Breast Imaging

Identification of a correlation between the sonographic appearance and molecular subtype of invasive breast cancer: A review of 311 cases

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ABSTRACT

Purpose: To identify the ultrasound and clinical features related to the different molecular subtypes of invasive breast cancer.
Methods: Sonographic and clinical data of 311 surgically confirmed breast cancer cases were retrospectively reviewed and compared based on various subtypes.
Results: Luminal A (LA) breast cancers were associated with a low histologic grade, spiculated margins, an echogenic rim and posterior acoustic attenuation. The human epidermal growth factor receptor 2-positive (HER2 +) subtype was characterized by a high grade, indistinct and spiculated margins, enhanced posterior acoustics, calcifications, and vascularity. Triple negative breast cancers (TNBCs) were more likely to present with a high tumor grade, circumscribed and microlobulated margins, and the absence of an echogenic rim and calcifications; to be markedly hypoechoic; and to have posterior acoustic enhancement and hypovascularity. Luminal B (LB) cancers were more likely to be associated with an indistinct margin and relative vascularity.
Conclusion: Our study demonstrated that the sonographic and clinical features of breast cancer were significantly correlated with the molecular subtype. The imaging findings of the different subtypes and their biological implications may provide additional auxiliary information for clinical diagnosis, systemic treatment and prognosis prediction.

1. Introduction

Breast cancer is one of the most frequently diagnosed malignancies and the leading cause of cancer-related death in women worldwide. There are an estimated 1.67 million newly diagnosed patients and 0.52 million deaths due to breast cancer annually [1]. Breast carcinoma is a highly heterogeneous disease and is classified into luminal A (LA), luminal B (LB), human epidermal growth factor receptor 2-positive (HER2 +) and triple-negative (TN) subtypes [2,3]. The various molecular subtypes of breast cancer have distinct biological characteristics, therapeutic modalities and clinical prognoses [2–9]. Generally, the LA subtype has a relatively good prognosis and a favorable response to endocrine therapy, while the LB subtype has a relatively poorer prognosis when treated with a combination of endocrine therapy and chemotherapy. The development of targeted therapy is of great significance for HER2 + cancers. Triple-negative breast cancer (TNBC) is

an extremely aggressive subtype characterized by a poor prognosis and limited treatment options. Therefore, effectively differentiating molecular subtypes of breast cancer would be beneficial for the clinical diagnosis, treatment and prognosis assessment.

The concept of a breast cancer molecular subtype based on gene expression profiling (GEP) was first proposed by Perou [4]. Soon thereafter, a simplified immunohistochemical (IHC) approach was suggested as a substitute for GEP [5]. The panel of the St. Gallen Consensus of 2013 concluded that a progesterone receptor (PR) expression cutoff of ≥ 20% best corresponded to the LA subtype and that a high Ki-67 value or a low PR value was correlated with the LB (HER2 −) subtype [6]. However, although GEP has been the gold standard for identifying molecular subtypes of breast cancer, 52.9% of newly diagnosed cases occur in less-developed areas, and the wider application of GEP has therefore been limited [1,4]. The IHC approach using pathological tissue is an invasive operation with some time delay; therefore,

radiologic imaging findings have become an important research topic for breast lesions. With the benefits of being non-invasive and cost-effective and having high sensitivity and specificity, ultrasound has been a preferred diagnostic modality for breast abnormalities [10,11]. The new technology of elastography also has been widely used in clinical settings [12]. Both can effectively differentiate benign and malignant breast lesions, but they are far from satisfactory in terms of clinical requirements [10,12]. Therefore, many studies have focused on the correlation between sonographic findings and the molecular classification of breast cancer [11,13–17]. For example, Kim et al. reported that estrogen receptor (ER)-negative and PR-negative breast cancers are more likely to show a hypoechoic or complex mass, while HER2+ cancers are associated with calcifications [11]. According to Krizmanich-Conniff and colleagues, TNBCs are associated with an irregular shape, hypoechoic or complex echoes, and non-circumscribed margins on ultrasound [13]. However, only few studies have explored the sonographic features related to each molecular subtype of breast cancer. There are two representative studies. Au-Yong and colleagues categorized breast cancers into six main clusters according to 25 IHC biomarkers and suggested that luminal tumors often had ill-defined margins, an irregular shape and distal shadowing. HER2+ tumors were also ill defined, while basal tumors were less characterized by the presentation of an echogenic halo [18]. Lei Zhang et al. used an ensemble-decision approach to differentiate the ultrasound and clinical features among four molecular subtypes according to the St. Gallen Consensus of 2011 and then created models based on their results [19]. Therefore, the comprehensive study of the correlation between ultrasound features and molecular subtypes is complicated and requires more extensive exploration.

Our study aimed to identify the differences between the sonographic and clinicopathological features of breast cancer related to each of the molecular subtypes according to the St. Gallen Consensus of 2013 using multi-modal ultrasonography, including B-mode ultrasound, color Doppler ultrasound and elastography [6]. In addition, a comprehensive analysis and biological interpretation of the sonographic findings among different molecular subtypes are described, which may help radiologists better understand imaging features.

2. Materials and methods

2.1. Patients

This retrospective study was approved by the institutional review board of Harbin Medical University, and the requirement for informed consent was waived. All patient information was anonymized. Data from 350 consecutive patients with primary invasive breast cancer who had undergone surgery at the Second Affiliated Hospital of Harbin Medical University between September 2013 and December 2015 were collected. All patients had standard ultrasound imaging and complete clinical-pathological data. However, of the 350 patients, 39 individuals who had bilateral breast carcinoma, preoperative neoadjuvant chemotherapy, a family history of cancer, pregnancy or evidence of distant metastases were excluded.

2.2. B-mode and color Doppler ultrasound examinations

Ultrasound images were obtained using a HITACHI Vision 900 system (Hitachi Medical System, Tokyo, Japan) equipped with a 12–15 MHz linear-array transducer. The optimal static images and cine clips of breast masses from more than two standard views of B-mode and color Doppler ultrasound were recorded during the real-time examination. These data was saved in separate files and then reviewed by two radiologists with at least 5 years of experience in interpreting breast ultrasound images, who were blinded to the pathological results. In cases of disagreement, a consensus was reached. Sonographic features of the breast masses, including tumor shape (oval/round,

irregular), orientation (parallel, not parallel), margins (circumscribed, indistinct, angular, microlobulated, spiculated), boundaries (abrupt interface, echogenic rim), echo patterns (hypoechoic, complex, markedly hypoechoic), posterior acoustic features (indifferent, enhancement, shadowing, combined pattern), calcification status, and blood flow signal grading, were retrospectively analyzed according to the fifth edition of the Breast Imaging Reporting and Data System (BI-RADS) and the Adler grading method [20,21].

2.3. Strain elastography ultrasound examination

Strain elastography (SE) was also performed by the same radiologists using the same equipment mentioned previously. The transducer was positioned perpendicular to the patients' skin with appropriate compression and frequency. The static images and cine clips were recorded until the color of the entire region of interest (ROI) was completely stable. ROI was recommended to include some of the subcutaneous tissue and pectoralis muscle. [22]. The elasticity data were converted into color-coded images, which were overlaid on the corresponding B-mode images to form a mapped image of tissue stiffness. Blue was assigned to hard tissue and red to soft tissue, and green indicated the average strain. The SE imaging was visually graded with an elasticity score defined by Itoh et al. [12,22].

2.4. Histological and IHC analyses

We used the postoperative pathology results to determine the histological tumor type, histologic grade, and lymphatic metastasis status. Tumor grading was classified as grade 1 (well differentiated), grade 2 (moderately differentiated) and grade 3 (poorly differentiated) [23]. Grades 1 and 2 were considered as low grade, while grade 3 was considered high grade. ER, PR and HER2 expression levels were tested by standard IHC staining. ER and PR expression were considered positive, with nuclear staining of $\geq 1\%$ and $\geq 20\%$, respectively. HER2 positivity was indicated by cell membrane staining of 3+ or by a fluorescence in situ hybridization analysis with a threshold ratio of ≥ 2.2 [6,24,25]. The cutoff value of the Ki-67 index was 14% [5]. Based on the expression of ER, PR, HER2, Ki-67, breast cancers were classified into four molecular subtypes: (1) LA subtype (ER+, PR+, HER2−, Ki-67 < 14%); (2) LB, which was further divided into the HER2− subtype (ER+, HER2- and at least one of Ki-67 $\geq$ 14% or PR < 20%) and the HER2+ subtype (ER+, HER2+, any Ki-67 value, any PR value); (3) HER2+ subtype (HER2+, ER−, PR−); and (4) TN subtype (ER−, PR−, HER2−) according to the St. Gallen consensus of 2013 [6].

2.5. Statistical analysis

Data analyses were performed using SPSS version 17.0 statistical software. The differences in sonographic and clinicopathological features among the different molecular subtypes of breast cancer were analyzed using the chi-square test or Fisher's exact test. The data are presented as the number (frequency). A P-value < 0.05 was considered statistically significant.

3. Results

Of 311 breast cancer cases included in the study, the LA subtype accounted for 24.1% (n = 75), LB for 44.4% (n = 138), HER2+ for 14.5% (n = 45) and TN for 17.1% (n = 53). Of the 138 LB type breast cancers, the proportions of the LB (HER2−) and LB (HER2+) subtypes were 63.0%(n = 87) and 37.0%(n = 51), respectively. The mean age at diagnosis was 51.0 years (range, 22–80 years), and the mean size of the tumors on ultrasound was 25.7 mm (range, 8–75 mm). The clinicopathological and sonographic correlations with four molecular subtypes of breast cancer were showed in Table 1.

Table 1
Sonographic and clinicopathological features among four subtypes of breast cancer.

Parameters	LA (n = 75)	LB (n = 138)	HER2+ (n = 45)	TN (n = 53)	P-value
Age (years)					0.1570
≤50	34 (45.3)	67 (48.6)	21 (46.7)	34 (64.2)	
> 50	41 (54.7)	71 (51.4)	24 (53.3)	19 (35.8)	
Tumor size (mm)					0.1568
≤20	39 (52.0)	67 (48.6)	15 (33.3)	21 (39.6)	
> 20	36 (48.0)	71 (51.4)	30 (66.7)	32 (60.4)	
Lymph node status					0.3611
Negative	47 (62.7)	74 (53.6)	25 (55.6)	25 (47.2)	
Positive	28 (37.3)	64 (46.4)	20 (44.4)	28 (52.8)	
Histologic grade					< 0.0001
1	28 (37.3)	14 (10.2)	2 (4.4)	2 (3.8)	
2	32 (42.7)	66 (47.8)	13 (28.9)	12 (22.6)	
3	15 (20.0)	58 (42.0)	30 (66.7)	39 (73.6)	
Shape					0.1877
Oval/round	7 (9.3)	18 (13.0)	7 (15.6)	12 (22.6)	
Irregular	68 (90.7)	120 (87.0)	38 (84.4)	41 (77.4)	
Orientation					0.1330
Parallel	39 (52.0)	93 (67.4)	26 (57.8)	35 (66.0)	
Not parallel	36 (48.0)	45 (32.6)	19 (42.2)	18 (34.0)	
Margin					< 0.0001
Circumscribed	5 (6.6)	10 (7.3)	6 (13.4)	18 (34.0)	
Indistinct	20 (26.7)	49 (35.5)	15 (33.3)	8 (15.1)	
Angular	13 (17.3)	18 (13.0)	4 (8.9)	7 (13.2)	
Microlobulated	11 (14.7)	25 (18.1)	6 (13.3)	15 (28.3)	
Spiculated	26 (34.7)	36 (26.1)	14 (31.1)	5 (9.4)	
Boundary					0.0496
Abrupt interface	41 (54.7)	90 (65.2)	32 (71.1)	41 (77.4)	
Echogenic rim	34 (45.3)	48 (34.8)	13 (28.9)	12 (22.6)	
Echo pattern					0.0201
Hypoechoic	58 (77.3)	97 (70.3)	27 (60.0)	26 (49.1)	
Complex	6 (8.0)	10 (7.2)	3 (6.7)	5 (9.4)	
Markedly hypoechoic	11 (14.7)	31 (22.5)	15 (33.3)	22 (41.5)	
Posterior features					0.0002
Indifferent	21 (28.0)	46 (33.3)	16 (35.6)	20 (37.7)	
Enhancement	11 (14.7)	38 (27.6)	18 (40.0)	24 (45.3)	
Shadowing	40 (53.3)	50 (36.2)	10 (22.2)	8 (15.1)	
Combined pattern	3 (4.0)	4 (2.9)	1 (2.2)	1 (1.9)	
Calcification					0.0152
Absent	44 (58.7)	75 (54.4)	19 (42.2)	39 (73.6)	
Present	31 (41.3)	63 (45.6)	26 (57.8)	14 (26.4)	
Adler blood grade					0.0383
0	9 (12.0)	15 (10.9)	4 (8.9)	13 (24.5)	
I	18 (24.0)	31 (22.4)	8 (17.8)	17 (32.1)	
II	25 (33.3)	44 (31.9)	11 (24.4)	14 (26.4)	
III	23 (30.7)	48 (34.8)	22 (48.9)	9 (17.0)	
BI-RADS category					0.0772
3	4 (5.3)	5 (3.6)	4 (8.9)	9 (17.0)	
4	31 (41.3)	56 (40.6)	18 (40.0)	21 (39.6)	
5	40 (53.4)	77 (55.8)	23 (51.1)	23 (43.4)	
Elasticity score					0.5243
2	1 (1.3)	5 (3.6)	2 (4.4)	3 (5.7)	
3	15 (20.0)	27 (19.6)	7 (15.6)	6 (11.3)	
4	37 (49.4)	67 (48.5)	19 (42.2)	21 (39.6)	
5	22 (29.3)	39 (28.3)	17 (37.8)	23 (43.4)	

LA, luminal A subtype; LB, luminal B subtype; HER2+, human epidermal growth factor receptor 2-positive subtype; TN, triple-negative subtype. Data are presented as the number of subjects, with percentages in parentheses.

3.1. The clinicopathological findings in four molecular subtypes of breast cancer

Although there was no statistical significance in the age at diagnosis, a young age was an important characteristic of TNBC tumors (64.2% at ≤50 years old vs. 45.3% in LA, 48.6% in LB and 46.7% in HER2+ cancers at ≤50 years old). A tumor size of > 2 cm was more common in HER2+ (66.7%) and TN cancers (60.4%) and less common in LB (51.4%) and LA cancers (48.0%) (P = 0.1568). Lymph node positivity was identified in TNBC (52.8%), LB (46.4%), HER2+ (44.4%) and LA cancers (37.3%), in descending order (P = 0.3611). From these results, we found statistically significant differences in tumor histologic grading; a high grade was generally observed in TNBC (73.6%) and HER2+ (66.7%) cancers and was less frequently observed in LA cancers (20.0%).

3.2. The ultrasound findings in four molecular subtypes of breast cancer

The shape of the breast cancers was more often irregular in the LA (90.7%), LB (87.0%), HER2+ (84.4%) and TN (77.4%) subtypes and was less frequently oval or round. No statistically significant differences in tumor growth orientation were observed (P = 0.1330). Regarding the tumor margin, TNBCs were more likely circumscribed (34.0%) and microlobulated (28.3%) and less likely to be indistinct (15.1%), angular (13.2%) or spiculated (9.4%). In LA, LB and HER2+ cancers, there was a greater association with an indistinct (26.7%, 35.5%, and 33.3%, respectively) and spiculated margin (34.7%, 26.1%, and 31.1%, respectively) and a lesser association with an angular, microlobulated or

circumscribed margin. In particular, spiculation was a significant characteristic in LA and HER2+ cancers. From this result, we also confirmed that echogenic rims were observed most frequently in LA cancers (45.3%) and least frequently in TN cancers (22.6%) (P = 0.0496). The echo pattern of tumors is the most basic feature on ultrasound, and the difference between subtypes was statistically significant (P = 0.0201). The internal echoes of the TN masses were hypoechoic (49.1%), complex echoic (9.5%) and markedly hypoechoic (43.4%). In particular, the sonographic presentation of markedly hypoechoic echoes was more common in TNBC (43.4%) than in HER2+ (33.3%), LB (22.5%) and LA cancers (14.7%). However, hypoechogenicity was the most important echo feature in the other three subtypes, which were less likely to be complex or markedly hypoechoic. For the posterior features with statistically significant differences (P = 0.0002), TN and HER2+ cancers comprised 45.3% and 40.0% of the posterior acoustic enhancement, respectively, compared with 27.6% for LB and 14.7% for LA. While LA was more often associated with posterior acoustic attenuation (53.3%), it was less frequently indifferent (28.0%) or enhanced (14.7%) than the other three subtypes. For the analysis of calcification, HER2+ cancers were more likely to be associated with the presence of calcifications (57.8%) than the other types (45.6% of LB, 41.3% of LA, and 26.4% of TN), and TNBC was the most likely to be associated with the absence of calcifications. On color Doppler ultrasound, Adler grades II and III were more commonly seen in HER2+ cancers (73.3%), but Adler grades 0 and I were more often seen in TNBC (56.6%) than in the other three subtypes (P = 0.0383). There were no statistically significant difference in tumor BI-RADS category (P = 0.0772) and elasticity score (P = 0.5243).

3.3. The clinicopathological and sonographic findings between two luminal B subtypes of breast cancer

Based on the analysis related to the four main subtypes of breast carcinoma, we further distinguished the clinicopathological and sonographic features between the LB (HER2−) and LB (HER2+) subtypes (Table 2). Unfortunately, there were no statistically significant differences between these two subtypes. However, we observed that the LB (HER2+) subtype trended toward the presentation of calcification (43.7% of LB HER2− subtype vs. 49.0% of LB HER2+ subtype) and vascularity (64.4% of LB HER2- subtype vs. 70.6% of LB HER2+ subtype with II and III of Adler blood grade).

4. Discussion

Ultrasound has been a preferred examination method for breast lesions and has been widely used to effectively differentiate malignant and benign lesions [10,12]. Different molecular subtypes of breast cancer are associated with various biological characteristics, clinical therapies and prognoses [2–9]. We have a good understanding of the relationship between the sonographic characteristics and the molecular background of breast cancer. Most previous studies generally correlated ultrasound features with traditionally clinical indicators, such as tumor size, histological type/grade, lymphatic metastases, and certain biological markers (ER, PR, HER2) or simple IHC classifications [10,11,13–17,27,28], but a comprehensive analysis is lacking [18,19]. Our study was conducted to fully analyze the distinctions between sonographic and clinicopathologic features in each molecular subtype of breast cancer and to provide more auxiliary imaging information and clinical implications for early diagnosis, systemic treatment and prognosis evaluation. In addition, better results and more clinical value may be obtained using the updated criteria of the St. Gallen Consensus of 2013 [6].

The LA subtype of cancers is associated with a relatively favorable prognosis, and most are low-grade tumors [14]. Our study found that LA is associated with some significant features, including low histologic grade, spiculated margins, an echogenic rim and posterior acoustic

Table 2

Clinicopathological and sonographic features between two luminal B subtypes of breast cancer.

Parameters	LB (HER2−) (n = 87)	LB (HER2+) (n = 51)	P-value
Age (years)			0.9328
≤50	42 (48.3)	25 (49.0)	
> 50	45 (51.7)	26 (51.0)	
Tumor size (mm)			0.7883
≤20	43 (49.4)	24 (47.1)	
> 20	44 (50.6)	27 (52.9)	
Lymph node status			0.9021
Negative	47 (54.0)	27 (52.9)	
Positive	40 (46.0)	24 (47.1)	
Histologic grade			0.7905
1	10 (11.5)	4 (7.8)	
2	41 (47.1)	25 (49.0)	
3	36 (41.4)	22 (43.2)	
Shape			0.7327
Oval/round	12 (13.8)	6 (11.5)	
Irregular	75 (86.2)	45 (88.5)	
Orientation			0.3727
Parallel	61 (70.1)	32 (63.5)	
Not parallel	26 (29.9)	19 (36.5)	
Margin			0.0944
Circumscribed	7 (8.0)	3 (5.9)	
Indistinct	30 (34.5)	19 (37.3)	
Angular	16 (18.4)	2 (3.9)	
Microlobulated	12 (13.8)	13 (25.5)	
Spiculated	22 (25.3)	14 (27.4)	
Echo pattern			0.9453
Hypoechoic	62 (71.3)	35 (69.2)	
Complex	6 (6.9)	4 (7.7)	
Markedly hypoechoic	19 (21.8)	12 (23.1)	
Posterior features			0.9629
Indifferent	28 (32.2)	18 (35.3)	
Enhancement	25 (28.7)	13 (25.5)	
Shadowing	31 (35.6)	19 (37.2)	
Combined pattern	3 (3.5)	1 (2.0)	
Boundary			0.7843
Abrupt interface	56 (64.4)	34 (67.3)	
Echogenic rim	31 (35.6)	17 (32.7)	
Calcification			0.5432
Absent	49 (56.3)	26 (51.0)	
Present	38 (43.7)	25 (49.0)	
Adler blood grade			0.7793
0	9 (10.3)	6 (11.8)	
I	22 (25.3)	9 (17.6)	
II	27 (31.1)	17 (33.3)	
III	29 (33.3)	19 (37.3)	
BI-RADS category			0.7325
3	4 (4.6)	1 (2.0)	
4	36 (41.4)	20 (39.2)	
5	47 (54.0)	30 (58.8)	
Elasticity score			0.2528
2	5 (5.8)	0 (0)	
3	19 (21.8)	8 (15.4)	
4	41 (47.1)	26 (51.9)	
5	22 (25.3)	17 (32.7)	

LB, luminal B type; HER2+, human epidermal growth factor receptor 2 positive subtype; HER2−, human epidermal growth factor receptor 2 negative subtype. Data are presented as the number of subjects, with percentages in parentheses.

attenuation (Fig. 1). According to previous studies, a low histologic grade of ductal breast carcinoma is generally associated with ER+ or PR+ masses and spiculation because low-grade tumors have a stromal reaction that presents as spicules and a perilesional hyperechogenic rim [27,29,30]. Another study demonstrated that the presence of echogenic rims represents the spiculated margins of masses on ultrasound, and the detection of these features were improved with the development of a more advanced ultrasound machine [10]. Posterior acoustic shadowing is more likely in low-grade breast carcinomas that are associated with desmoplastic reactions, which lead to excessive sound reflection or attenuation in masses compared to the surrounding tissue [27,29].
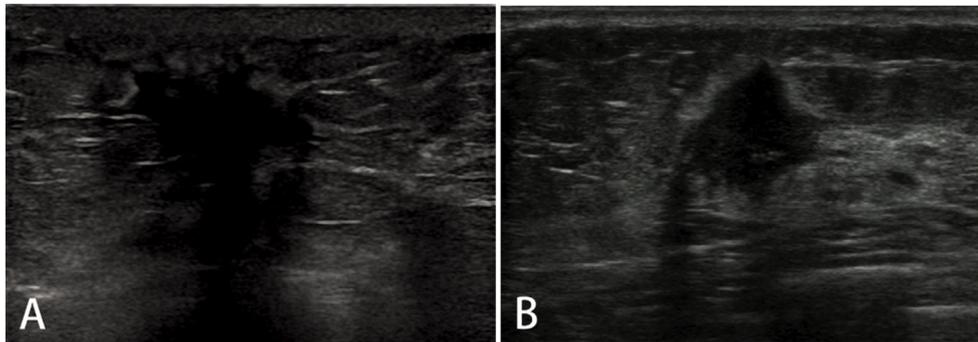
Fig. 1. Luminal A subtype breast cancers on ultrasound. (A, B) These two masses showed a spiculated margin, an echogenic rim, posterior acoustic attenuation, and a spiculated margin, an echogenic rim and calcifications on ultrasound.
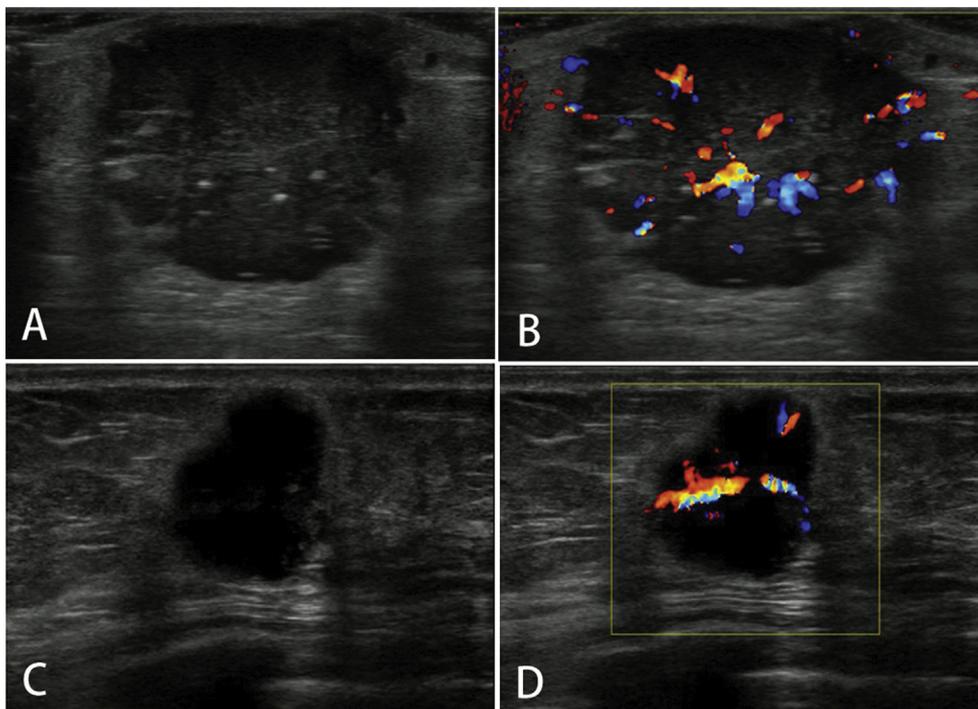

Fig. 2. HER2 + subtype breast cancers on ultrasound. (A, B) This large mass exhibited posterior acoustic enhancement, calcifications on B-mode ultrasound and marked blood flow signals (vascularity) on color Doppler ultrasound (Adler grade III). (C, D) Indistinct margins, echogenic rim and posterior acoustic enhancement on B-mode ultrasound with obvious blood flow signals on color Doppler ultrasound (Adler grade III). Abbreviations: HER2 +, human epidermal growth factor receptor 2-positive subtype.

We found that HER2 + breast cancers are characterized by a high grade, indistinct and spiculated margins, enhanced posterior acoustics, calcifications, and vascularity (Fig. 2). The HER2/neu gene plays an important role in tumorigenicity [31,32]. Over-expression of the HER2/neu gene is associated with some adverse prognostic indicators, such as large tumor size, high tumor grade, axillary lymph node metastasis and the absence of hormone receptors [32]. Spiculated margins and the presence of calcifications are significantly associated with HER2 + status [11,15]. According to the Yang's report, ER − /PR − /HER2 + breast cancers are more often accompanied by ductal carcinoma in situ (DCIS), which is known to be related to the formation of calcifications [33]. In addition, over-expression of HER2 is an independent factor for poor prognosis; consequently, the presence of spiculation or calcifications on ultrasound might be related to adverse prognosis [31]. High-grade and highly cellular circumscribed carcinomas tend to increase the transmission of ultrasound waves, and therefore, HER2 + cancers that have a high grade may show posterior acoustic enhancement [28]. Indistinct margins and marked vascularity are associated with a high histological grade as well [16,34]. In addition, HER2 + status is closely linked to angiogenesis, which may be due to the increased expression of vascular endothelial growth factor [26]. Therefore, HER2 + cancers are more likely to be vascular on color Doppler ultrasound.

Compared with other breast cancer subtypes, TNBCs are associated with aggressive biological characteristics, poor clinical outcomes and limited therapeutic methods [7,8,13,17]. Therefore, the early diagnosis and treatment of TNBCs are essential. Our study showed that TNBCs are more likely to present with a high tumor grade, circumscribed and microlobulated margins, and the absence of an echogenic rim and calcifications; to be markedly hypoechoic; and to have posterior acoustic enhancement and hypovascularity (Fig. 3). TNBCs are higher-grade tumors than non-TNBCs [7,13]. The circumscribed and micro-lobulated tumor margins are associated not only with a high grade and a negative hormone receptor status but also with the rapidly aggressive proliferating pattern of TNBCs, which is described as pushing margins without any infiltrating stromal reaction [16,33]. However, Elkabetset's study demonstrated that a desmoplastic response and inflammation, which present as echogenic rims on ultrasound, regularly occur in TNBCs on pathology [35]. However, our ultrasound findings showed the opposite, namely, that TNBCs are more likely to present with the absence of an echogenic rim, and we suspect the rapid process of carcinogenesis directly related to invasive carcinoma may play an important role. Therefore, desmoplasia might truly exist in TNBCs but not show on ultrasound imaging. Due to the above-mentioned rapid growth pattern without a precancerous stage, TNBCs are accompanied by DCIS
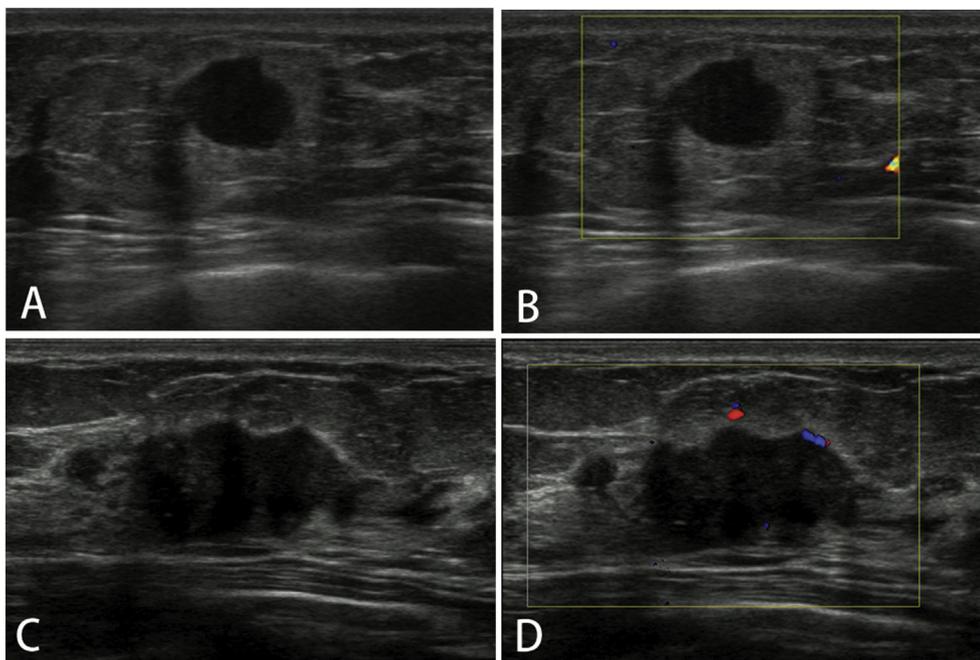
Fig. 3. Triple negative breast cancers on ultrasound. (A, B) Circumscribed margins, marked hypoechogenicity and posterior acoustic enhancement, without the presentation of an echogenic rim or calcifications, and color Doppler ultrasound reveals obvious hypovascularity (Adler grade 0). (C, D) Hypoechoic with microlobulated margins on B-mode ultrasound and color Doppler ultrasound shows few blood flow signals (Adler grade I).

less often (82% of TN cancers are invasive ductal carcinoma without DCIS), which relates to the formation of calcifications [33]. Therefore, TNBCs are more likely to present with the absence of calcifications. A previous study from Shin and colleagues showed a correlation between hypoechogenic masses and high grade but did not further categorize the degree of hypoechogenicity [16]. Our results found that marked hypoechogenicity is most common in TNBCs, which was in agreement with the results of Ko's study (48% in TNBCs vs. 14% in ER+/PR−/ HER2− cancers and 27% in ER−/PR−/HER2+ cancers) [17]. In addition, a significantly increased rate of posterior acoustic enhancement was found in TNBCs, which may be associated with internal necrosis of masses formed by the rapid growth pattern, thereby reducing the attenuation of the ultrasound waves compared to the surrounding tissue [36]. Another pathological explanation for posterior enhancement is a syncytial growth pattern, which is formed by tumor cells arranged closely without distinct cytoplasmic membranes, and it exists in approximately 56% of TNBCs. This growth pattern has fewer layers than does the trabecular pattern, which improves the propagation of the sonographic waves [28]. Consequently, these two aspects may both contribute to the posterior acoustic enhancement. However, in our study, TNBCs unexpectedly demonstrated hypovascularity, which is in contrast to the previous conclusion that marked vascularity on color Doppler is correlated with a high grade and negative hormone receptors [16]. We speculate that tumor necrosis causes the destruction of the tumor blood vessel component and leads to the lack of vascularity. Ultrasound elastography technique can effectively improve overall diagnostic performance in the differentiation of benign and malignant lesions when combined with B-mode ultrasound [37]. Although the elasticity score in this study didn't have significant differences among the four subtypes of breast cancer, the proportion of elastic score of 5 in TN breast cancer is more than in other three subtypes, indicating that TNBC is more aggressive and tends to infiltrate surrounding tissue.

LB cancers are associated with a relatively poor prognosis, and most are high grade and are characterized by indistinct margins and vascularity (Fig. 4) [2,3]. These results are supported by the above-mentioned studies [16,34]. Clinically, the LB (HER2−) subtype is usually treated with endocrine therapy and chemotherapy, while the LB (HER2+) subtype requires the addition of another anti-HER2 therapy. Therefore, it is necessary to make a sonographic and clinicopathological distinction between the two LB subtypes. Despite the lack of a

statistically significant difference, some trends in calcification and vascularity have been observed in the LB (HER2+) subtype because HER2 positivity is closely related to the formation of these features, which is in agreement with previous conclusions [11,15,26]. We speculate that these negative results were caused by the insufficient sample size or because there was little intrinsic difference between the two LB subtypes. Therefore, further study of LB-subtype breast cancers is required.

Our study had several limitations. First, the sample size was relatively small for a study on various molecular subtypes of breast cancer. Our study was retrospective, and only patients who had available clinicopathologic and ultrasound imaging data were included. Second, although we tried to avoid the subjectivity of an ultrasound examination, it still exists in clinical practice. Third, we did not use other imaging modalities, such as mammography and magnetic resonance imaging, which may be more favorable for a study comparing the features of different subtypes of breast cancer. Fourth, we did not find significant differences between the two LB subtypes of breast cancer. Aiming to determine such differences will be the emphasis of a future study.

5. Conclusion

We have demonstrated that the sonographic and clinical features of breast cancer show some significant correlations with different molecular subtypes. Our study involved using multi-mode ultrasound assessments based on the BI-RADS criterion and molecular classification using IHC markers. Distinguishing the various features in each molecular subtype of breast cancer on ultrasound may help provide additional auxiliary clinical information. In addition, the biological mechanisms for these findings and the implications regarding the imaging features may improve the detection of breast lesions, which are of great significance for early diagnosis, appropriate treatment and prognosis prediction in clinical practice. Further studies are warranted and should include larger sample sizes.
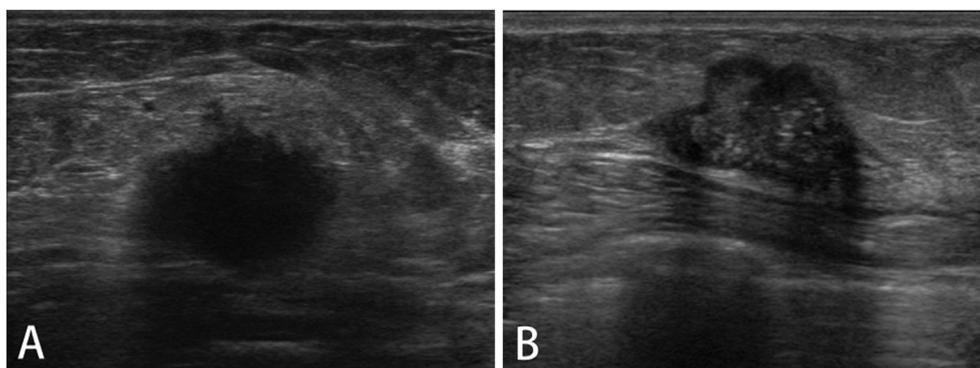
Fig. 4. Luminal B subtype breast cancers on ultrasound. (A) This LB (HER2−) tumor displayed indistinct margins and posterior acoustic shadowing. (B) This LB (HER2+) tumor exhibited calcifications and posterior acoustic shadowing. There were no significant differences between these two LB subtypes.
Abbreviations: HER2, human epidermal growth factor receptor 2.

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Conflict of interest

The author reports no conflicts of interest in this work.

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