Evaluating the response of neoadjuvant chemotherapy for treatment of breast cancer: are tumor biomarkers and dynamic contrast enhanced MR images useful predictive tools?

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Objective: In order to evaluate the therapeutic response to neoadjuvant chemotherapy (NAC) for breast cancer, this research focused on the changes in expression of tumor biomarkers and the correlations associated with changes of magnetic resonance imaging (MRI) pre- and post-NAC. We also compared the accuracy of MRI and pathology in terms of residual tumor extent after NAC.

Methods: MRI was performed before and after four courses of cyclophosphamide, epirubicin and paclitaxel (CET) NAC on 114 patients treated in Huashan Hospital (Fudan University) from December 2009 to January 2013. All patients were pathologically diagnosed with invasive breast cancer via core needle biopsy. A series of tumor biomarkers, including P-glycoprotein (P-gp) and Ki-67, was tested by immunohistochemistry in both core needle biopsy and surgical specimens. The changes in tumor biomarker expression and the shrinkage of tumor on MRI were observed. The residual tumor extent after NAC was compared in terms of MRI and histopathology, and the accuracy of MRI was evaluated by both residual tumor extent and by NAC therapeutic effect. Together, these methods enabled a prognostic estimate of NAC.

Results: The P-gp expression before NAC was used to evaluate the therapeutic effect of NAC. The upregulation of P-gp expression after NAC was associated with poor therapeutic effect (P=0.0011). The expression of Ki-67 was significantly down-regulated (P<0.0001) but it had no association with NAC response (P=0.9645). The mean extent of residual tumor after NAC as seen on MRI was 20.83 mm (\pm 4.14 mm, 95% CI) and that of surgically removed specimens, 18.89 mm (\pm 3.71 mm, 95% CI). The sensitivity of MRI was 95.1%, the specificity was 28.6%, the positive predictive value was 79.6%, and the negative predictive value was 66.7%.

Conclusions: P-gp status was an important factor affecting the pathological complete response (pCR) rate. The change in P-gp expression, from negative to positive following NAC treatment, indicated the emergence of drug resistance resulting from chemotherapy. The down-regulation of Ki-67 was associated with the decline of tumor proliferation. However, compared to the pre-NAC P-gp status, the pre-NAC Ki-67 status had little prognostic value. Additionally, the evaluation of the efficacy of NAC by either MRI or histopathology was inconclusive.

Keywords: Breast cancer; neoadjuvant chemotherapy (NAC); Ki-67; P-glycoprotein (P-gp); pathological complete response (pCR)

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Introduction

In recent years, a number of clinical research results have indicated that neoadjuvant chemotherapy (NAC) has failed to increase overall survival (OS) in patients with early stage breast cancer (1). However, OS of patients with local advanced breast cancer has increased after NAC if they achieved complete pathological response (pCR) (2,3). It would be beneficial to clinical practice if an evaluation system for NAC therapeutic effect could be identified.

Compared with clinical examination, ultrasonography and mammography, dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) is considered the most accurate method for evaluating the extent of residual breast tumor after NAC (4,5). Moreover, the tumor biomarkers Ki-67 (a tumor proliferation marker) and P-glycoprotein (P-gp) are also widely recognized as important histopathologic NAC evaluation tools (6,7).

In order to improve prognostic predictions of patients with breast cancer, we evaluated MRI and tumor biomarkers as indicators of therapeutic response to NAC. We also investigated the correlation of the two methods.

Materials and methods

Patients

Between December 2009 and January 2013, 114 breast cancer patients treated at Huashan Hospital (Fudan University) were enrolled in this study. We used the AJCC (American Joint Committee on Cancer) Breast Cancer Staging System (7th edition, 2009) (8), to classify tumors by TNM staging. All patients were diagnosed with breast cancer based on the tumor tissues obtained by core needle biopsy before NAC. Patient demographics, clinical and imagining examinations, and tumor characteristics were collected.

The inclusion criteria of this study were as follows:

- (I) Operable female patients;
- (II) AJCC stage II (exclude T0N1M0 and T1N1M0) and AJCC stage III;
- (III) No adjuvant treatments (chemotherapy, endocrine therapy, radiology therapy, targeting therapy) before NAC.

The exclusion criteria of this study were as follows:

- (I) Pregnancy;
- (II) Patients who had a previous history of breast cancer;
- (III) AJCC stage IV patients.

Chemotherapy

All patients receiving NAC were given four courses of CET regimen chemotherapy at three week intervals [cyclophosphamide (500 mg/m²), epirubicin (75 mg/m²) and paclitaxel (175 mg/m²)]. All were given by intravenous bolus injection (9).

Pathological examination

The breast cancer tissue was collected by core needle biopsy (Mammotome System, 8 Gauge, Ethicon Inc., USA) before NAC treatment. After four courses of NAC, the surgically removed breast tissue was fixed in neutral buffered formalin and then embedded in paraffin. The specimens were cut into thin sections and stained with hematoxylin and eosin. Determination of P-gp and Ki-67 expression was made immunohistochemically. Standard immunohistochemistry (IHC) techniques were used. P-gp grading was as follows: 0, negative for cell membrane or fewer than 10% of cancer cells positive; 1, slightly positive in more than 10% of cancer cells; 2, moderately positive in more than 10% of cancer cells; 3, markedly positive in more than 10% of cancer cells. Ki-67 positive staining was defined as Ki-67 staining of 14% or more of cancer cell nuclei; Ki-67 negative staining was defined as Ki-67 staining of fewer than 14% of cancer cell nuclei (10).

Histopathologic criteria for assessment of NAC therapeutic response

The histopathologic therapeutic effects were determined based on the histopathologic criteria for assessment of therapeutic response in breast cancer described in the 2007 version of the Japanese Breast Cancer Society (JBCS) (11). In this study, we grouped the histopathologic therapeutic effects into three categories: (I) no pathological response (pNR); (II) partial pathological response (pPR, grade 1 and grade 2); and (III) pCR (grade 3).

MRI acquisition and assessment

To be eligible for the study, all patients were required to receive MRI scans before and after NAC treatment. Two experienced radiologists calculated the MR tumor size measurements using Functool SER software with a AW43 imaging processing workstation (GE Inc., USA). The radiologists were blinded to patient information, including

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Table 1 Patient cohort							
	Pre-NAC patients			Consorrad	Post NAC patients		
	Pre-NAC	pCR	mCR	Censoled	POSI-INAO pallents		
Total patients	114	28		0	114		
P-gp	89	20		0	69		
Ki-67	88	19		3	66		
MRI	55		6	0	49		
NAC, neoadjuvant chemotherapy; pCR, pathological complete response; P-gp, P-glycoprotein; mCR, complete response as							

determined by MRI; MRI, magnetic resonance imaging.

breast cancer subtype, NAC regimen and final pathological outcome.

Results

Patient cobort

In accordance with the response evaluation criteria in solid tumors (RECIST) (12), MRI tumor size was determined by the longest tumor diameter. For multiple tumors, the maximal tumor diameters of the five largest lesions were measured. NAC treatment responses were based on DCE-MRI examinations. Responses were classified as follows: MRI complete response (mCR: no residual cancer); MRI partial response (mPR: \geq 30% size reduction); MRI no response (mNR: <30% size reduction, including stable disease (SD); and progressive disease (PD).

Time-signal intensity curves (TIC, a measure of peak intensity from the time of contrast material injection) in DCE-MRI were designed to differentiate benign from malignant enhancing lesions based on region of interest (ROI) analysis. TICs were classified according to the shapes as type I, steady enhancement with a straight or curved time-signal intensity line; type II, plateau of signal intensity; or type III, washout of signal intensity.

Statistical analysis

Correlations between Ki-67, P-gp and pCR were derived using chi-square tests. The Kruskal-Wallis test was used to analyze the correlation between the histopathologic therapeutic responses (pCR, pPR and pNR), and Ki-67/P-gp expression. The NAC therapeutic effect was evaluated using Spearman's rank correlation coefficient. The consistency between MRI responses and histopathologic therapeutic responses was tested using the Kappa test. Data was analyzed by SAS (Version 8.01; Statistical Analysis System Inc., North Carolina, USA). A Pvalue of less than 0.05 was considered statistically significant. A total of 114 NAC-treated patients were included in the study. Twenty-eight patients (24.6%) reached a pCR after NAC treatment. At the start of the study, Ki-67 and P-gp were not used as routine test biomarkers; thus, 88 and 89 patients were analyzed for Ki-67 and P-gp expression, respectively, before NAC treatment. There were 19 pCR patients who tested positively for Ki-67 and 20 pCR patients who tested positively for P-gp. Three non-pCR patients were excluded due to the failure of the Ki-67 test. Thus, Ki-67 and P-gp tests were run on 66 and 69 non-pCR patients, respectively, following NAC treatment. Fifty five patients underwent complete MRI therapy before NAC treatment. Among them, six patients achieved a mCR. Thus, the MRI therapeutic response was evaluated in 49 patients after NAC treatment (*Table 1*).

Patient characteristics

The clinical-pathologic characteristics of the 114 patients are shown in *Table 2*. The patients' ages ranged from 29 to 74 years (mean 51.9 years). In terms of menopausal status, 68 patients (59.7%) were postmenopausal, and 46 patients (40.4%) were premenopausal. With regard to the pathologic category, invasive ductal carcinoma (76.3%) was the most commonly observed. With a cut-off for Ki-67 positivity of \geq 14% positively stained tumor cells, most of the tumors (65.9%) were classified as having a high level of Ki-67. Fifty-two patients (58.4%) were P-gp positive before NAC. Twenty-eight patients (24.5%) achieved a pCR; 66 patients (57.9%) achieved a pPR, and 20 patients (17.5%) achieved a pPR.

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Table 2 Patients' characteristics and clinical characteristics							
Factor	pCR (%)	pPR (%)	pNR (%)	Sum (%)	P value		
	28 (24.56)	66 (57.89)	20 (17.54)	114			
Age					0.87		
<40 y	2 (25.00)	6 (75.00)	0 (0.00)	8 (7.02)			
40-49 y	7 (21.21)	20 (60.61)	6 (18.18)	33 (28.95)			
50-59 у	15 (26.79)	30 (53.57)	11 (19.64)	56 (49.12)			
60-69 y	4 (23.53)	10 (58.82)	3 (17.65)	17 (14.91)			
Menopausal status					0.17		
Postmenopausal	15 (22.06)	38 (55.88)	15 (22.06)	68 (59.65)			
Premenopausal	13 (28.26)	28 (60.87)	5 (10.87)	46 (40.35)			
Pathological category					0.0035		
Invasive ductal carcinoma	17 (19.54)	54 (62.07)	16 (18.39)	87 (76.32)			
Invasive lobular carcinoma	1 (16.67)	3 (50.00)	2 (33.33)	6 (5.26)			
Medullary carcinoma	2 (50.00)	2 (50.00)	0 (0.00)	4 (3.51)			
Mucinous adenocarcinoma	0 (0.00)	0 (0.00)	2 (100.00)	2 (1.75)			
DCIS with invasive tumor	4 (40.00)	6 (60.00)	0 (0.00)	10 (8.77)			
Others	4 (80.00)	1 (20.00)	0 (0.00)	5 (4.39)			
Tumor biomarker (before NAC)							
Ki67 ≥14%	10 (17.24)	37 (63.79)	11 (18.97)	58 (65.91)	0.196		
Ki67 <14%	9 (30.00)	16 (53.33)	5 (16.67)	30 (34.09)			
P-gp +	4 (7.69)	34 (65.38)	14 (26.92)	52 (58.43)	0.024		
P-gp –	16 (43.24)	19 (51.35)	2 (5.41)	37 (41.57)			
NAC response							
pCR				28 (24.5)			
pPR				66 (57.89)			
pNR				20 (17.54)			
DCIS, ductal carcinoma in situ; pCR, pathological complete response; pPR, partial pathological response; pNR, no pathological							

response; NAC, neoadjuvant chemotherapy; P-gp, P-glycoprotein.

Correlations between patient characteristics and NAC histopathologic therapeutic effects

Table 2 shows the results of the univariate analyses for the factors associated with NAC histopathologic therapeutic effects. The association between NAC response versus age (P=0.87) and menopausal status (P=0.17) were not statistically significant. Compared with postmenopausal patients, premenopausal patients achieved slightly higher pCR and pPR rates (pCR: 28.3% vs. 22.1%; pPR: 60.9% vs. 55.9%), but a lower pNR rate (10.9% vs. 22.1%).

The predominant tumor type was invasive ductal carcinoma (76.3%). Patients having medullary carcinoma, or ductal carcinoma in situ (DCIS) with invasive tumors, achieved promising response rates (50% pCR, 50% pPR,

and 40% pCR, 60% pPR, respectively). Two mucinous adenocarcinoma patients did not respond. These data indicated that pathologic type of tumor had a significant correlation with the NAC response (P=0.0035).

The pre-NAC Ki-67 expression was not a good predictor of NAC treatment response (P=0.196). P-gp negative patients achieved higher pCR rates than P-gp positive patients (43.2% *vs.* 7.7%, P=0.024).

Change in P-gp and Ki-67 status pre- and post-NAC

Figure 1A indicates that P-gp was up-regulated in 32 patients and down-regulated in 10 patients after NAC treatment. This change was statistically significant (P=0.001). Among the 16 pNR patients, P-gp was up-



Figure 1 P-gp change pre- and post-NAC and its predictive effect. Bubble plot for IHC score for P-gp before and after NAC in 69 patients with P-gp expression change. The figure added to the bubbles is the number of patients and each bubble's size is determined by the number of patients in the category: (A) the more the patients, the larger the bubble; (B) bar figure displayed the NAC therapeutic effect with P-gp change; white, P-gp down-regulated; light grey, P-gp unchanged; dark grey, P-gp up-regulated. NAC, neoadjuvant chemotherapy; IHC, immunohistochemistry; P-gp, P-glycoprotein; pPR, partial pathological response; pNR, no pathological response.



Figure 2 Ki-67 change pre- and post-NAC and its predictive effect. Bubble plot for IHC score for Ki-67 before and after NAC in 66 patients with Ki-67 expression change. The figures added to the bubbles are the number of patients and each bubble's size is determined by the number of patients in the category: (A) the more the patients, the larger the bubble; (B) bar figure displayed the NAC therapeutic effect with Ki-67 change; white, Ki-67 ≥14% both pre-NAC and post-NAC; light grey, Ki-67 ≥14% pre-NAC while <14% post-NAC; medium grey, Ki-67 <14% pre-NAC while ≥14% post-NAC; dark grey, Ki-67<14% both pre-NAC and post-NAC. NAC, neoadjuvant chemotherapy; IHC, immunohistochemistry; pPR, partial pathological response; pNR, no pathological response.

regulated in 13 patients, down-regulated in 1 patient and remained unchanged in 2 patients. Of the 53 pPR patients, P-gp was upregulated in 19, down-regulated in 9 and unchanged in 25. The up-regulation of P-gp was associated with a negative NAC therapeutic effect (P=0.024) (*Figure 1B*). *Figure 2A* indicates that Ki-67 was down-regulated in 31 patients, and up-regulated in 2 patients after NAC treatment. The down-regulation of Ki-67 after NAC treatment was statistically significant (P<0.001), but it had no correlation with NAC therapeutic effect (P=0.9645) (*Figure 2B*).

Table 3 Pre-NAC TIC type and NAC therapeutic effect										
	MRI therapeutic effect					Histopathologic therapeutic effect				
	mCR	mPR	mNR	Sum	P value	PCR	pPR	pNR	Sum	P value
I	2	7	3	12		4	6	2	12	
II	2	13	6	21		2	16	3	21	
III	2	17	3	22		8	11	3	22	
Total	6	37	12	55	0.637	14	33	8	55	0.503

NAC, neoadjuvant chemotherapy; TIC, Time-signal intensity curves; MRI, magnetic resonance imaging; mCR, complete response as determined by MRI; mPR, MRI partial response; mNR, MRI no response; pCR, pathological complete response; pPR, partial pathological response; pNR, no pathological response.



Figure 3 Bubble plot for MRI TIC type conversion before and after NAC in 49 patients with TIC change. The figures added to the bubbles are the number of patients and each bubble's size is determined by the number of patients in the category: the more the patients, the larger the bubble. NAC, neoadjuvant chemotherapy; TIC, Time-signal intensity curves; MRI, magnetic resonance imaging.

Table 4 MRI therapeutic effect compared with histopathologic							
therapeutic effect							
	pCR	pPR	pNR	Sum	P value		
mCR	4	1	1	6			
mPR	9	23	5	37			
mNR	1	9	2	12			
Total	14	33	8	55	0.056		
pCP, pathological complete response; pPP, partial pathological							

response; pNR, no pathological response; pPR, partial pathological response; pNR, no pathological response; mCR, complete response as determined by MRI; mPR, MRI partial response; mNR, MRI no response; MRI, magnetic resonance imaging.

Correlations between MRI TIC type and NAC MRI/ histopathologic therapeutic effect

Table 3 indicates that the pre-NAC TIC categories were not predictive for NAC therapeutic effects, by either MRI analysis (P=0.637) or by histopathologic analysis (P=0.503). *Figure 3* shows the change of TIC categories pre-and-post NAC treatment: 21 patients (42.9%) remained unchanged; 19 patients (38.8%) moved into a higher TIC category; and 9 (18.4%) patients moved into a lower TIC category. However, the change of TIC category pre-and-post NAC treatment was not statistically significant (P=0.0872). It had no significant correlation with therapeutic effect, by either MRI analysis (P=0.948) or by histopathologic analysis (P=0.792).

The equivalence of therapeutic effects as analyzed by MRI or histopathology

Table 4 indicates overlap of 4 mCR with 14 pCR patients (28.6%), and 2 mNR with 8 pNR patients (25.0%). Thus, MRI and the pathology response grades did not match well. In patients achieving a partial response, there was a better overlap of 23 mPR with 33 pNR patients (69.7%). However, although the data approached significance, it failed to reach statistical significance (P=0.056).

In *Figure 4*, we classified NAC treatment responses as pCR and non-pCR. Within the pCR group, we found 4 mCR in 14 pCR patients. This specificity was 28.6%. Within the group of 41 non-pCR patients, there were only 2 mCR patients. This sensitivity was 95.1%. Overall, the positive predictive value was 79.6% and the negative predictive value was 66.7%. However, the correlation between mCR and pCR was not statistically significant (P=0.29).

Non-pCR		pCR	
True positive	39	False negative	2
False positive	10	True negative	4
Sensitivity		95.12%	
Specificity		28.57%	
Positive predictive	e value	79.59%	
Negative predictiv	e value	66.67%	
P value		P=0.29	

Figure 4 The consistency between pCR and mCR. pCR, pathological complete response; mCR, complete response as determined by MRI; MRI, magnetic resonance imaging.



Figure 5 Correlation of pathology as measured by longest diameter of residual tumor (mm) (y-axis) and preoperative MRI as measured by MRI longest diameter (mm) (x-axis). MRI, magnetic resonance imaging.

The average extent of the longest diameter in residual tumor by MRI analysis was 20.83 ± 4.14 mm, and that of the surgically removed specimen, 18.89 ± 3.71 mm. In 18 patients, the residual tumor extent was smaller by MRI analysis than the actual surgically excised specimen (max: -31.0 mm; mean: -10.67 mm). However, in 22 patients, the residual tumor extent by MRI analysis was larger than the actual surgically excised specimen (max: +43.0 mm; mean: +12.77 mm) (*Figure 5*).

Discussion

Earlier studies of the prognostic assessment of breast cancer

following NAC have focused on one of two aspects: (I) the biological and pathological analysis of surgical specimens pre/post treatment of NAC (13) or; (II) magnetic resonance imaging (MRI) to evaluate residual tumor extent after NAC (14). Traditional prognostic tumor biomarkers for breast cancer have been well studied. Imaging assessment of NAC treatment response, such as MRI, can provide valuable information about the residual tumor extent and enable better surgical plans to achieve a tumor-free margin. Complete response determined by MRI overlaps with complete response determined by pathological methods, for evaluating NAC response and predicting prognosis. In this current study, we combined the predictive use of pathological biomarkers with imaging findings, to evaluate their consistency and to identify discrepancies. The factors that influence accuracy were elucidated, to better guide clinical practice in the treatment of breast cancer.

Our study investigated DCE-MRI and the expression of tumor biomarkers, in an effort to explore their combined assessment value in NAC-treated breast cancer patients. IHC results indicated that lower pre-NAC P-gp expression was associated with better pCR rates. This suggests that P-gp is a good predictive tool for the efficacy of NAC. In addition, P-gp was significantly up-regulated after NAC treatment, the tendency of which was even more obvious in pNR patients. In other studies, it has been shown that P-gp expression is induced by NAC and its expression confers some degree of drug resistance (15). In this current study, Ki-67 expression was lower after NAC treatment, suggesting a decrease of tumor proliferation; however, its prognostic value for pCR is limited. The accuracy of MRI for evaluating NAC outcome and its consistency with pathological findings are inconclusive. Here, complete response as determined by MRI (mCR) was compared to complete response as determined by IHC (pCR). MRI analysis for pCR prediction was observed with high sensitivity, but with low specificity.

In recent years, proliferative tumor markers, including Ki-67, have been widely investigated as predictive and prognostic factors following NAC treatment. It has been shown that, in the neoadjuvant setting, a high level of Ki-67 expression predicts response to chemotherapy regimens (16), which may explain the high sensitivity of proliferating tumor cells to NAC compounds. Many studies have studied the correlation of Ki-67 to overall response rates (17-19), but with varying and conflicting results. de Azambuja *et al.* (20) analyzed the data from 29 studies, using patients with node-negative and node-positive breast cancer, and

demonstrated that Ki-67 expression is associated with poor OS and disease-free survival. While other studies found that patients with high Ki-67 expression were more likely to achieve pCR (21), or Ki-67 shows no significant trend in relation to OS (22), Klintman *et al.* (23) have concluded that the value of Ki-67, as a prognostic tool, is restricted to ER-positive patients and to those with histological grade 2 tumors. There are some plausible reasons for these discordant results, including varying chemotherapy protocols, heterogeneous patient subtypes, and different Ki-67 standards and scoring systems.

Our study investigated the value of Ki-67 as a predictive factor and found that patients with low Ki-67 status pre-NAC were somewhat more likely to achieve pCR, although these results were not statistically significant. Interestingly, in this current study, most patients demonstrated a decrease of Ki-67 from high pre-treatment levels to low post-treatment levels. Our findings are consistent with previous studies that show Ki-67 low level expression (<3%) in healthy breast tissue (23), and a progressive increase of Ki-67 expression from benign breast disease, to DCIS, to invasive breast cancer (24). Our finding of Ki-67 reduction change suggests that NAC treatment induces a gradual downgrading from invasive carcinoma to DCIS, and may even promote eventual tumor disappearance.

The drug resistance of breast cancer is widely observed during chemotherapy, and various mechanisms have been characterized. Several xenobiotic transporter-related genes have been most frequently implicated as modifiers of NAC in breast cancer (25), and P-gp (encoded by MDR-1) is one of the key factors. P-gp is one member of the transmembrane efflux pump group, which is normally involved in the excretion of toxins from cells. P-gp has a broad range of substrates and exports a number of chemotherapeutics, inducing drug resistance (26). Some studies have investigated the relationship between P-gp expression levels and its prognostic value in breast cancer. Although a number of different conclusions have been drawn, the broad consensus is that P-gp expression can be induced by NAC (27,28). In our study, we found that 46.4% of patients showed P-gp up-regulation following chemotherapy for disease progression, a finding consistent with other studies (28,29). The reasons for this P-gp up-regulation include at least two possibilities. The first is our chemotherapy regimen of anthracycline and taxanes, which are both known substrates for P-gp (26). Here, P-gp negative cells are more sensitive and are killed by NAC

treatment, while the primary high P-gp expressing tumor cells survive. The second possibility is acquisition of drug resistance after exposure to NAC treatment. Seven P-gp negative patients became P-gp positive, an indication of drug resistance. Regardless of whether P-gp expression is primary or acquired, high P-gp expression in either the pre- or post-treatment setting predicts poor patient outcome.

Some studies have reported that the decrease of tumor volume and enhancement on DCE-MRI is correlated with histopathologic response (30). Results from the I-SPY 1 trial (31) revealed that MRI findings of tumor reduction are better indicators of pathology than clinical examinations. In contrast, one study by Ko *et al.* (14) reported that ER positive breast cancer of a low nuclear grade might be associated with a poor response following NAC, and MRI might be less accurate in predicting the residual lesion extent of this kind of tumor.

In our study, the residual tumor extent on MRI and the surgically removed specimen size were analyzed. Based on the longest diameter of residue tumor, Pearson's correlation coefficient between the two methods was 0.513 (P=0.0003), indicating a moderate correlation between the two measurements. However, the measurements by MRI included ten false-positive diagnoses. There are several possible reasons for this overestimation. (I) The residual tumor extent on MRI is in vivo. The tumor may shrink when fixed in formalin after surgical removal; (II) DCIS is often manifested as enhanced lesions on MRI images, which are similar to invasive tumors and difficult to differentiate from invasive tumors. Small residual lesions after NAC may often be misinterpreted as pCR, and residual DCIS tissue may lead to a false-positive diagnosis. Two of the 10 false positive lesions in our study were revealed to be DCIS, and were included in the pCR category. Although the impact of a DCIS diagnosis might be negligible in terms of prognosis, it may be an important risk factor for local recurrence after breast conserving surgery (32). Therefore, with DCIS tumors, overestimation on MRI is inevitable; (III) In another study, Ko et al. reported that a taxaneinduced chemotherapy regimen may increase the vascular permeability, and capillary protein leakage could lead to augmented gadolinium uptake by the tumor, producing an over-or underestimation (14).

In conclusion, by studying the change of P-gp and Ki-67 expression from pre- to post-NAC, our investigation demonstrated that NAC treatment may arrest tumor proliferation, but may also potentially increase drug

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resistance. The pre-NAC P-gp status provided independent prognostic information for NAC treatment, while the pre-NAC Ki-67 status had little prognostic value. With rapidly evolving MRI technology, imaging assessment has been more widely applied to the evaluation of therapeutic effectiveness; it provides valuable information about the residual tumor. However, since the accuracy of MRI might be affected by many factors-the NAC-induced reactive change in tumor, the molecular character of the tumor, and the influence of chemotherapeutic agents-the discrepancies between imaging results and pathologic findings remain to be settled. In this retrospective study, we did not compare our chemotherapy regimen with more recently developed NAC chemotherapeutics. Therefore, our findings may not be applicable to newer therapeutics and will require further investigation.

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