

Early prediction of response to neoadjuvant chemotherapy using contrast-enhanced ultrasound in breast cancer

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Abstract

Early prediction of non-response is essential in order to avoid inefficient treatments. The objective of this study was to determine the contrast-enhanced ultrasound (CEUS) for early predicting pathologic complete response (pCR) to neoadjuvant chemotherapy (NAC) in breast cancer patients.

Between March 2018 and October 2019, 93 consecutive patients with histologically proven breast cancer scheduled for NAC were enrolled. Conventional ultrasound and CEUS imaging were performed before NAC and after two cycles of NAC. CEUS parameters were compared with pathologic response. Multiple logistic regression analyses were utilized to explore CEUS parameters to predict pCR, and receiver operating characteristic analysis was used to evaluate the predictive ability.

Therapeutic response was obtained from 25 (27%) patients with pCR and 68 (73%) with non-pCR. Compared to non-pCR, pCR cases have a significantly higher proportion of homogeneous enhancement feature (56% vs 14%, P<.001) and centripetal enhancement (52% vs 23%, P=.012). A significant decrease in peak intensity (PI) was observed after two cycles of NAC. Compared with non-pCR patients, the kinetic parameters PI change (PI%) was higher in pCR patients (P<.001). Multiple logistic regression demonstrated two independent predictors of pCR: internal homogeneity (odds ratio, 4.85; 95% confidence interval: 1.20–19.65; P=.027) and PI% (odds ratio, 1.08; 95% confidence interval: 1.02–1.15; P=.007). In receiver operating characteristic curve analysis, internal homogeneity and PI%, with area under curve of 0.71 and 0.84, predicted pCR with sensitivity (56%, 95%) and specificity (85%, 70%), respectively.

Internal homogeneity and PI% of CEUS may be useful in the noninvasive early prediction of pCR in patients with breast cancer.

Abbreviations: AS = ascending slope, AUC = area under curve, CEUS = contrast-enhanced ultrasound, CI = confidence interval, NAC = neoadjuvant chemotherapy, pCR = pathologic complete response, PI = peak intensity, ROC = receiver operating characteristic, TTP = time to peak, WIT = wash-in time.

Keywords: breast cancer, contrast-enhanced ultrasound, neoadjuvant chemotherapy, predictor

1. Introduction

Neoadjuvant chemotherapy (NAC) before surgery is widely used in patients with locally advanced breast cancer. A pathological complete response (pCR) after NAC is assumed to be associated

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with better outcomes, including increased overall survival and free disease survival rates.^[1,2] Recently, pCR rates have increased because of the wide availability of more effective chemotherapy agents and targeted drugs. Accurate identification of tumor response to NAC would be of significant clinical relevance for a refined therapy decision, individually tailored surgical approaches, as well as for improving patient's prognosis.^[3–5] After the final NAC, non-responders inevitably suffered from the side-effects of large doses of chemotherapeutic agents. Therefore, accumulating studies have sought to identify early predictive factors of pCR in breast cancer, including clinic-pathological factors and imaging parameters.

Contrast-enhanced ultrasound (CEUS) has gained extensive interest in recent years for its ability to collect macro- and microvascular information in various tumors.^[6–9] Imaging of blood flow serves as a crucial alternative for monitoring the treatment effects of chemotherapeutic drugs. Early alteration in neovascular net of breast tumors during NAC may reflect in altered pharmacokinetics of contrast agents detected by CEUS, making it possible to assess early response to therapeutic drugs. To our knowledge, few studies have determined the performance of CEUS in predicting pathologic outcomes in patients with breast cancer, and most of those studies used quantitative parameters only, including a relatively small study population.^[10–13]

The purpose of our study was to investigate whether CEUS qualitative and quantitative parameters acquired baseline and

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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following two cycles of NAC can be utilized to predict pathologic response in breast cancer patients.

2. Methods

2.1. Patient population

The clinical and imaging records of 93 breast cancer patients with the approval of the ethics committee of the Renmin hospital of Wuhan University, China and informed consent provided were retrospectively reviewed. The 93 consecutive patients were enrolled, who proven breast cancer according to positive results on core needle biopsy and scheduled for NAC from March 2018 to October 2019. Exclusion criteria were contraindications to the contrast agent, such as a history of cardiac failure, respiratory disorder, or hypersensitivity.

2.2. NAC regimen

All patients received 6 or 8 cycles of NAC prior to breast surgery, and each chemotherapy cycle was 21 days. The NAC regimens were either taxane-based, anthracycline-based or anthracycline and taxane-based. The course of treatment includes 6 or 8 chemotherapy cycles. The chemotherapy regimen comprised 3 or 4 cycles of a taxane-based antitumor agent followed by 3 or 4 cycles of FEC (fluorouracil, 500 mg/m²; epirubicin, 75–100 mg/m²; cyclophosphamide, 500 mg/m²). Patients received PEC for 6 or 8 cycles (paclitaxel, 175 mg/m²; epirubicin, 60 mg/m²; cyclophosphamide, 60 mg/m²). Additionally, human epidermal growth factor receptor 2-positive (HER2+) patients were treated with trastuzumab.

2.3. Conventional ultrasound and CEUS

All patients underwent conventional ultrasound and CEUS within a week prior to the initiation of NAC (baseline) and after two cycles of NAC. The flow chart of NAC is shown (Fig. 1). The examinations were performed by two experienced breast radiologists. Conventional ultrasound images were initially acquired using Arietta 70 ultrasonic device (Hitachi-Aloka Medical, Tokyo, Japan) with a 5–13 MHz transducer. CEUS was performed subsequently using the same transducer at a low mechanical index (MI < 0.10). The contrast agent, SonoVue (Bracco, Milan, Italy) was intravenously administered at a dose of 4.8 mL as bolus and was subsequently flushed with 10 mL of saline. The examination was documented in a 3-minutes long clip, starting at the beginning of the bolus injection.

Classification of the enhancement patterns was defined based on the previous literature and our clinical experience.^[14] Enhancement degree was assessed compared to surrounding normal breast tissue at the peak time and was classified as hypo-, iso-, or hyper-enhancement. The enhancement order was also assessed and was classified as centripetal, centrifugal or diffused enhancement. Centripetal enhancement, intensity of enhancement was more apparent in the center than the periphery and center of lesion site was enhanced. Centrifugal enhancement, the periphery of the lesion site was enhanced, however, the center of the lesion was not marked enhancement. Diffused enhancement was the proper classification if the lesion was diffusely enhanced without obvious order. Enhancement margin was either distinct (>50% of the lesion circumference was clearly visible) or indistinct (>50% of the lesion was spiculated or angular). Internal homogeneity was classified as homogeneous or heterogeneous enhancement. Homogeneous enhancement, all areas in the lesion site were homogeneously and enhanced with almost the same enhancement intensity. Heterogeneous enhancement, the enhancement areas were distributed unevenly in the lesion with different intensities. The presence or absence of perfusion defect and radial or penetrating vessels were recorded.

Quantitative parameters of the time intensity curve were obtained with Arietta 70 ultrasonic device software (Hitachi-Aloka Medical) (Fig. 2). A region of interest (ROI) was manually drawn in the area of the strongest enhancement. Quantitative parameters were defined using previously described criteria as follows:^[15] Wash-in time (WIT) was the time when the first microbubble was seen entering the lesion. Peak intensity (PI) was the maximum intensity of the time-intensity curve. PI in breast cancer lesions on CEUS images was calculated as maximum intensity minus baseline intensity. Time to peak (TTP) was the time needed to reach peak intensity beginning from the moment the first microbubble reached the lesion. Ascending slope (AS) was the maximum wash-in velocity of the contrast medium, which was calculated as PI divided by TTP.

2.4. Pathologic assessment

The postoperative specimens were examined by dedicated pathologists in breast cancer, and a binary outcome was assessed: pCR versus non-pCR. The pCR was defined as the absence of any invasive component, or residual ductal carcinoma in situ (DCIS) component in the specimen obtained from surgery.^[16,17] A non-pCR was the presence of microscopic invasive tumor in the final pathology,^[16] lymph node status was not taken into account. After the course of NAC, all patients underwent either



Figure 1. Flow chart of NAC. Each cycle lasted for 21 days and surgical excision was performed within 10 days after 6 or 8 cycles. The parameters of CEUS were recorded. CEUS = contrast-enhanced ultrasound, NAC = neoadjuvant chemotherapy.



Figure 2. Graph of the TIC generated after placement of region of interest in the area of the most rapid and strongest enhancement within the breast lesion. TIC= time intensity curve.

mastectomy (73 patients) or breast-conserving therapy (20 patients) and pathological postoperative evaluation was performed at our institution in all cases. Tumors were classified into three subgroups according to their receptor markers: HR +/HER2- (Luminal), HR-/HER2+ (HER2 positive) and HR-/ HER2- (Triple-receptor negative).^[18] Hormone receptor (HR) status was defined using immunohistochemistry according to Harvey et al and Leake et al.^[19–20] HER2 status was determined according to the American Society of Clinical Oncology/College of American Pathologists guideline recommendations using immunohistochemistry and fluorescence in situ hybridization (FISH).^[21]

2.5. Statistical analysis

Categorical variables were compared using the Chi-square test or Fisher exact test between patients with pCR and non-pCR. The continuous variables were compared using unpaired t-test between the two groups. Changes in the quantitative parameters before and after 2 cycles of NAC were calculated using the following formula: ([value before NAC - value after 2 cycles of NAC]/value before NAC) \times 100%. The continuous variables were compared using Wilcoxon rank sum test between two time points. Multiple logistic regression analyses were performed to test the independent factors for prediction of pCR. Models were adjusted for age, and we excluded variables from univariable analysis if their between-group differences were not significant. The receiver operator characteristic (ROC) curve analysis was performed to test the ability of each parameter to predict pCR. Statistical analysis was performed IBM SPSS Statistics, version 21.0 (IBM, Armonk, NY). A P value < .05 was considered statistically significant.

Table 1

Summary of	clinical and	pathological	characteristics	of patients
with breast c	ancer.			

	pCR	non-pCR	
Characteristic	(n=25) (%)	(n=68) (%)	P value
Age(yr)			.639
Mean \pm SD	47.7 ± 10.2	48.8±10.2	
Menopause status			.456
Premenopausal	9 (10)	19 (20)	
Postmenopausal	16 (17)	49 (53)	
Tumor diameter (cm)			.122
<2	2 (2)	9 (10)	
2–5	23 (24)	50 (54)	
>5	0 (0)	9 (10)	
Tumor histology			1.000
IDC	23 (24)	61 (66)	
ILC	2 (2)	7 (8)	
Clinical stage			.417
	8 (9)	15 (16)	
III	17 (18)	53 (57)	
Subtype			.337
Luminal	4 (4)	20 (22)	
HER2 positive	12 (13)	23 (24)	
Triple-receptor negative	9 (10)	25 (27)	
Ki67 labeling index			.338
≤20%	2 (2)	12 (13)	
>20%	23 (24)	56 (61)	
Lymph node status			.004
Positive	16 (17)	20 (22)	
Negative	9 (10)	48 (51)	

 $\label{eq:HER2} HER2 = human epidermal growth factor receptor 2, IDC = invasive ductal carcinoma, ILC = invasive lobular carcinoma, pCR = pathologic complete response, SD = standard deviation.$

Table 2

Distribution of	enhancement	pattern for	different	pathological	response.

Enhancement pattern	pCR (n=25) (%)	non-pCR (n=68) (%)	P value
Enhancement degree			.616
Hyper-enhancement	16 (17)	48 (51)	
lso- or hypo-enhancement	9 (10)	20 (22)	
Enhancement order			.012
Centripetal	13 (14)	16 (17)	
Centrifugal or diffuse	12 (13)	52 (56)	
Enhancement margin			.150
Distinct	13 (14)	23 (24)	
Indistinct	12 (13)	45 (49)	
Internal homogeneity			.000
Homogeneous	14 (15)	10 (11)	
Heterogeneous	11 (12)	58 (62)	
Perfusion defect			.292
Present	9 (10)	16 (17)	
Absent	16 (17)	52 (56)	
Radial or penetrating vessel			.610
Present	8 (9)	18 (20)	
Absent	17 (18)	50 (53)	

pCR = pathologic complete response.

3. Results

3.1. Clinical and pathological characteristics of breast cancer patients

The mean age of the 93 women with breast cancer was 48 years (range, 29–70 years). 84 (90%) patients were invasive ductal carcinomas and 9 (10%) were invasive lobular carcinomas. 36 (39%) patients had axillary lymph node involvement, 79 (85%) of whom had a Ki-67 labeling index of more than 20%. HER2 positive is the most common subtype and accounts for 37% of all breast cancer, it is followed by triple-receptor negative 34 (37%) and luminal subtype 24 (26%) in our study. The non-pCR cases have a much higher proportion of lymph node metastasis compared to pCR cases (71% vs 35%, P=.004). There were no differences in age, menopause status, tumor diameter, clinical tumor stage, immunohistochemical subtype and level of Ki-67 expression between pCR and non-pCR groups (P >.05). The patient's characteristics are summarized (Table 1).

3.2. CEUS before NAC

Of the 93 breast lesions, 64 (68%) demonstrated signs of hyperenhancement, and 29 (32%) showed signs of iso- or hypoenhancement. There were 29 (31%) lesions that showed centripetal enhancement and 64 (69%) that presented centrifugal or diffuse enhancement. Distinct tumor margin was observed in 36 (38%) lesions. Heterogeneous enhancement was seen in 69 (74%) lesions, and homogeneous enhancement was found in 24 (26%) lesions. A perfusion defect was observed in 25 (27%) lesions. Radial or penetrating vessels were found in 26 (29%) lesions. Compared to non-pCR cases, pCR cases have a significantly higher proportion of homogeneous enhancement feature (56% vs 14%, P<.001) and centripetal enhancement (52% vs 23%, P=.012). No differences were observed between pCR and non-pCR patients in terms of other CEUS features (all P>.05). Pre-NAC CEUS pattern findings are presented (Table 2).

3.3. CEUS quantitative parameters after two cycles of NAC

63 patients underwent CEUS after two cycles of NAC. A significant decrease in PI was present after two cycles of NAC. And a trend to decrease in tumor diameter and AS, and a trend to increase in TTP and WIT were observed, those changes are not statistically different (Table 3). Compared with non-pCR patients, the kinetic parameters PI % was higher in pCR patients. However, parameters of WIT, PI, TTP, AS, diameter, WIT%, TTP%, AS% and diameter% showed no differences between pCR and non-pCR patients (all P > .05). The change of quantitative parameters was shown in pCR and non-pCR patients, respectively (Figs. 3 and 4).

Table 3

Relationship between quantitative parameters and pathological complete response

	Pre-NAC			2 cy	cles NAC		% Change		
Parameter	pCR (n=25)	non-pCR (n=68)	P value	pCR (n=20)	non-pCR (n=43)	P value	pCR (n=20)	non-pCR (n=43)	P value
WIT (s)	12.3±4.9	10.8 ± 5.5	.070	12.7±2.9	11.0 ± 3.0	.055	36.2±25.1	31.2 ± 20.0	.535
PI	38.1 ± 6.9	40.0 ± 7.9	.207	32.8±3.4	34.3 ± 4.7	.031	22.1 ± 10.5	10.7±10.8	.000
TTP(s)	12.4±3.2	12.1±5.7	.397	13.0±3.4	14.0±3.8	.280	22.5±19.1	36.8±32.9	.058
AS	3.1 ± 0.7	3.6 ± 1.2	.151	2.6 ± 0.6	2.7 ± 0.8	.701	29.6±12.2	31.9±28.0	.701
Diameter (cm)	3.2 ± 1.0	3.8±1.8	.323	2.4±0.9	2.9±1.4	.095	26.3 ± 17.5	24.9±16.7	.751

AS = ascending slope, PI = peak intensity, TTP = time to peak, WIT = wash-in time.



Figure 3. A 35-year-old woman with breast cancer. (A) Before NAC, a CEUS image obtained 34s after contrast agent injection showed a homogeneous hyperenhancement lesion with a distinct margin. The largest diameter of the tumor was 3.5 cm. The TIC at CEUS showed strong and rapid enhancement. WIT=8.2, PI= 75.1, TTP=9.6, AS=7.8. (B) After 2 cycles NAC, CEUS showed a decrease in the tumor size to 3.2 cm, homogeneous hypo-enhancement with relative distinct margin. The TIC at CEUS showed weak and slow enhancement. WIT=16.6, PI=21.1, TTP=9.8, AS=2.2. (C-D) The pathological specimen after surgery with pCR, disappearance of neoplastic cells (magnification in left panel, × 100; the right panel showed the windows from the left panel at a magnification of × 400). AS= ascending slope, CEUS=contrast-enhanced ultrasound, NAC=neoadjuvant chemotherapy, pCR=pathologic complete response, PI=peak intensity, TIC=time intensity curve, TTP=time to peak, WIT=wash-in time.

3.4. Predictors of pCR and ROC analysis

The pCR was achieved in 25 (27%) patients, as determined by pathologic specimens. In univariable logistic regression analysis, internal homogeneity (P < .001), centripetal enhancement order (P=.012), PI (after two cycles of NAC, P=.031) and PI% (P < .001) were associated with pCR. After adjusting for age, the multivariable model showed that PI% (odds ratio, 1.08; 95% confidence interval [CI], 1.02–1.15; P=.007) and internal homogeneity (odds ratio, 4.85; 95% CI, 1.20–19.65; P=.027) were independent predictors of pCR (Table 4).

To evaluate the contribution of CEUS parameters to the prediction of pCR, the ROC curve analysis was performed. The area under curve of ROC for PI% after two cycles of NAC in the prediction of pCR was 0.84 (95% CI: 0.73–0.94), that yielded 95% sensitivity and 70% specificity. The area under curve for internal homogeneity was 0.71 (95% CI: 0.61–0.81) with 56% sensitivity and 85% specificity (Table 5 and Fig. 5).

4. Discussion

The accurate prediction of the response to NAC at an earlier stage has the potential to contribute to breast cancer patient prognosis. Among the independent factors extracted from CEUS examinations in the present study, an early PI change (PI%) and internal homogeneity were independent predictors for pCR. Regarding the feasibility of clinical practice in China, the experts strongly recommends that ultrasound should be utilized to assess the primary breast mass and regional lymph nodes every two cycles of NAC.^[22] Consequently, we selected the completion of two cycles of NAC as the timing of ultrasound imaging for predicting for pCR in this study.

PI reflects the quantity of contrast agent microbubbles in the vascular bed of the lesion, which is associated with the degree of vascularization. Our results are consistent with previous studies, better tumor response was associated with a larger decrement in PI in patients with breast cancer undergoing NAC.^[10] The time point for CEUS assessment in our study was much earlier than previous CEUS studies (after two cycle of NAC vs completion of NAC). Theoretically, our results are more helpful for NAC of breast cancer. The possible explanation of the PI% in pCR is that in the early stages of NAC demonstrated a reduction in blood flow, which in turn led to slower wash-in of contrast agent reflecting good efficacy of chemotherapeutic agents.^[23]

The multivariable logistic regression analysis also revealed that homogeneous enhancement pattern before NAC was a significant predictor for pCR. This result is consistent with previous dynamic contrast-enhanced magnetic resonance imaging (MRI) study. The study reported that patients in the pCR were more likely to present homogeneous enhancement than were those in the non-pCR group.^[24] However, caution is indicated when



Figure 4. A 56-year-old woman with breast cancer. (A)Before NAC, a CEUS image obtained 34s after contrast agent injection showing heterogeneous hyperenhancement with an indistinct margin. The largest diameter of the tumor was 4.8 cm. The TIC at CEUS showed strong and rapid enhancement. WIT = 13.0, PI = 44.5, TTP = 10.2, AS = 4.4. (B) After 2 cycles NAC, CEUS showed a decrease in the tumor size to 4.5 cm, heterogeneous hyper-enhancement with indistinct margin. The TIC at CEUS also showed strong and rapid enhancement. WIT = 11.2, PI = 42.5, TTP = 12.4, AS = 3.4. (C-D) The pathological specimen with non-pCR, revealed nest of tumor cells and surrounding dense fibrous tissue (magnification in left panel, \times 100; the right panel showed the windows from the left panel at a magnification of \times 400). AS = ascending slope, CEUS = contrast-enhanced ultrasound, NAC = neoadjuvant chemotherapy, pCR = pathologic complete response, PI = peak intensity, TIC = time intensity curve, TTP = time to peak, WIT = wash-in time.

comparing results from CEUS with those from MRI perfusion study, as the parameters are dependent on different pharmacokinetics of contrast agents and mathematical models. The possible explanation for a heterogeneous enhancement feature being more associated with non-pCR than homogeneous enhancement is that its pathologic features, including its higher histologic tumor grade or increased vascular endothelial growth factor expression, which is associated with a poor prognosis, may have been reflected in the enhancement feature.^[25,26] Previous research suggested chemotherapeutic drugs acted directly on cancer cells and microvasculature to affect a clinically relevant alteration in tumor size. Reduction in tumor size is a late demonstration of effective treatment and may take several weeks to occur.^[27] Consistent with the findings of previous studies, our data also showed at an earlier stage tumor diameter remains unchanged despite a positive functional response to treatment. We found no significant association between the other CEUS quantitative parameters excluding PI% and pCR in our study

Table 4

Logistic regression analysis for	independence forecast factors.
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Variable	Univariabl	e	Multivariab	ble
	OR (95%Cl)	P value	OR (95%CI)	P value
Enhancement order	3.52 (1.34–9.23)	.011		
Internal homogeneity	7.38 (2.61–20.81)	.001	4.85 (1.20-19.65)	.027
PI%	1.09 (1.03–1.15)	.002	1.08 (1.02–1.15)	.007

CI = confidence interval, OR = odds ratio, PI = peak intensity.

Table 5

ROC	curve	results	of	factors	for	CEUS	parameters.
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Predictive factor	Sensitivity (%)	Specificity (%)	AUC	95%CI
Internal homogeneity	56	85	0.71	0.61–0.81
PI%	95	70	0.84	0.73-0.94

AUC = area under the curve, CEUS = contrast-enhanced ultrasound, CI = confidence interval, PI = peak intensity, ROC = receiver operating characteristic.



Figure 5. The ROC curves of internal homogeneity and Pl%. The sensitivity of internal homogeneity was 56% and the specificity was 85%, with the area under the ROC curve (AUC) of 0.71 (95% CI, 0.61–0.81; P < .001). The sensitivity of Pl% was 95%, and the specificity was 70%, with AUC of 0.84 (95% CI, 0.73–0.94; P < .001). AUC=area under curve, CI=confidence interval, PI=peak intensity, ROC=receiver operating characteristic.

population. However, Wan et al reported that CEUS quantitative parameters (after completion of NAC) were risk factors for predicting pCR in breast cancer patients.^[10] The reason for this discrepancy is not completely clear, but it may be ascribed to differences in imaging time, or patient selection.

Our study had some limitations. First, our study included patients with various subtypes of breast cancer. There were some different chemotherapy regimens among the different subtype patients. Second, selection of the ROI and classification of the enhancement patterns were subjective. Third, the small sample size reduced the stability of the regression models, and the role of different CEUS parameters on the reflection of predictors could not be thoroughly investigated. Further extensive study is needed. Fourth, the PI% also suffers from overlapping error margins in the before and after NAC groups.

5. Conclusion

Internal homogeneity and PI% on CEUS may provide early identification of pathologic responsiveness to NAC, which may be used as biomarkers of tumor response in breast cancer patients who are undergoing NAC.

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