Research paper

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Ultrasonography: an aid in molecular subtyping of breast carcinoma

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breast cancer; ultrasound; posterior acoustic features; molecular subtypes; immunohistochemistry (IHC)

Abstract

Introduction: The recognition of molecular subtypes of breast cancer has initiated a new regimen of targeted therapy. Early diagnosis is a key step in improving survival. Therefore, a cost-effective and widely available imaging tool is needed for the timely detection and prediction of the molecular profile of breast cancer. **Aim:** To study the predictive value of ultrasonographic features in identifying the estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2 neu (HER2/neu) expression status, and molecular subtypes of breast cancer. **Material and methods:** We conducted a study on 51 histopathologically proven invasive breast carcinoma cases over a period of one and a half years. The patients underwent ultrasonography followed by tissue biopsy. Sonographic parameters were assessed based on BI-RADS imaging features. The molecular subtypes of breast cancer were grouped into four subtypes based on the St. Gallen International Expert Consensus Panel. The predictive value of ultrasonographic features was then studied in relation to the hormone receptor status and molecular subtypes of breast cancer. **Results:** A significant association between posterior acoustic features and molecular subtypes was seen. Posterior acoustic shadowing was associated with progesterone receptor status with an odds ratio (OR) of 36.58, confidence interval (CI) of 5.527–866.1, and p <0.001. The luminal type A molecular subtype was significantly higher in the posterior acoustic shadowing group (10 cases; 52.63%) with an OR of 3.85, CI of 1.12–13.98, and p of 0.02. The proportion of patients with triple-negative molecular subtype (9 cases, 50%) was significantly higher in the posterior acoustic enhancement group, with an OR of 29.42, CI of 4.117–725.4, and p <0.001. Tumors with circumscribed margins were also highly suggestive of the triple-negative molecular subtype [OR of 5.12, CI of 1.16–24.85, and p of 0.03]. The association between the presence or absence of vascularity and its type with molecular subtypes failed to show statistical significance in our study, although vascularity was more frequently observed in triple-negative molecular subtype and luminal type B Her+ve cases. **Conclusion:** Certain sonographic features are associated with the estrogen/progesterone receptor hormone receptor status and molecular subtypes of breast cancer. With validation of this association, ultrasound may serve as a basic imaging modality for predicting molecular subtypes of breast cancer even in remote areas, where immunohistochemistry hormone receptor and HER2 testing are not available.

Introduction

Breast cancer, in a broader sense, encompasses a diverse group of diseases characterized by variable natural history, morphological appearance, imaging features, histological and morphological classifications, and response to treatment[1]. Treatment decisions for breast cancer used to be based on the conventional TNM classification that relies on the histopathological features and grading[2]. However, with the advent of molecular subtyping of breast cancer, this classification has become suboptimal, as it fails to fully capture the diversity of breast cancer and its associated genetic aberrations. These genetic insights enable clinicians to provide patients with the best therapeutic options[3]. Each molecular subtype of breast cancer exhibits distinct biological behavior, which governs the treatment plan and affects the prognosis and disease-free survival[4].

Breast cancer is pathologically subcategorized by the St. Gallen International Expert Consensus Panel into four molecular subtypes on the basis of the gene expression status of tumor markers, including es-

trogen receptor (ER); progesterone receptor (PR); Ki 67 and human epidermal growth factor receptor 2 (HER2; proto-oncogene neu; receptor tyrosine-protein kinase erbB-2 ERRB2) overexpression[5]. The latest generation of anticancer systemic therapies for breast cancer depends on its molecular profile. Therefore, comprehensive molecular characterization is essential before starting the management plan.

The imaging features of breast cancer have been studied for a long time, with significant advances made in understanding the role of ultrasound in differentiating between benign and malignant tumors with a degree of certainty, as outlined in the current imaging criteria used in the BI-RADS (Breast Imaging-Reporting and Data System) lexicon[6]. Among these features, non-circumscribed margins, vascularity, and posterior acoustic features are among the imaging characteristics which have been shown to be associated with receptor status.

However, there is scarcity of sufficient research establishing the association between ultrasonographic features and the molecular profiling of breast tumors. If strong evidence emerges that imaging features of breast cancer correlate well with receptor status, a response-based anti-cancer therapy can be initiated on an empirical basis. This approach may serve as a cost-effective substitute for expensive genetic tests, particularly in settings where detailed and costly histopathologic analysis is not readily available[7].

Given this context, the study aimed to determine whether ultrasound features of breast cancer could predict the hormone receptor status (ER/PR), HER2/neu expression, and molecular subtypes of breast cancer.

Material and methods

This prospective observational study was conducted over one and a half years at a tertiary care institute. Approval from the institutional ethical committee and informed consent from the patients were obtained before the examinations. The inclusion criteria encompassed females over 18 years of age, who had a suspicious breast mass on screening mammography, who presented with a breast lump, or who came for staging with already pathologically diagnosed invasive breast carcinoma based on core biopsy. The exclusion criteria were pregnant patients and those with in-situ breast cancer, patients who had undergone neoadjuvant chemotherapy or had recurrent breast cancer, lesions identifiable only on MRI and with inadequate tissue sample for IHC analysis. Ultimately, a total of 51 female patients (each with a single breast mass and no multifocal cancer) meeting the inclusion and exclusion criteria were enrolled in the study.

Ultrasonographic analysis

Ultrasonography (USG) of the breast was performed with a linear transducer 10–13 mHz on LOGIQ P6 (GE Healthcare) and Samsung RS80EVO ultrasound units by two radiologists with 12 and 18 years of experience in breast imaging. In case of disagreement between the radiologists, a consensus was reached through joint review of the images. The imaging features of the breast mass that were assessed on ultrasound included size (<2 cm, ≥2 cm), shape (irregular/oval/round), margins (circumscribed/non-circumscribed), echo pattern (heterogeneous/hyperechoic/hypoechoic), posterior acoustic features (absent/enhancing/shadowing/mixed), calcifications (absent/present), and vascularity (absent/internal/peripheral). Although mass size is not part of the BI-RADS sonography lexicon, masses with a size ≥2 cm were arbitrarily classified as large for the purposes of this study. Also, the tumors with indistinct, spiculated, microlobulated, or angular margins were grouped together as having non-circumscribed margins and compared with the tumors having circumscribed margins. A default setting of the USG machine for color Doppler breast imaging was used; a medium wall filter of around 150–170 Hz, a scale of 5 to 7 cm/s, and a pulse repetition frequency of 1.3 kHz.

Pathologic analysis

The patients underwent core or excision biopsy of the breast mass (if not previously performed), and the specimens were sent for histopathological examination. On IHC examination for ER and PR expression based on the Allred scoring system as per the 2020 American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines, tumor cell nuclear staining of ≥1% was considered positive. The Allred score combined the proportion of cells stained and intensity of staining, with a total score of >2 (proportion + intensity score) classified as positive[8].

The assessment of HER2 IHC slides was done using the ASCO/CAP 2023 and graded as follows[9]:

- **Positive: IHC 3+** (strong positive): tumor displays complete, intense circumferential membranous staining in >10% of tumor cells (easily identifiable under low power magnification and observed within a homogenous and contiguous invasive cell population);
- **Equivocal: IHC 2+:** weak to moderate complete membrane staining observed in >10% of invasive tumor cells;
- **Negative: IHC 1+:** incomplete, faint membrane staining observed in >10% of invasive tumor cells;
- **IHC 0:** no staining observed, or incomplete, faint/barely perceptible membrane staining in ≤10% of invasive tumor cells.

Scores of 0 and 1+ were considered negative (unamplified), 2+ as equivocal, and 3+ as positive [10]. Cases with HER2-score 2+ (equivocal) were considered HER2-positive if fluorescent in situ hybridization (FISH) showed HER2 gene amplification. Since FISH was not available at our institute, these cases were excluded from the study.

Based on ER, PR, Ki67 % (proliferative index), and HER2-expression status, breast cancers were categorized into four molecular subtypes based on the St. Gallen International Expert Consensus Panel 2013[5]:

- **Luminal A subtype:** ER, PR-positive, HER2-negative and Ki 67% <20%.
- **Luminal B subtype (HER2-negative):** ER+, HER2-negative and at least one of the following – Ki 67 % ≥20% and PR–/low (<20%); **luminal B subtype (HER2-positive):** ER+, HER2-positive, any Ki 67, any PR.
- **HER2-enriched type (HER2):** ER-, PR-negative and HER2-positive.
- **Triple-negative type (TN):** ER-, PR- and HER2-negative.

Statistical analysis

The categorical variables were presented as numbers and percentages (%). On the other hand, the quantitative data were expressed as means ± SD and as medians with 25th and 75th percentiles (interquartile range). The statistical tests applied for the results were as follows. The variables which were quantitative in nature were analyzed using the independent t-test, while the variables which were qualitative in nature were analyzed using the chi-square test. If any cell had an expected value of less than 5, Fisher's exact test was used. Data entry was done in a Microsoft EXCEL spreadsheet, and the final analysis was conducted using the Statistical Package for Social Sciences (SPSS) software, IBM manufacturer, Chicago, USA, ver 25.0. For statistical significance, a p-value of less than 0.05 was considered statistically significant.

Results

A total of 51 females with histopathologically proven invasive breast carcinoma and a mean age of 51.63 ± 10.5 years were included in the study. No significant family history was reported in any of the patients. In most of them [50 (98.04%)], past history of breast cancer was absent, with only one having a history of cancer in the contralateral breast. The majority presented with a palpable breast lump. No other relevant complaints, such as nipple discharge or breast pain, were present in any of the patients.

The most common histopathological tumor type in the study was invasive ductal carcinoma, which accounted for 94.12% of cases (Tab. 1, Fig. 1), and the most common molecular subtype was Luminal type A [17 (33.33%)]. The triple-negative type was the second most common molecular subtype [10 (19.61%)]. Luminal type B, HER2-negative was seen in 6 (11.76%) patients. Nine patients (17.65%) showed Luminal type B, HER2 positivity (Fig. 2), and a similar number showed HER2/neu-enriched expression (Fig. 3) (Tab. 1).

Tab. 1. Distribution of pathological types, receptor status, and molecular subtypes of breast cancer

Type of tumor	**Frequency**	**Percentage**
Invasive ductal carcinoma	48	94.12%
Invasive lobular carcinoma	2	3.92%
Mucinous carcinoma	1	1.96%
Total	51	100.00%
Receptor status	**Frequency**	**Percentage**
Estrogen receptor	35	68.63%
Progesterone receptor	28	54.90%
HER2/neu receptor	18	35.29%
Molecular subtype	**Frequency**	**Percentage**
HER2/neu-enriched type	9	17.65%
Luminal type A	17	33.33%
Luminal type B (HER-ve)	6	11.76%
Luminal type B (HER+ve)	9	17.65%
Triple-negative type	10	19.61%
Total	51	100.00%

The size of the mass was large in 37 (72.55%) of the 51 patients. This was because the majority of the patients presented with a palpable breast lump. Irregular shape of the mass was the most common

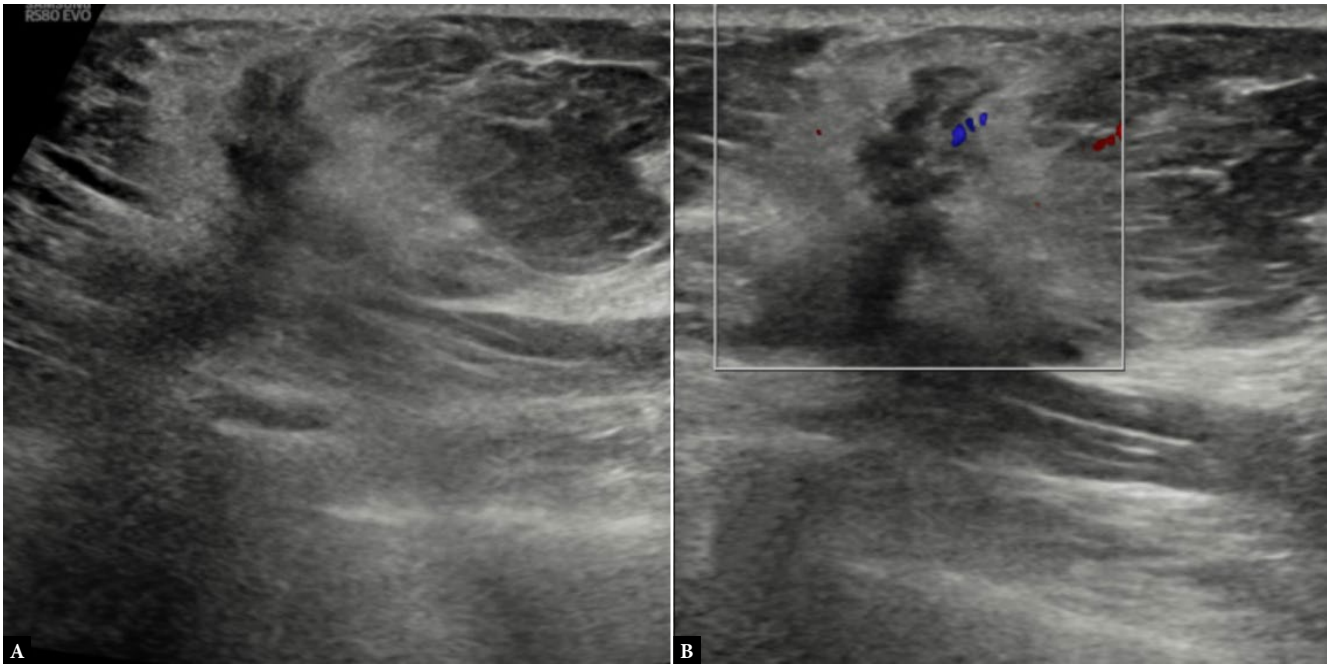

Fig. 1. *A 45-year-old female with invasive ductal carcinoma and luminal type A molecular subtype of breast cancer. Ultrasound image* **A.** *gray scale shows an irregular hypoechoic lesion, taller than wider, having spiculated margins, thick echogenic rim, and posterior acoustic shadowing,* **B.** *color Doppler shows minimal peripheral vascularity*

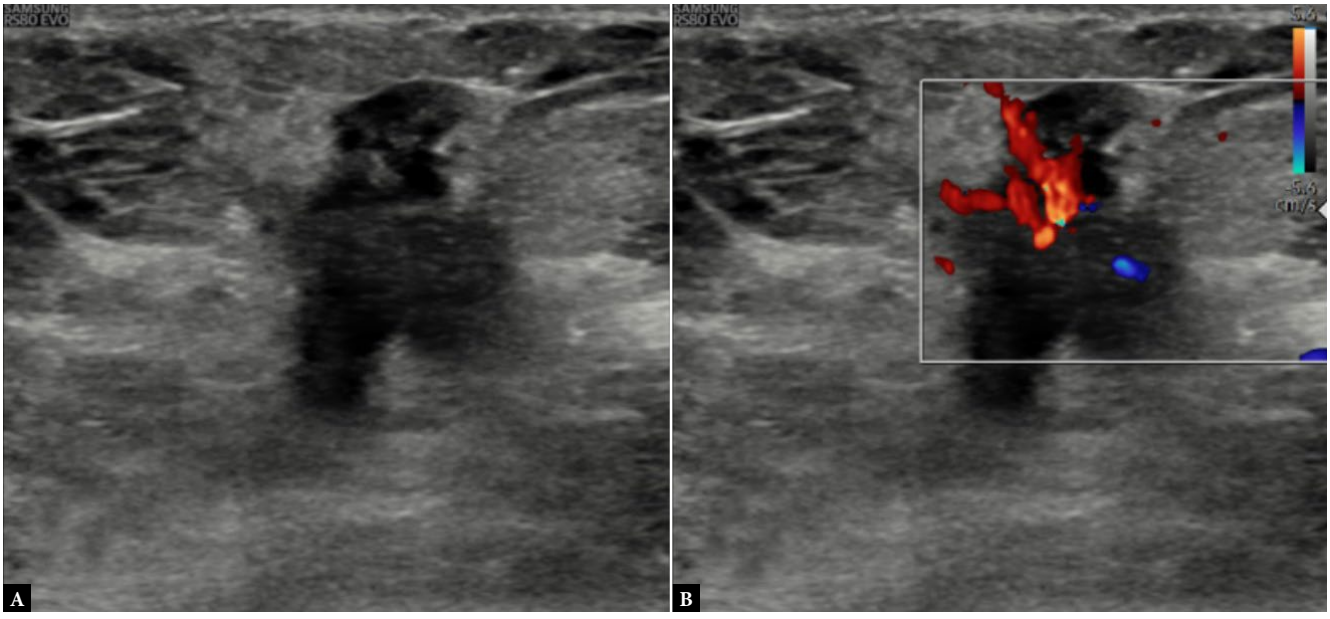

Fig. 2. *A 55-year-old female with invasive lobular carcinoma and luminal type B HER2-positive molecular subtype of breast cancer. Ultrasound image* **A.** *gray scale shows an irregular hypoechoic lesion, taller than wider, having spiculated and microlobulated margins, and posterior acoustic shadowing* **B.** *color Doppler shows internal vascularity*

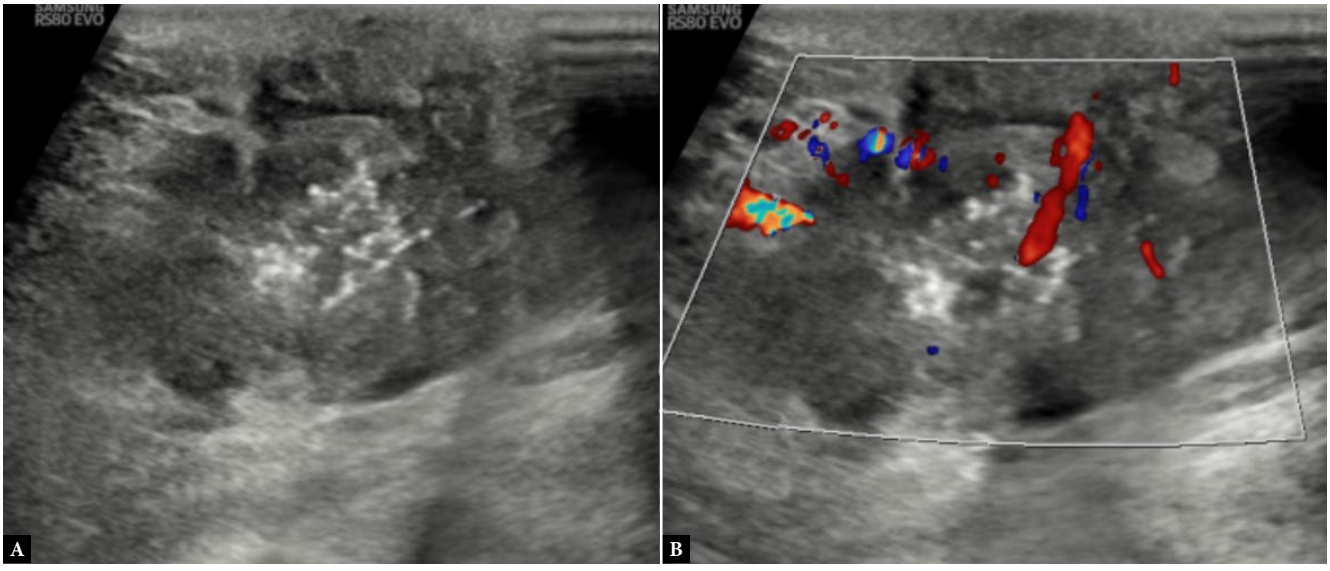

Fig. 3. *A 45-year-old female with invasive ductal carcinoma and HER2/neu-enriched type molecular subtype of breast cancer. Ultrasound image* **A.** *gray scale shows a heterogeneously hypoechoic mass with multiple echogenic foci of calcification* **B.** *color Doppler shows internal vascularity*

shape, seen in 39 (76.47%) patients. In 36 (70.59%) patients, the margin was non-circumscribed. The echo pattern was hypoechoic in the majority of patients. Posterior acoustic shadowing 19 (37.25%), followed by enhancement 18 (35.29%), were the most common acoustic features. Calcification was absent in 28 (54.90%) patients, and internal vascularity was present in 24 (47.06%) patients (Tab. 2).

There was no statistically significant association between ER receptor positivity and the margin of the mass. However, a statistically significant association was seen between posterior acoustic enhancement and ER receptor negativity (66.67%), and posterior acoustic shadowing with ER receptor positivity (100%) (p <0.0001). Similarly, there was no statistically significant association between PR receptor positivity and the margin of the mass. However, a statistically significant association was found between posterior acoustic enhancement and PR receptor negativity (83.33%), and posterior acoustic shadowing with PR receptor positivity (94.74%) (p <0.0001) (Tab. 3). There was no statistically significant association between HER2/neu receptor positivity and the margin or posterior acoustic features of the mass (Tab. 4).

No statistically significant association was observed between ER, PR, HER2/neu receptor positivity or negativity and the presence or absence of vascularity in the mass (Tab. 5). Also, no statistically sig-

Tab. 2. Distribution of ultrasound findings of breast mass in the study subjects

Ultrasound findings	Frequency (n = 51)	Percentage
Size		
Large (≥2 cm)	37	72.55%
Small (<2 cm)	14	27.45%
Shape		
Irregular	39	76.47%
Oval	11	21.57%
Round	1	1.96%
Margin		
Circumscribed	15	29.41%
Non-circumscribed	36	70.59%
Echo pattern		
Heterogenous	16	31.37%
Hyperechoic	5	9.80%
Hypoechoic	30	58.82%
Posterior acoustic features		
No posterior features	10	19.61%
Enhancing	18	35.29%
Shadowing	19	37.25%
Mixed	4	7.84%
Calcifications		
Absent	28	54.90%
Present	23	45.10%
Vascularity		
Absent	17	33.33%
Internal vascularity	24	47.06%
Vessels in rim	10	19.61%

nificant association was seen between ER, PR, or HER2/neu receptor positivity or negativity and the type of vascularity in the mass (Tab. 6).

A significant association between the posterior acoustic features and the molecular subtypes was observed in the study (Tab. 7). The number of patients with luminal type A molecular subtype (10 cases; 52.63%, Fig. 1), luminal type B HER2-ve (3 cases; 15.79%), and luminal type B HER2+ve (5 cases; 26.32%) were significantly higher in the posterior acoustic shadowing group. The proportion of patients with the triple-negative type (9 cases, 50%, Fig. 4) was significantly higher in the posterior acoustic enhancing group (Tab. 7). The association of the presence or absence of vascularity and its type with molecular subtypes failed to show statistical significance (Tab. 8). Moreover, posterior acoustic shadowing was associated with progesterone receptor status [odds ratio (OR) of 36.58, confidence interval (CI) of 5.527–866.1, and p <0.001] (Tab. 9) and luminal A status (OR of 3.85, CI of 1.12–13.98, and p <0.02) (Tab. 10). Tumors with circumscribed margins (OR of 5.12, CI of 1.16–24.85, and p = 0.03) and posterior acoustic enhancement (OR of 29.42, CI of 4.117–725.4, and p <0.001) were highly suggestive of TNBC (Tab. 10).

Discussion

With the expanding knowledge of intrinsic molecular subtypes of breast cancer, a new era in breast cancer research has begun, with the development of targeted treatments that allow for the avoidance of cytotoxic therapies and associated comorbid conditions, offering patients a better quality of life. Given the distinctly different treatment and prognosis of the molecular subtypes, it is clinically important to distinguish patients with these subtypes. Luminal A tumors are more common and have the best prognosis, whereas luminal B, HER2-enriched and TNBC are associated with poorer prognosis[10]. Determining the intrinsic molecular subtype of breast cancer requires gene expression profiling (GEP), which is expensive, time-consuming, and not widely available. IHC analysis and fluorescent in situ hybridization (FISH)[5] are commonly used in the

Tab. 3. Association of margins and posterior acoustic features with estrogen and progesterone receptors

Variables	ER-negative (n = 16)	ER-positive (n = 35)	Total	p value	PR-negative (n = 23)	PR-positive (n = 28)	Total	p value
Margin								
Circumscribed	7 (46.67%)	8 (53.33%)	15 (100%)	0.129†	8 (53.33%)	7 (46.67%)	15 (100%)	0.446†
Non-circumscribed	9 (25%)	27 (75%)	36 (100%)		15 (41.67%)	21 (58.33%)	36 (100%)	
Total	16 (31.37%)	35 (68.62%)	51 (100%)		23 (45.10%)	28 (54.90%)	51 (100%)	
Posterior acoustic features								
No posterior features	3 (30%)	7 (70%)	10 (100%)	<0.0001*	5 (50%)	5 (50%)	10 (100%)	<0.0001*
Enhancing	12 (66.67%)	6 (33.33%)	18 (100%)		15 (83.33%)	3 (16.67%)	18 (100%)	
Shadowing	0 (0%)	19 (100%)	19 (100%)		1 (5.26%)	18 (94.74%)	19 (100%)	
Mixed	1 (25%)	3 (75%)	4 (100%)		2 (50%)	2 (50%)	4 (100%)	
Total	16 (31.37%)	35 (68.62%)	51 (100%)		23 (45.10%)	28 (54.90%)	51 (100%)	

† Chi-square test; * Fisher's exact test
ER – estrogen receptor; PR – progesterone receptor

Tab. 4. Association of margin and posterior acoustic features with HER2/neu receptor

Margin	HER2/neu-negative (*n* = 33)	HER2/neu-positive (*n* = 18)	Total	*p* value
Circumscribed	12 (80%)	3 (20%)	15 (100%)	0.202*
Non-circumscribed	21 (58.33%)	15 (41.67%)	36 (100%)	
Total	33 (64.71%)	18 (35.29%)	51 (100%)	
Posterior features				
No posterior features	4 (40%)	6 (60%)	10 (100%)	0.37*
Enhancing	13 (72.22%)	5 (27.78%)	18 (100%)	
Shadowing	13 (68.42%)	6 (31.58%)	19 (100%)	
Mixed	3 (75%)	1 (25%)	4 (100%)	
Total	33 (64.71%)	18 (35.29%)	51 (100%)	
* Fisher's exact test				

Tab. 5. Association of vascularity with estrogen, progesterone and HER2/neu receptor

Vascularity	Absent	Present	Total	*p* value
ER-negative (*n* = 16)	6 (35.29%)	10 (29.41%)	16 (31.37%)	0.67†
ER-positive (*n* = 35)	11 (64.71%)	24 (70.59%)	35 (68.63%)	
Total	17 (100%)	34 (100%)	51 (100%)	
PR-negative (*n* = 23)	7 (41.18%)	16 (47.06%)	23 (45.10%)	0.691†
PR-positive (*n* = 28)	10 (58.82%)	18 (52.94%)	28 (54.90%)	
Total	17 (100%)	34 (100%)	51 (100%)	
HER2/neu-negative (*n* = 33)	11 (64.71%)	22 (64.71%)	33 (64.71%)	1†
HER2/neu-positive (*n* = 18)	6 (35.29%)	12 (35.29%)	18 (35.29%)	
Total	17 (100%)	34 (100%)	51 (100%)	
† Chi-square test				

Tab. 6. Association of type of vascularity with estrogen, progesterone and HER2/neu receptor

Type of vascularity	Internal vascularity	Vessels in rim	Total	*p* value
ER-negative (*n* = 10)	7 (29.17%)	3 (30%)	10 (29.41%)	1†
ER-positive (*n* = 24)	17 (70.83%)	7 (70%)	24 (70.59%)	
Total	24 (100%)	10 (100%)	34 (100%)	
PR-negative (*n* = 16)	12 (50%)	4 (40%)	16 (47.06%)	0.715†
PR-positive (*n* = 18)	12(50%)	6(60%)	18 (52.94%)	
Total	24 (100%)	10 (100%)	34 (100%)	
HER2/neu-negative (*n* = 22)	13 (54.17%)	9 (90%)	22 (64.71%)	0.061†
HER2/neu-positive (*n* = 12)	11 (45.83%)	1 (10%)	12 (35.29%)	
Total	24 (100%)	10 (100%)	34 (100%)	
† Chi square test				

clinical setting. However, even IHC may not be readily available in certain remote areas of developing countries, so an improved understanding of how the various imaging features of breast cancer correlate with molecular subtype would help guide treatment in the cost-constrained regions.

The study reported here showed that ultrasonographic parameters, such as posterior acoustic features and tumoral margins, were significantly associated with molecular subtype. The proportion of patients with ER positivity was significantly higher in the shadowing group (100%). Luminal A and luminal B subtypes were more often associated with posterior acoustic shadowing, and triple-negative breast cancers commonly showed posterior acoustic enhancement (p <0.0001). A prior study by Irshad *et al.*[11], investigating the association of imaging features with molecular subtypes, found evidence that cancers with posterior acoustic shadowing have higher odds of hormone-receptor positivity (greater than nine times), while those with posterior acoustic enhancement are likely to have negative receptor expression, which was consistent with the present study.

The distribution of triple-negative breast cancer (TNBC) was comparable with the mass margin, although it did not turn out to be statistically significant in our study when all the molecular subtypes were studied together [circumscribed (40%) vs non-circumscribed (11.11%), p = 0.138; Tab. 7]. Non-TNBC had the majority of patients with non-circumscribed margins (32 out of 36). However, when the individual molecular subtypes were studied separately, the circumscribed margins were found to be strongly associ-

Tab. 7. Association of margin and posterior acoustic features with molecular subtype

Margin	**HER2/neu-enriched type (*n* = 9)**	**Luminal type A (*n* = 17)**	**Luminal type B HER2-ve (*n* = 6)**	**Luminal type B HER2+ve (*n* = 9)**	**Triple-negative type (*n* = 10)**	**Total**	***p***
Circumscribed	2 (13.33%)	4 (26.66%)	2 (13.33%)	1 (6.67%)	6 (40%)	15 (100%)	0.138*
Non-circumscribed	7 (19.44%)	13 (36.11%)	4 (11.11%)	8 (22.22%)	4 (11.11%)	36 (100%)	
Total	9 (17.65%)	17 (33.33%)	6 (11.76%)	9 (17.65%)	10 (19.61%)	51 (100%)	
Posterior features	**HER2/neu-enriched type (*n* = 9)**	**Luminal type A (*n* = 17)**	**Luminal type B HER2-ve (*n* = 6)**	**Luminal type B HER2+ve (*n* = 9)**	**Triple-negative type (*n* = 10)**	**Total**	***p***
No posterior features	2 (20%)	2 (20%)	1 (10%)	4 (40%)	1 (10%)	10 (100%)	<0.0001*
Enhancing	5 (27.78%)	3 (16.67%)	1 (10%)	0 (0%)	9 (50%)	18 (100%)	
Shadowing	1 (5.26%)	10 (52.63%)	3 (15.79%)	5 (26.32%)	0 (0%)	19 (100%)	
Mixed	1 (25%)	2 (50%)	1 (25%)	0 (0%)	0 (0%)	4 (100%)	
Total	9 (17.65%)	17 (33.33%)	6 (11.76%)	9 (17.65%)	10 (19.61%)	51 (100%)	
* Fisher's exact test							

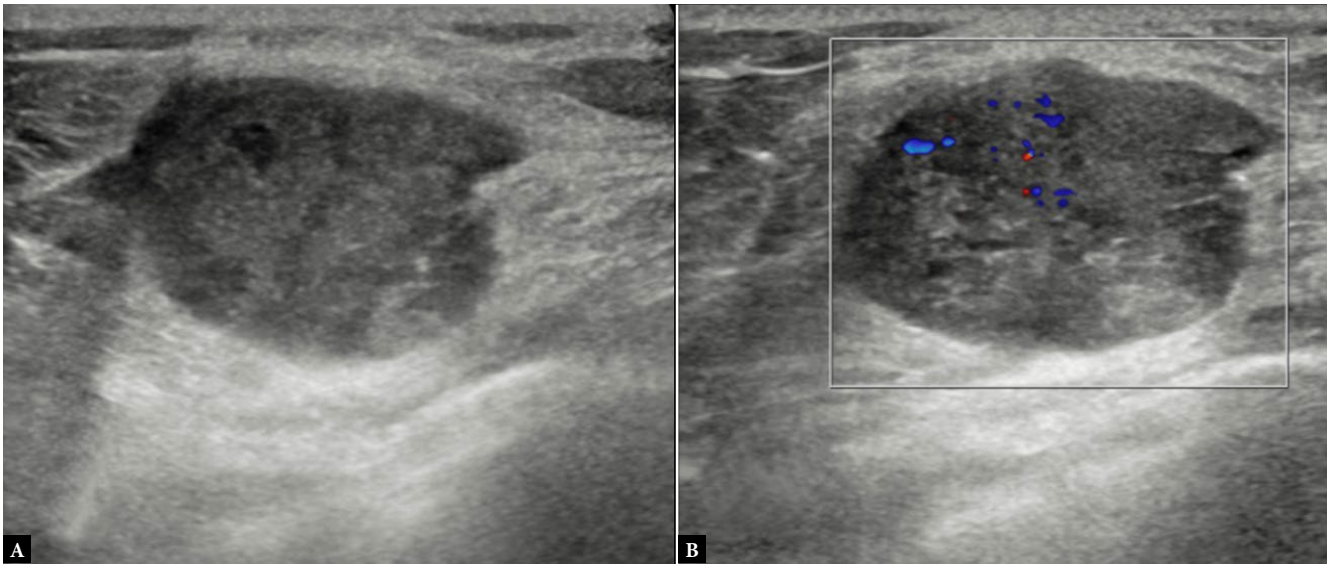

Fig. 4. *A 48-year-old female with invasive ductal carcinoma and triple-negative molecular subtype of breast cancer. Ultrasound image* **A.** *gray scale shows a well-circumscribed hypoechoic mass with macrolobulations and posterior acoustic enhancement* **B.** *color Doppler shows internal vascularity*

ated with TNBC [OR of 5.12 and $p = 0.03$; Tab. 10]. The results of the present study were consistent with prior studies[12,13]. Tandon *et al.*[14] reported that tumors with posterior acoustic shadowing had 25 times higher chances, and tumors with non-circumscribed margins with surrounding architectural distortion had 9.5 times higher chances of having hormone receptor positivity. In their study, the likelihood of having TNBC status was 12 times higher with posterior acoustic enhancement and 16 times higher with circumscribed margins. In a study by Celebi *et al.*[13], circumscribed margins were more often associated with the TNBC subtype (OR of 6.72, CI of 2.56–17.65, $p < 0.001$). The authors also found that tumors with combined findings of non-circumscribed margins and posterior shadowing had 10.58 times higher association with Luminal A and Luminal B subtypes. Since luminal cancers grow at a slower rate, they create a desmoplastic reaction resulting in non-circumscribed, spiculated, angulated, and microlobulated margins. The desmoplastic reaction also affects the acoustic impedance of the tumor to the healthy tissue interface, causing excessive sonographic attenuation by the tumor, resulting in posterior shadowing[11,13,15].

Tab. 8. Association of vascularity and its type with molecular subtype

Vascularity	**HER2/neu-enriched type (*n* = 9)**	**Luminal type A (*n* = 17)**	**Luminal type B HER2-ve (*n* = 6)**	**Luminal type B HER2+ve (*n* = 9)**	**Triple-negative type (*n* = 10)**	**Total**	***p***
Absent	4 (23.53%)	6 (35.29%)	2 (11.76%)	2 (11.76%)	3 (17.65%)	17 (100%)	0.823*
Present	5 (14.71%)	11 (32.35%)	4 (11.76%)	7 (20.59%)	7 (20.59%)	34 (100%)	
Total	9 (17.65%)	17 (33.33%)	6 (11.76%)	9 (17.65%)	10 (19.61%)	51 (100%)	
Type of vascularity	**HER2/neu-enriched type (*n* = 5)**	**Luminal type A (*n* = 11)**	**Luminal type B HER2-ve (*n* = 4)**	**Luminal type B (*n* = 7)**	**Triple-negative type (*n* = 7)**	**Total**	***p***
Internal vascularity	4 (16.67%)	7 (29.16%)	3 (12.5%)	6 (25%)	4 (16.67%)	24 (100%)	0.312*
Vessels in rim	1 (10%)	4 (40%)	1 (10%)	1 (10%)	3 (30%)	10 (100%)	
Total	5 (14.71%)	11 (32.35%)	4 (11.76%)	7 (20.59%)	7 (20.59%)	34 (100%)	
* Fisher's exact test							

Tab. 9. Association of ultrasonographic features with hormone receptor and HER2/neu status

Imaging feature (*n* = 51)	**Outcome characteristic**	**Odds ratio (OR)**	**Confidence interval (CI)**	***p* value**
Circumscribed margins	Estrogen receptor	0.38	0.1–1.4	0.129
	Progesterone receptor	0.625	0.18–2.1	0.45
	HER2/neu receptor	0.356	0.07–1.43	0.15
Posterior features	Estrogen receptor	0.92	0.17–4.15	0.94
	Progesterone receptor	1.272	0.298–5.428	0.741
	HER2 neu receptor	0.28	0.06–1.218	0.09
Enhancement	**Estrogen receptor**	**0.074**	**0.016–0.298**	**<0.001**
	Progesterone receptor	**0.068**	**0.013–0.280**	**<0.001**
	HER2/neu receptor	0.59	0.157–2.073	0.43
Shadowing	Estrogen receptor	¥		
	Progesterone receptor	**36.58**	**5.527–866.1**	**<0.001**
	HER2 neu receptor	0.77	0.218–2.598	0.69
Mixed	Estrogen receptor	1.356	0.133–38.17	0.857
	Progesterone receptor	0.81	0.078–8.343	0.850
	HER2/neu receptor	0.59	0.021–6.022	0.72
Vascularity (*n* = 34)	Estrogen receptor	1.30	0.356–4.588	0.68
	Progesterone receptor	0.79	0.232–2.611	0.705
	HER2/neu receptor	1	0.291–3.592	0.75
Internal vascularity	Estrogen receptor	5.65	1.656–21.12	0.003
	Progesterone receptor	1	0.315–3.17	0,77
	HER2/neu receptor	0.72	0.224–2.282	0.56
Vessels in rim	Estrogen receptor	4.949	0.746–40.82	0.07
	Progesterone receptor	2.158	0.348–4.71	0.37
	HER2/neu receptor	**0.019**	**0.0005–0.234**	**<0.001**

A statistically significantly relationship exists between TNBC, an aggressive molecular subtype, and posterior acoustic enhancement and circumscribed margins. The more regular interface between the tumor and surrounding tissue likely results in a circumscribed margin, while internal necrosis and high cellularity probably attenuate the sound wave to a lesser degree, manifesting as posterior acoustic enhancement on ultrasound[16,17]. On sonography, the smooth, well-circumscribed margin in TNBC is considered secondary to the rapid

Tab. 10. Association of ultrasonographic features with molecular subtypes of breast cancer

Margin	HER2/neu-enriched type Odds ratio [CI] (*p*)	Luminal type A Odds ratio [CI] (*p*)	Luminal type B HER2-ve Odds ratio [CI] (*p*)	Luminal type B HER2+ve Odds ratio [CI] (*p*)	Triple-negative type Odds ratio [CI] (*p*)
Circumscribed margins	0.64 [0.081–3.365] (0.60)	0.64 [0.151–2.436] (0.51)	1.22 [0.142–7.791] (0.82)	0.25 [0.01–1.83] (0.21)	**5.12* [1.16–24.85] (0.03)**
Posterior features	0.82 [0.147–6.761] (0.80)	2.274 [0.453–17.45] (0.35)	1.24 [0.147–32.88] (0.84)	0.21 [0.041–1.14] (0.07)	2.5 [0.337–61.82] (0.44)
Enhancement	2.72 [0.597–13.18] (0.19)	0.278 [0.055–1.099] (0.06)	0.335 [0.013–2.683] (0.36)	¥	**29.42* [4.117–725.4] (<0.001)**
Shadowing	0.17 [0.007–1.21] (0.08)	**3.85* [1.12–13.98] (0.02)**	1.79 [0.278–11.53] (0.52)	2.45 [0.539–11.78] (0.24	¥
Mixed	1.608 [0.055–17.2] (0.69)	2.099 [0.202–21.8] (0.50)	2.72 [0.089–31.27] (0.46)	¥	¥
Vascularity	0.567 [0.123–2.727] (0.46)	0.8791 [0.253–3.181] (0.83)	1 [0.158–8.562] (0.97)	1.92 [0.37–14.98] (0.47)	1.20 [0.270–6.519] (0.83)
Internal vascularity	1.77 [0.189–49.21] (0.68)	0.62 [0.127–3.22] (0.56)	1.27 [0.118–37.42] (0.89)	2.91 [0.354–76.72] (0.37)	0.47 [0.0783–3.131] (0.42)
Vessels in rim	0.56 [0.02–5.272] (0.68)	1.59 [0.31–7.838] (0.56)	0.78 [0.026–8.417] (0.89)	0.34 [0.013–2.818] (0.37)	2.09 [0.319–12–76] (0.42)

* significant *p* value
¥ – cannot be calculated, as one of the cell values is zero; CI – confidence interval

growth and high proliferation rate of malignant cells, which leads to the lack of both stromal desmoplastic reaction and fibrosis. Cooper ligaments are believed to be displaced but not significantly disrupted in TNBC[16,18,19]. TNBC is notorious for having a benign appearance on multimodality imaging. The orderly and nestled growth of tumor cells, as seen in benign masses, is also seen in TNBC, which creates fewer layers, leading to improved enhanced through transmission[18]. Li *et al.*[19] established that "pseudo fibroadenoma"-type benign features can often be seen in TNBC. However, there are wide variations in imaging features for TNBC. The present study showed that TNBC can less commonly share imaging features similar to non-TNBC, such as a mass with irregular margins and posterior shadowing. TNBC is also known to lack the presence of suspicious microcalcifications on mammograms[2,20].

El-Maadaw *et al.* conducted a study on 105 patients with pathologically proven breast cancer. Their study concluded that breast masses with non-circumscribed margins and posterior acoustic shadowing had a statistically significant association with luminal A or luminal B subtypes. Masses with circumscribed margins and posterior acoustic enhancement were more likely to be TNBC[21]. Zhu *et al.* studied multimodal sonographic parameters of breast cancer in 85 patients with histologically proven breast cancer. The patients underwent B-mode sonography, real-time elastography, color Doppler flow imaging, and contrast-enhanced ultrasound for the breast masses. However, in their study, the tumor ultrasound shape and posterior acoustic features did not significantly correspond to any molecular subtypes, unlike in our current study[22]. Li *et al.* also studied conventional gray-scale sonographic features and contrast-enhanced sonographic features of 86 breast cancers. However, they could not correlate sonographic features like shape, margins, orientation, echointensity, posterior acoustic features, calcifications, and vascularity with different molecular subtypes. They demonstrated that on contrast-enhanced ultrasonography of these breast masses, the enhancement speed, enhancement degree, and size after enhancement were statistically different for different molecular subtypes, especially the diagnostic efficiency of peak intensity, which was much better for detecting luminal A and HER2-enriched subtypes[23].

In the present study, tumors with circumscribed margins (OR = 5.12, $p = 0.03$) and posterior acoustic enhancement (OR = 29.42, and $p < 0.001$) were highly suggestive of TNBC, indicating that circumscribed margins and posterior acoustic enhancement were 5 times and 29 times more likely to occur in TNBC cases. This finding aligns with the study by Rashmi *et al.*, in which circumscribed tumor margins (OR = 8.0, $p < 0.0001$) and posterior enhancement (OR = 12.7, $p < 0.0001$) were also strongly associated with TNBC[24]. Moreover, in the present study, posterior acoustic shadowing was associated with progesterone receptor status (OR = 36.58, $p < 0.001$) and luminal A status (OR = 3.85, $p < 0.02$). This was again consistent with the study by Rashmi *et al.*, which concluded that tumors with posterior acoustic shadowing were likely to be luminal A or luminal B subtype (OR = 6.2 and 4.2, respectively, both with $p < 0.0001$)[24].

Posterior acoustic shadowing was characteristic of the luminal A subtype, as this subtype is typically low-grade and ER (+) breast cancer. In contrast, posterior acoustic enhancement, calcification,

older age, and vascularity were the characteristics of the HER2 subtype. Although ultrasound is not as sensitive as mammography for detecting microcalcifications, some of which might have even been missed, calcifications detected on ultrasound were more frequent in HER2-enriched tumors than in other subtypes, as also reported by Seo *et al.*[25] and Zhang *et al.*[26]

A study by Tandon *et al.*[14] found that triple-negative cancers were hypervascular compared with non-triple-negative cancers. Another study, by Zhu *et al.*, concluded that the TNBC subtype was significantly associated with rich tumoral vascularity ($p = 0.007$) [22]. Our study revealed that TNBC molecular subtypes (seven out of a total of 10 cases), as well as luminal type B HER2+ve subtypes (seven out of a total of nine cases), had a greater number of cases with vascularity. However, no significant correlation was observed.

The limitation of this study was the small sample size. The non-inclusion of all imaging features from the BI-RADS lexicon was another shortcoming. The results described above indicate that not a single parameter, but a constellation of ultrasonographic features, is required for the molecular profiling of breast cancer.

Conclusion

Exploring the genetic heterogeneity of breast cancer is crucial for developing targeted therapies that will lead to improved treatment outcomes. Some of the ultrasonic parameters show association with the molecular profile of breast cancer and, if strengthened and validated through large-scale studies, they may be utilized as supplementary tools for molecular subtyping of breast cancer in the near future.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Author contributions

Original concept of study: ST, CST. Writing of manuscript: ST, CST. Analysis and interpretation of data: ST, SK. Collection, recording and/or compilation of data: ST, CST, NR. Critical review of manuscript: VT, AJ.

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