUs of CEA, CA12-5, and CA15-3 were measured at three time points in this study. Unfortunately, all three tumor markers failed to predict the favorable response according to the analyses of statistical significance performed herein. These results suggest that no such detection should be performed in clinical practice during NAC for patients with locally advanced breast cancer. No sufficient evidence was found in our study to indicate that serum tumor biomarkers should be routinely used to monitor the response of preoperative systemic treatment.

This study has several limitations. First, this single-center study was too small to cover all the characteristics

of the entire breast cancer population. Second, the missing records influence the final results even we chose statistical methods suited to avoiding errors generated by these conditions. Finally, this study only analyzed the condition of biomarkers in a Chinese population, which might not represent the situation in other countries. Multi-center studies are therefore needed to confirm the conclusion drawn from this study.

Acknowledgements

We gratefully thank GuangyuLiu,Jiong Wu, Zhimin Shao, and Zhenzhou Shen for their guidance of this study. We also sincerely thank Miao Mo, Jianwei Li, and Xiaoqing Jia for their active participation throughout this study.

The authors declare no conflict of interest. This study was supported in part by a grant from Shanghai Science and Technology (No. 12410707700).

References

Al-azawi D, Kelly G, Myers E, et al (2006). CA 15-3 is predictive of response and disease recurrence following treatment in locally advanced breast cancer. *BMC Cancer*, **6**, 220.

Cho JH, Park JM, Park HS, et al (2013). Oncologic safety of breast-conserving surgery compared to mastectomy in patients receiving neoadjuvant chemotherapy for locally advanced breast cancer. *J Surg Oncol*, **108**, 531-6.

Cortazar *P*, Zhang L, Untch M, et al (2014). Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. *Lancet*, **384**, 164-72.

Coudert B, Pierga JY, Mouret-Reynier MA, et al (2014). Use of [(18)F]-FDG PET to predict response to neoadjuvant trastuzumab and docetaxel in patients with HER2-positive breast cancer, and addition of bevacizumab to neoadjuvant trastuzumab and docetaxel in [(18)F]-FDG PET-predicted non-responders (AVATAXHER): an open-label, randomised phase 2 trial. *Lancet Oncol*, **15**, 1493-502.

de Ronde JJ, Bonder MJ, Lips EH, et al (2014). Breast cancer subtype specific classifiers of response to neoadjuvant chemotherapy do not outperform classifiers trained on all subtypes. *PLoS One*, **9**, 88551.

Denkert C, Loibl S, Muller BM, et al (2013). Ki67 levels as predictive and prognostic parameters in pretherapeutic breast cancer core biopsies: a translational investigation in the neoadjuvant GeparTrio trial. *Ann Oncol*, **24**, 2786-93.

Duffy MJ (2006). Serum tumor markers in breast cancer: are they of clinical value? *Clin Chem*, **52**, 345-51.

Duffy MJ (2007). Role of tumor markers in patients with solid cancers: A critical review. *Eur J Intern Med*, **18**, 175-84.

Edge SB, Compton CC (2010). The American joint committee on cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol*, **17**, 1471-4.

Hatt M, Groheux D, Martineau A, et al (2013). Comparison between 18F-FDG PET image-derived indices for early prediction of response to neoadjuvant chemotherapy in breast cancer. *J Nucl Med*, **54**, 341-9.

Houssami N, Macaskill *P*, von Minckwitz G, et al (2012). Meta-analysis of the association of breast cancer subtype and pathologic complete response to neoadjuvant chemotherapy. *Eur J Cancer*, **48**, 3342-54.

Khatcheressian JL, Hurley *P*, Bantug E, et al (2013). Breast cancer follow-up and management after primary treatment: American society of clinical oncology clinical practice guideline update. *J Clin Oncol*, **31**, 961-5.

Koolen BB, Pengel KE, Wesseling J, et al (2014). Sequential (18)F-FDG PET/CT for early prediction of complete pathological response in breast and axilla during neoadjuvant chemotherapy. *Eur J Nucl Med Mol Imag*, **41**, 32-40.

Lee JS, Park S, Park JM, et al (2013). Elevated levels of preoperative CA 15-3 and CEA serum levels have independently poor prognostic significance in breast cancer. *Ann Oncol*, **24**, 1225-31.

Lips EH, Mulder L, de Ronde JJ, et al (2013). Breast cancer subtyping by immunohistochemistry and histological grade outperforms breast cancer intrinsic subtypes in predicting neoadjuvant chemotherapy response. *Breast Cancer Res Treat*, **140**, 63-71.

Liu Y, Huang X, Bi R, et al (2014). Similar prognoses for invasive micropapillary breast carcinoma and pure invasive ductal carcinoma: a retrospectively matched cohort study in China. *PLoS One*, **9**, 106564.

Park BW, Oh JW, Kim JH, et al (2008). Preoperative CA 15-3 and CEA serum levels as predictor for breast cancer outcomes. *Ann Oncol*, **19**, 675-81.

Ramsey SD, Henry NL, Gralow JR, et al (2015). Tumor marker usage and medical care costs among older early-stage breast cancer survivors. *J Clin Oncol*, **33**, 149-55.

Schnipper LE, Smith TJ, Raghavan D, et al (2012). American society of clinical oncology identifies five key opportunities to improve care and reduce costs: the top five list for oncology. *J Clin Oncol*, **30**, 1715-24.

Shin HC, Han W, Moon HG, et al (2013). Breast-conserving surgery after tumor downstaging by neoadjuvant chemotherapy is oncologically safe for stage III breast cancer patients. *Ann Surg Oncol*, **20**, 2582-9.

Takada M, Sugimoto M, Ohno S, et al (2012). Predictions of the pathological response to neoadjuvant chemotherapy in patients with primary breast cancer using a data mining technique. *Breast Cancer Res Treat*, **134**, 661-70.

Wolff AC, Hammond ME, Hicks DG, et al (2014). Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *Arch Pathol Lab Med*, **138**, 241-56.

Wolmark N, Wang J, Mamounas E, et al (2001). Preoperative chemotherapy in patients with operable breast cancer: nine-year results from national surgical adjuvant breast and bowel project B-18. *J Natl Cancer Inst Monogr*, 96-102.

Yerushalmi R, Tyldesley S, Kennecke H, et al (2012). Tumor markers in metastatic breast cancer subtypes: frequency of elevation and correlation with outcome. *Ann Oncol*, **23**, 338-45.

Yoshioka T, Hosoda M, Yamamoto M, et al (2015). Prognostic significance of pathologic complete response and Ki67 expression after neoadjuvant chemotherapy in breast cancer. *Breast Cancer*, **22**, 185-91.

Zhang SJ, Hu Y, Qian HL, et al (2013). Expression and significance of ER, PR, VEGF, CA15-3, CA125 and CEA in judging the prognosis of breast cancer. *Asian Pac J Cancer Prev*, **14**, 3937-40.