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Ultrasound features combined with tumor-infiltrating lymphocytes for prediction of pathological response to neoadjuvant chemotherapy in breast cancer

Yingying Jia[1-4], Xin Zhou[1,3,4], Yangyang Zhu[1,3,4], Xuewen Song[5], Ying Duan[1,3,4], Kundi Chen[1,3,4], Fang Nie[1,3,4]

[1]Ultrasound Medical Center, Lanzhou University Second Hospital, Lanzhou, [2]Department of Ultrasound, People's Hospital of Ningxia Hui Nationality Autonomous Region, Yinchuan, Ningxia, [3]Gansu Province Clinical Research Center for Ultrasonography, Lanzhou, [4]Gansu Province Medical Engineering Research Center for Intelligence Ultrasound, Lanzhou, [5]Pathology Department, Lanzhou University Second Hospital, Lanzhou, China

Abstract

Aim: Neoadjuvant chemotherapy (NAC) plays an important role in the treatment and prognosis of breast cancer. The early identification of patients who can truly benefit from preoperative NAC is crucial in clinical practice. The purpose of this study was to explore whether the ultrasound features and clinical characteristics combined with tumor-infiltrating lymphocyte (TIL) levels can improve the performance of predicting NAC efficacy in breast cancer patients. **Material and methods***:* In this retrospective study, 202 invasive breast cancer patients who underwent NAC followed by surgery were included. The baseline ultrasound features were reviewed by two radiologists. Miller-Payne Grading (MPG) was used to assess pathological response, and MPG 4-5 was defined as major histologic responders (MHR). Multivariable logistic regression analysis was used to evaluate independent predictors for MHR and build the prediction models. The receiver operating characteristic (ROC) curve was used to evaluate the performance of the models. **Results***:* Of the 202 patients, 104 patients achieved MHR and 98 patients achieved non-MHR. Multivariate logistic regression analysis showed the US size ($p=0.042$), molecular subtypes ($p=0.001$), TIL levels ($p<0.001$), shape ($p=0.030$), and posterior features ($p=0.018$) were independent predictors for MHR. The model combined the US features, clinical characteristics, and TIL levels had a better performance with an area under the curve (AUC) of 0.811, a sensitivity of 0.663, and a specificity of 0.847. **Conclusion***:* The model combined US features, clinical characteristics, and TIL levels had a better performance in predicting pathological response to NAC in breast cancer.

Keywords: breast neoplasms; tumor-infiltrating lymphocytes; neoadjuvant therapy; ultrasound

Introduction

Neoadjuvant chemotherapy (NAC) has become one of the most important therapeutic strategies for breast cancer patients with the advantage of reducing the tumor load, downgrading the clinical staging, improving the possibility of breast-conserving surgery, and decreasing the extent of axillary surgery performed [1]. It has been widely confirmed that patients who achieved pathological complete response (pCR) were associated with higher disease-free and overall survival rates [2,3]. However, not all patients benefit from NAC and this can lead to cytotoxicity and delays in surgery [4]. The early prediction of NAC efficacy especially before surgical treatment is crucial to the prognosis and survival of patients. Therefore, an early, non-invasive, and comprehensive evaluation method to predict NAC response earlier, especially before the initiation of treatment, is of great importance.

There was a continuous search for biological and medical imaging biomarkers to help in the prediction of

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Corresponding author: Fang Nie
Ultrasound Medical Center,
Lanzhou University Second Hospital,
Cuiyingmen No. 82, Chengguan District,
Lanzhou 730030, China
Phone: 13993163088 (China)
E-mail: Ery_nief@lzu.edu.cn

the therapeutic response and avoid unnecessary treatment [5-7]. Tumor-infiltrating lymphocytes (TILs) levels had been proven to be an efficient biomarker for predicting treatment response and prognosis of breast cancer [8]. Studies revealed that TIL levels were associated with response to neoadjuvant chemotherapy in all breast cancer subtypes and with longer survival time in patients with HER-2 enriched breast cancer and triple-negative breast cancer (TNBC) [9]. Also, it is known that higher TIL levels are associated with higher pCR rates, which is known to be a strong predictor of excellent long-term outcomes [10]. However, the value of TIL levels alone in predicting treatment response was limited [11]. Therefore, any additional information that could help improve the prediction accuracy would be of great help.

Ultrasound (US) had been commonly used in monitoring NAC treatment response, with advantages of easy access and low cost. Previous studies revealed that baseline US features were associated with pCR rates in breast cancer patients receiving NAC [12]. However, pre-treatment imaging features had been reported to be less accurate [13]. Imaging features combined with clinical information and pathological findings take advantage of gaining additional information from both imaging and pathology, which were proven to improve the accuracy [14,15]. Therefore, we hypothesized that baseline US features combined with TIL levels could better reflect NAC efficacy. In this study, we aimed to develop a predictive model combining US features, clinical characteristics, and TIL levels to improve the performance of predicting response to NAC in breast cancer patients.

Material and methods

Study population

The Ethics Committee of our hospital approved this retrospective study (approved number：2022A-256) and waived the necessity for formal consent.

A total of 426 patients with invasive breast cancer confirmed by needle biopsy pathology from January 2018 to September 2022 were enrolled. The inclusion criteria were as follows: patients (i) with invasive breast cancer confirmed by core biopsy pathology pretreatment, and the pathological specimens were enough for the assessment of TIL levels; (ii) have completed pretreatment US images to reflect the characteristics of the lesion; (iii) had a complete NAC cycle (all therapies consisted of anthracycline and taxane-containing chemotherapy agents), finished in 1 year; (v) received surgery after NAC, and had definite pathology results. The exclusion criteria were as follows: patients (i) received radiotherapy or endocrine therapy before US examination; (ii) a non-mass lesion. As shown in figure 1, 202 breast cancer patients with NAC treatment and surgical pathology were included in the final analysis. According to pathological results, 104 patients were in the major histologic responders (MHR) group and 98 were in the non-MHR group.

Conventional US

US was performed by either iU22 (Philips Healthcare, Seattle, WA, USA) equipped with a 5 to 12 MHz linear probe or the ACUSON Sequoia scanner (Siemens Medical Solutions, Michigan, USA) with a 4 to 10 MHz linear probe. The conventional US procedures were finished by one radiologist (YY.Z. who had five years of experience in breast US). Conventional US images of breast lesions were obtained according to a uniform standard. The images stored included at least a longitudinal section, transverse section, and malignant signs of lesions. The definition of malignant signs was according to the Breast Imaging Reporting and Data System (BI-RADS) lexicons. Lesions were measured with the maximal diameter on the longitudinal section.

Two radiologists (Y.D. and KD.C. with more than 5 years of experience in breast US) who were blinded to the patients' clinical information and pathological results, reviewed and analyzed the ultrasound features according to the ACR BI-RADS Atlas 5th edition ultrasound lexicon [16]. If the two radiologist had different views, a third radiologist (F.N. with >15 years of experience in breast US) was consulted to reach a consensus.

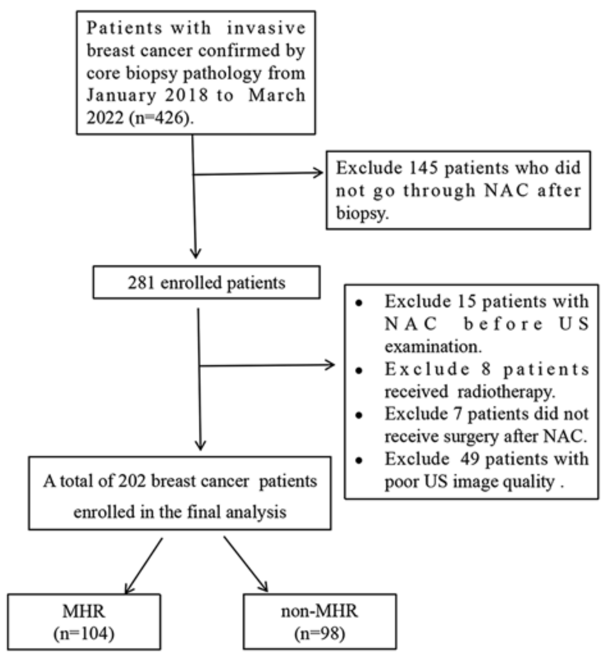

Fig 1. Flowchart shows the patients inclusion process used in this study.

US imaging features documented included shape (oval or round, irregular), orientation (parallel, not parallel), margin (indistinct, circumscribed), echo pattern (isoechoic, hypoechoic, hyperechoic or heterogeneous), posterior features (enhanced, no posterior features, attenuated or combined pattern), micro-calcification (present, absent).

Pathological analysis

Pretreatment core biopsies specimens were used to evaluate the pathological results and TIL levels. Pathological information such as histology, histological grade, and immunohistochemical (IHC) marker status was acquired from pathology reports. Breast cancer was divided into four molecular subtypes reported by the "St. Gallen International Expert Panel" consensus. These are the followings: Luminal A (ER+, PR ≥ 20%, HER2− and Ki-67 < 20%), Luminal B (ER+ and PR < 20% or HER2+ or Ki-67 ≥ 20%), HER-2 enriched (HER2+ and ER−, PR−), and triple-negative (PR− and ER−/HER2−) [17].

TILs include both stromal TILs (sTILs) and intratumoral TILs (iTILs) in tumor tissue; however, sTILs are more abundant than iTILs. Besides, it is hard to separate iTILs from apoptosis or cell necrosis in hematoxylin and eosin (HE) stained sections. Therefore, the preferred approach is to assess sTILs levels. The sTILs score of breast cancers was defined based on the proportion of the area infiltrated by lymphocytes within the tumor itself plus the adjacent stroma according to recommendations provided by the International Immuno-Oncology Biomarker Working Group in Breast Cancer [18]. The sTIL levels for this study were categorized as low ≤10% and high＞10%. All of the specimens were classified into high and low TILs groups by a 5 years experienced pathologist who was blinded to the patients' US features.

Definition of major histologic responders (MHR)

The response to NAC was determined by a pathologist with 10 years of experience in breast pathology using the Miller-Payne Grading (MPG) system 1 of 5 grades based on malignant cell changes between the surgical and biopsy specimen [19]. The grades were as follows: MPG 1 -no change or some alteration in individual malignant cells but no reduction in overall cellularity; MPG 2 - no loss of tumor cells but overall cellularity still high, up to 30% loss; MPG 3 - 30-90% loss of malignant cells; MPG 4 - more than 90% loss of malignant cells; MPG 5 -no malignant cells were found in the sections with or without ductal carcinoma in situ. Similar to previous studies [20], MPG 4–5 was classified as major histologic responders (MHR), and MPG 1-3 was classified as non-major histologic responders (non-MHR).

Statistical analysis

Statistical analysis was performed with SPSS 26.0 software (IBM Corp., Armonk, NY, USA). Continuous variables were described as means ± standard deviations (SDs), and comparisons between two groups were made using the Mann–Whitney U test or student's t-test. Categorical variables were expressed as numbers with percentages and were compared using the chi-square test or Fisher exact test. A two-tailed *P* value < 0.05 was accepted as statistically significant. A multivariable logistic regression analysis was used to select independent predictors. The area under the receiver operating characteristic curves (ROC-AUC) was used to evaluate discriminative ability. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated at the optimal cut-off points.

Results

Clinicopathological characteristics

Of the 202 patients with invasive breast cancer, 104/202 (51.5%) patients were MHR and 98/202 (48.5%) patients were non-MHR. The clinicopathological characteristics of the 202 patients were summarized in Table I. There were significant differences in TIL levels and molecular subtypes between the two groups (p<0.001). The MHR group had higher TIL levels than the non-MHR group (fig 2). HER-2 enriched and triple negative breast cancer had a higher rate in the MHR group than non-MHR. There was no significant difference in ki-67 (p=0.106), histological grade (p=0.555), axillary lymph node status (p=0.703), and histological type between the two groups (p=0.691).

Conventional US

The ultrasound features of MHR and non-MHR groups were summarized in Table II. The mean US size of the MHR and non-MHR groups were 3.5±0.9 cm and 3.0 ±1.4 cm, respectively. There were significant differences in tumor size between the two groups (p=0.020）. The shape (p=0.003), margin (p=0.002）, and posterior features of the lesions (p<0.001) were significantly dif-

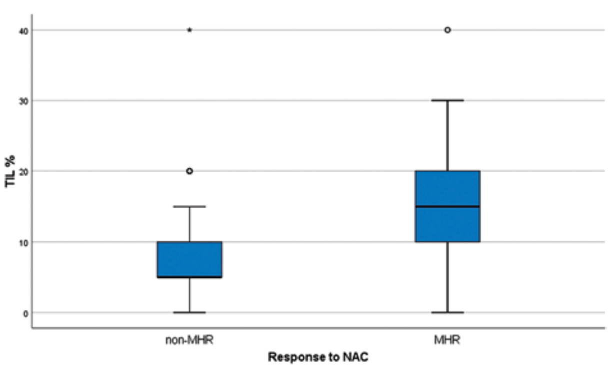

Fig 2. TILs levels of major histologic responders (MHR) and non-MHR groups.

Table I. Baseline clinical and pathological characteristics of MHR and non-MHR

Characteristics	non-MHR (n = 98, 48.5%)	MHR (n = 104, 51.5%)	p value
Age, years，mean ± SD	51.5±9.9	51.5±9.3	0.495
Axillary lymph node metastasis			0.703
Positive	73 (74.5 %)	75 (72.1%)	
Negative	25 (25.5%)	29 (27.9%)	
Histological grade			0.555
1	4 (4.1%)	4 (3.8%)	
2	90 (91.8%)	92 (88.5%)	
3	4 (4.1%)	8 (7.6%)	
Ki-67			0.106
⩽14	12 (12.2%)	6 (5.8%)	
＞14	86 (87.8%)	98 (94.2%)	
Molecular subtypes			<0.001*
Luminal A	42 (42.9%)	26 (25.0%)	
Luminal B	39 (39.8%)	29 (27.9%)	
HER-2 enriched	11 (11.2%)	30 (28.8%)	
Triple negative	6 (6.1%)	19 (18.3%)	
Histology type			0.691
Invasive ductal carcinoma	91 (92.9%)	98 (94.2%)	
Others	7 (7.1%)	6 (5.8%)	
TIL levels			
High	25 (25.5%)	67 (64.4%)	<0.001*
Low	73 (74.5%)	37 (35.6%)	

Other types of histology were included: ductal carcinoma in situ (DCIS), invasive lobular carcinoma, intraductal papillary carcinoma, mucinous carcinoma. MCR - major histologic responders

Table II. Differences in ultrasound imaging features pre-NAC between MHR and non-MHR

Features	Total (n=202)	non-MHR (n =98)	MHR (n = 104)	p value
US tumor size, cm, mean±SD	3.3±1.1	3.5±0.9	3.0±1.4	0.020*
Shape				0.003*
Oval/round	57 (28.2%)	18 (18.4%)	39 (37.5%)	
Irregular	145 (71.8%)	80 (81.6%)	65 (62.5%)	
Orientation				0.216
Parallel	156 (77.2%)	72 (73.5%)	84 (80.8%)	
Not parallel	46 (22.8%)	26 (17.3%)	20 (19.2%)	
Margin				0.002*
Indistinct	109 (53.9%)	63 (63.3%)	44 (42.3%)	
Circumscribed	95 (47.1%)	35 (35.7%)	60 (57.7%)	
Echo pattern				0.476
Hypoechoic	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Isoechoic	51 (25.2%)	21 (21.4%)	30 (28.8%)	
Hypoechoic	87 (43.1%)	44 (44.9%)	43 (41.3%)	
Heterogeneous	64 (31.6%)	33 (33.7%)	31 (29.8%)	
Posterior features				＜0.001*
Enhanced	68 (33.7%)	19 (19.4%)	49 (47.1%)	
No posterior features	56 (27.7%)	27 (27.6%)	28 (26.9%)	
Attenuated	52 (25.7%)	35 (35.7%)	17 (16.3%)	
Combined pattern	27 (13.4%)	17 (17.3%)	10 (9.6%)	
Micro-calcification				0.068
Present	144 (71.3%)	64 (65.3%)	80 (76.9%)	
Absent	58 (28.7%)	34 (34.7%)	24 (23.1%)	

MHR, major histologic responders; NAC, neoadjuvant chemotherapy

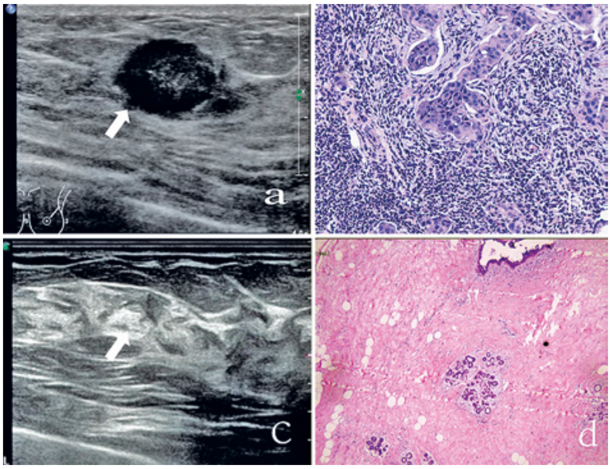

Fig 3. These were B-mode ultrasound (a,c) and pathological (H&E, ×200) (b,d) images of a patient treated with NAC and eventually achieved major histologic response (MHR). (a) Baseline ultrasound showed round, hypoechoic lesion with circumscribed margin and enhanced posterior echoes. (b) Photomicrograph of biopsy specimen before treatment showed high infiltration of lymphocytes in tumor stroma. (c) Post-NAC ultrasound showed no focal lesion in the primary area. (d) Tumor bed resected after surgery showed no tumor cells exist, Miller-Payne G 5.

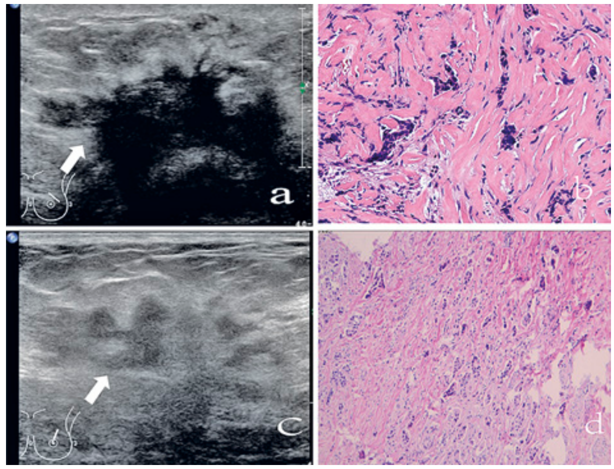

Fig 4. These were B-mode (a,c) and pathological (H&E, ×200) (b,d) images of a patient who was treated with NAC and who eventually achieved non-MHR. (a) Baseline ultrasound showed irregular, hypoechoic lesion with indistinct margin and attenuated posterior echoes. (b) Photomicrograph of biopsy specimen before treatment showed low infiltration of lymphocytes in tumor stroma. (c) Post-NAC ultrasound showed there was still a focal lesion in the primary area with irregular shape, heterogeneous echo and indistinct margin. (d) Tumor bed resected after surgery showed no reduction in overall cellularity, Miller-Payne G2.

ferent between the MHR and non-MHR groups. Most of the lesions were irregular shape in both groups (145/202, 71.8%), while it had a higher rate of oval or round shape in the MHR group than non-MHR group (p=0.003). In the MHR group, circumscribed margins were more common (p=0.002). In terms of posterior features, tumors in the MHR group were more likely to have enhanced posterior features (n =49/104, 47.1%; p<0.001) (fig 3), while tumors in the non-MHR group were more likely to have attenuated posterior features (n=35/98, 35.7%; p<0.001) (fig 4). Besides, lesion orientation (p=0.216), echo pattern (p=0.476), and micro-calcification (p=0.068) showed no significance between the two groups.

Identifying Independent Predictors of MHR Using Multivariate Logistic Regression

Based on multivariate logistic regression, six independent predictors of MHR were US size [OR=0.71, 95%CI (0.46, 0.98); p=0.042], TIL levels [OR=4.58, 95%CI (2.29, 9.17); p<0.001], molecular subtypes [OR=1.88, 95%CI (1.31, 2.69); p=0.001], shape [OR=0.43, 95%CI (0.20, 0.92); p=0.030], and posterior features [OR=0.67, 95%CI (0.48, 0.93); p=0.018] (Table III).

Evaluation of the performance of various models

The ROC curve of the TIL model showed a sensitivity of 64.4%, a specificity of 74.5%, and an AUC of 0.695 (95%CI 0.621–0.768). The US model combined the shape, posterior features, and the US size had a sensitivity of 67.3%, a specificity of 77.6%, and an AUC of 0.758 (95%CI 0.691–0.824). The combined model adding TIL levels and molecular subtypes to the US model showed a sensitivity of 66.3%, a specificity of 84.5%, and an AUC of 0.811 (95%CI 0.753–0.870) (Table IV). The combined model performed better than either the

Table III. Multivariate logistic regression analyses of the features predictive of MHR

Variables	B(SD)	OR	95%CI	P
Size	-0.392	0.67	0.46-0.98	0.042*
TIL level	1.523	4.58	2.29-9.17	<0.001*
Molecular subtypes	0.633	1.88	1.31-2.69	0.001*
Shape	-0.843	0.43	0.20-0.92	0.030*
Margin	0.422	1.52	0.77-3.03	0.229
Posterior features	-0.394	0.67	0.48-0.93	0.018*

MHR, major histologic responders; TIL, tumor-infiltrating lymphocytes

Table IV. Performance of various models for predicting MHR

Features	AUC	95%CI	SEN	SPE	PPV	NPV
TILs model	0.695	0.621-0.768	0.644	0.745	0.728	0.664
US model	0.758	0.691-0.824	0.673	0.776	0.761	0.691
Combined model	0.811	0.753-0.870	0.663	0.847	0.821	0.703

AUC, area under the curve; SEN, sensitivity; SPE, specificity; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; MHR, major histologic responders; TIL, tumor-infiltrating lymphocytes

US model (p=0.019) or TIL levels alone (p<0.001) for the prediction of MHR, and there were significant differences between the performance of the combined model and the other two models (fig 5).

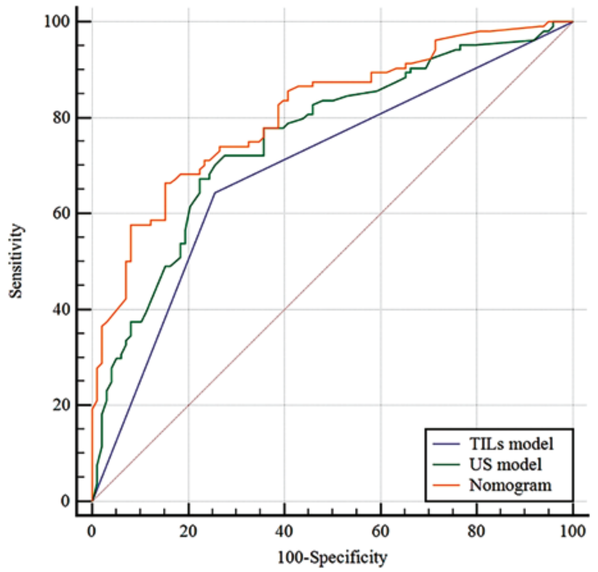

Fig 5. The comparison of ROC curves from different models.

Discussion

In this study, we found that baseline US imaging features, molecular subtypes, and TIL levels were associated with pathological response to NAC in breast cancer. Then we built three predictive models: the TIL model, the US model, and the combined model. The model combined baseline US features, clinical characteristics, and TIL levels had a better performance for the prediction of response to NAC. These preliminary findings suggest that combining baseline US features, molecular subtypes, and TIL levels has the potential to serve as an imaging biomarker to predict response to NAC for patients with breast cancer before treatment.

Early and accurate selection of patients most likely to benefit from NAC is essential and can help patients avoid ineffective treatment and seize the best time for surgery. Primary studies on the association between breast US and response to NAC have paid attention to the changes in lesion size [14]. Indeed, there were limits to this approach. Then, morphological response assessment had been used in clinical practice, which focused primarily on the early prediction of tumor response and preoperative assessment of residual tumor extent [20-22]. Moreover, predicting the response to NAC before treatment may provide greater help for clinical treatment planning.

In a retrospective study of pretreatment MR imaging of 132 patients with breast cancer who underwent NAC, the round or oval shape was more frequently observed in the pCR group than in the group without pCR [23]. The findings from another study reported that response was associated with the absence of posterior shadowing on US [12]. Also, this study demonstrated that shape, US size, and posterior acoustic pattern were identified as independent predictors for NAC response. The most statistically significant imaging biomarkers found in our study were almost consistent with those reported in the previous studies. Round or oval shape and posterior echo enhancement tumors were more frequently observed in the MHR group, whereas irregular shape and posterior echo attenuation tumors were more frequently observed in the non-MHR group. Posterior features were associated with internal tumor components. It has previously been demonstrated that high-grade tumors with many water-soluble components, mainly tumor cell components, demonstrate acoustic enhancement on ultrasound examination because of a high tumor proliferation status, while low-grade tumors tend to produce acoustic shadowing with a high percentage of collagenous fiber [24]. Meanwhile, previous studies revealed that tumors with more tumor cell components were more sensitive to NAC [25]. In contrast, a high amount of stroma is associated with a lower MPG and pCR rate [26]. These findings add to the evidence that tumor morphologic features captured on baseline US images may be associated with NAC response in patients with breast cancer. And then we built the US model based on independent predictors for MHR. However, the performance of the model was not satisfactory.

Many studies have confirmed that tumor size and molecular subtypes are related to the efficacy of NAC. Smaller size tumors were significantly more likely to achieve a complete response [27]. HER2 enriched and triple negative breast cancer had a higher rate of MHR. In agreement with previously published studies, we found that size and molecular subtypes were significant predictors for predicting MHR.

The occurrence, progress, metastasis, and response to a breast cancer drug is a complex process. Previous studies showed better performance in predicting pathological response when combined with US features and Ki-67 [14]. These findings further revealed that imaging biomarkers combined with pathological indicators may acquire better progress. TIL level was an established and highly reproducible biomarker that can reflect breast cancer prognosis and response to treatment. Higher TIL level was associated with higher pCR rates in TNBC [28]. In our study, 64.4% of patients with a high TIL level achieved MHR, compared with 35.6% of patients with low TIL levels. On multivariate logistic regression analysis, TIL level was an independent predictor for MHR; however, the correlation between TIL level and MHR is not strong enough to be used as a single predictor, and AUC was just 0.695.

To our knowledge, few studies have combined the baseline US features and TIL levels. In this exploratory study, we developed a predictive model that combined baseline US features, molecular subtypes, and TIL levels for the prediction of NAC efficacy. The model performed better (AUC=0.811, SEN=66.3%, and SPE=84.7%) than either US model (AUC=0.758, SEN=67.3%, and SPE=77.6%, p=0.019) or TIL levels alone (AUC=0.695, SEN=64.4%,and SPE=74.5%, p＜0.001) for prediction of MHR. As a result, the US features and clinical characteristics, combined with tumor-infiltrating lymphocyte (TIL) levels has the potential to preliminary screen the patients who can truly benefit from NAC.

Also, there were several studies which explored the feasibility of radiomics in the prediction of NAC response. Jiang et al proposed an integrated US radiomics model based on a multicenter dataset of 592 individuals that combined deep learning and machine learning to predict pCR to NAC for breast cancer patients. The deep learning radiomics nomogram model achieved an AUC of 0.94 in the validation cohort, with a significant improvement in predictive accuracy compared to two radiologists (p<0.01) [29]. Gu et al developed a deep learning radiomics pipeline based on the US images before NAC, after the second course, and after the fourth course. The model reached a satisfactory performance in predicting NAC efficacy with an AUC of 0.937. In our next studies, we will further explore the predictive value of radiomics features combined with TILs levels in the efficacy of NAC [30].

There were several limitations in our study. Firstly, this was a single-center retrospective study with limited patients. A larger study sample and multi-center are necessary to validate our results. Secondly, the study only included the baseline US features and there was no dynamic follow-up of NAC efficacy after every cycle. Thirdly, we just included the grayscale US features as this was the most common modality in daily work. Muti-modalities may provide more information and improve accuracy, which would be the priority in our future study. Lastly, as the number of patients increases, subgroup analysis based on molecular subtypes may have a further study.

In **conclusion**, the model combined baseline US features, molecular subtypes, and TIL levels may serve as a promising biomarker for predicting response to NAC in patients with breast cancer before treatment.

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