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Can Color and Spectral Doppler Ultrasound of Breast Cancers Help to Predict the Immunohistochemistry profile?

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ABSTRACT

Background: To determine the relationship between color and spectral Doppler features of breast cancers and their biomarkers.

Methods: From January 2017 to January 2018, 43 patients with breast cancer were enrolled. Age, the existence of color flow in the Doppler ultrasound, color flow pattern, tumor size, and immunohistochemistry (IHC) subtypes were recorded.

Results: Among 43 breast cancer patients, IHC profiles showed that 36 patients were estrogen receptor (ER) positive, 30 patients were progesterone receptor (PR) positive, and 12 patients were human epidermal growth factor receptor 2 (Her-2) positive. The prevalence of biomarker groups in this study were as follows: luminal A, 21 patients (48.83%); luminal B, 15 (34.88%); Her-2 amplifiers, 2 (4.65%); and triple negative, 5 (11.62%). Thirty-seven patients (86.04%) with malignant masses had detectable flow and six patients (13.95%) had no detectable flow. The ER-positive and PR-positive breast cancers had the highest vascular presence rate in color Doppler ultrasound, but it was not statistically significant. Maximum vessel diameter in the difference biomarker groups and Doppler color patterns with various biomarkers showed no significant the differences.

Conclusion: Based on the results of this article, we could not predict the breast cancer biomarker groups using available Color Doppler features and indexes, so pathology with IHC is still required.

Introduction

Breast cancer is the main cause of cancer-related deaths in women worldwide and it has a diverse heterogeneous natural history with variable responses to different treatments.[1]

Breast cancer is a highly heterogeneous disease with a wide variety of clinical presentations and behavior, which cause a range of responses to treatment and different prognosis.[2-4] Breast cancer is different on a molecular level and traditional histological classification has limitations. Thus, new molecular classifications have been identified such as luminal-A, luminal-B, human epidermal growth factor receptor 2 (Her-2) amplified, and triple-negative.[5] Current management of breast cancer has changed as molecular biology continues to evolve, and targeted therapies based on the genetic, hormonal, or immunohistochemical (IHC) subtypes of breast cancer are being used. Thus, determining IHC subtypes has great value.[6,7] Clinically, luminal-A is the most prevalent subtype and Her-2 positive patients generally show excellent clinical outcomes when they receive an effective targeted therapy

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including Her-2 receptor antibodies, such as trastuzumab. The triple-negative subtype is a group that has only chemotherapy options and these patients have the worst prognosis.[8,9]

Pathological diagnosis is the "gold standard" for distinguishing between the breast cancer molecular subtypes, but it is invasive and costly for patients and time consuming for clinicians. Thus, the development of a non-invasive method will significantly improve the diagnostic procedure.

Several studies have investigated different imaging modalities to differentiate between these biomarker groups such as mammography, magnetic resonance imaging (MRI), and positron emission tomography (PET).[10,11] Researchers have also extensively used gray scale ultrasound (US) to compare between receptor-positive and triple-negative tumors.[12-14] Thus, the relationship between the IHC panel of breast cancers and imaging features is important and promising.

We aimed to determine the relationship between color Doppler images of breast cancer and the molecular subtypes.

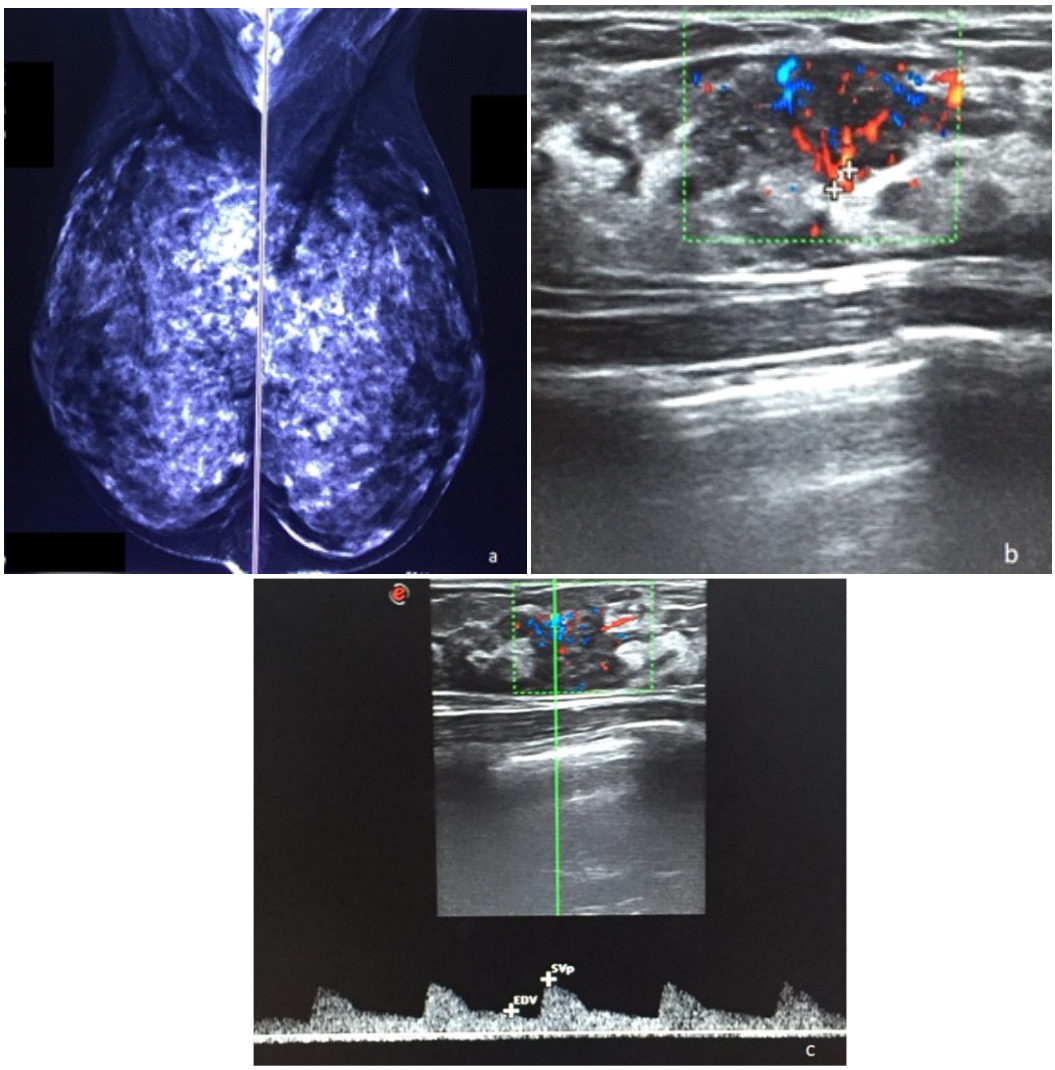

Figure 1. A 37-year-old woman with extremely dense breast tissue and an invasive ductal carcinoma in her right breast.
a) Mediolateral mammography of both breasts shows a dense irregular mass in the right breast deep upper area.
b) Color Doppler image of the same mass shows an irregular mass with a significant flow in the entire mass.
c) Spectral color Doppler of the mass and the peak systolic and end diastolic flow measurements.

Methods

The study protocole was approved by the ethics committee of Tehran University of Medical Science. Written informed consent was obtained from all patients before undergoing any procedures.

The lesions first were imaged with gray-scale sonography to evaluate their sonographic characteristics and to ascertain their Breast Imaging Reporting and Data System (BIRADS) score and the tumor diameter.15 Next, color and spectral Doppler sonography was performed on the suspicious (BIRADS 4 or 5) breast masses based on their grayscale characteristics with a 9- to 12-MHz linear array transducer of an Esaote My Lab Ultrasound machine. All the sonography procedures were performed by a single radiologist who was experienced in breast imaging and who is a faculty member at the university. Optimized parameters were set at a pulse repetition frequency of 750 to 1000 Hz and a low wall pass filter was used to detect low-velocity or low-volume blood flow. The color box included the lesion and a margin of normal

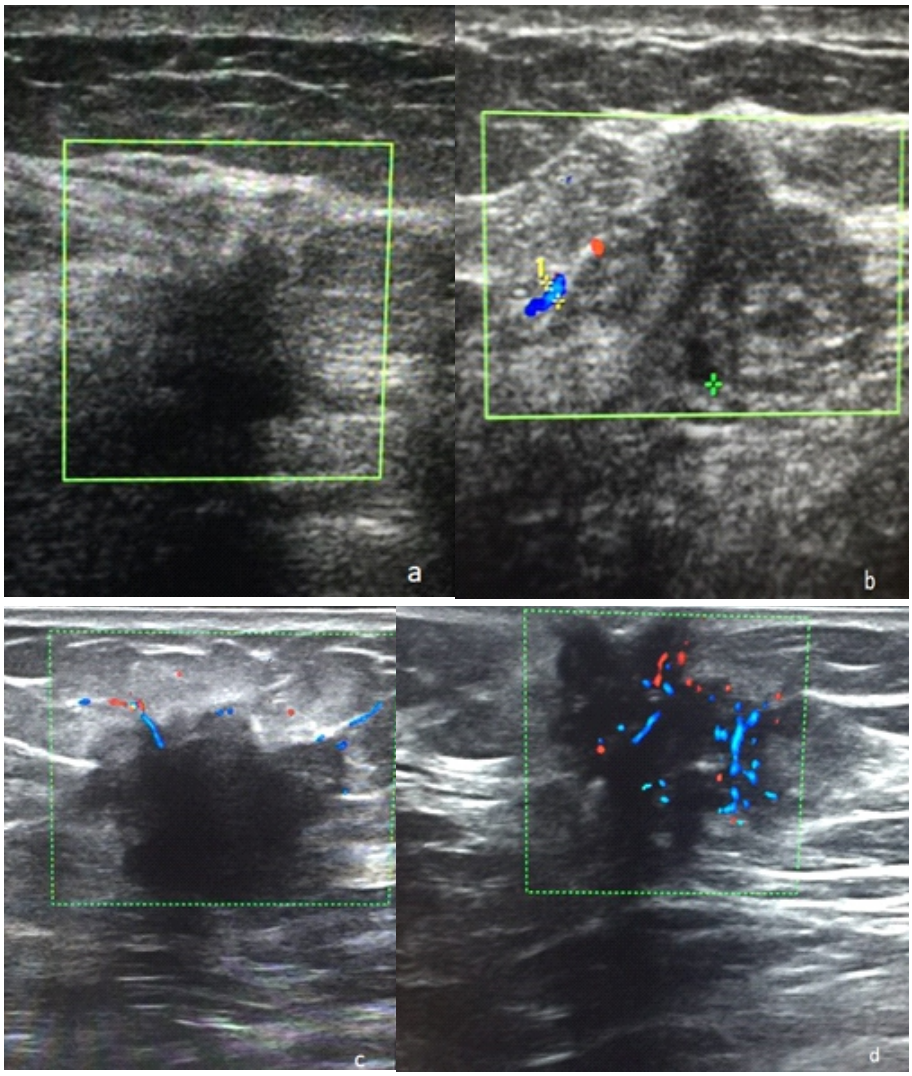

Figure 2. Different patterns of color Doppler flow were seen among the patients in this study. a) No flow; b) Feeder; c) Peripheral flow; and d) Entire mass flow.

neighboring breast tissue with slow scanning without probe pressure in the transverse plane. The color gain was adjusted to a level for the detection of small tumor vessels without background noise.

The existence of flow was considered to be a positive color Doppler test if at least one vessel was depicted inside the lesion or as a feeder beside it, and it showed an arterial flow pattern in simultaneous-pulsed Doppler imaging. If tumoral vascularization was detected, then a more thorough color Doppler study was performed. The cross-sectional size of the masses and the average vessel diameter were recorded. Spectral waveforms for up to three different vessels were measured and the resistive index (RI) was calculated. The calculations were performed by placing the machine's cursor on the flow obtained and selecting the best cycle from the Doppler waveform. The highest RI value obtained was used for analysis (Figure 1).

The color Doppler flow pattern in the patients was subjectively divided in to four groups as follows:

Group 1: no detectable flow (Fig. 2a);

Group 2: a feeder vessel (Fig. 2b);

Group 3: capsular peripheral flow (Fig. 2c); and

Group 4: vessels in nearly the entire part of the mass (Fig. 2d).

All the data, as mentioned above, were recorded before the results for the biopsies on the lesions were gathered. These patients were then followed, if they had accessible malignant pathology after biopsy and were under surgical treatment with IHC results, they kept in the study. IHC results, which were performed on surgical samples, were all obtained in the same pathology department.

We excluded patients who: 1) did not wish to participate in the study; 2) had benign pathology results after biopsy; 3) were transferred to another hospital and we did not have access to their pathology results; 4) underwent neoadjuvant chemotherapy before surgery; 5) had multifocal or multi-centric masses; 6) had a mass larger than 5 cm in diameter (previous studies showed that there might be a difference between detecting vessels in lesions based on their size, so we exclude masses larger than 50 mm in diameter[16]); 7) had cancer with malignant pathology other than invasive ductal; 8) did not have sufficient sufficient clinicopathologic data, and 9) had a non-mass appearance on

sonography. Ultimately, 43 patients with cancer were enrolled in this study.

Estrogen (ER), progesterone (PR), and Her-2 expression in primary tumors were analyzed by IHC staining of formalin-fixed, paraffin-embedded surgically removed breast cancers. ER and PR were considered to be positive if tumors had more than 10% of nuclear-stained cells. Her-2 staining was scored on a scale of 0 to 3+, according to the Herceptin Test guidelines[16]; Her-2 was considered to be positive when it was graded as 3+, while 0 to 1+ were considered to be negative. If the score was 2+, then the fluorescence in situ hybridization test (FISH test) was performed and samples with a >two-fold increase in expression were considered to be positive. ER or/and PR-positive, Her-2 negative tumors, which had Ki-67 ≥14% were considered to be luminal B.[17, 18] Based on the hormonal status, each patient was categorized into one of four distinct molecular subtypes: luminal A (ER and PR-positive), luminal B With positive (ER, PR and Her-2), Her-2-enriched (ER and PR-negative with Her-2), and triple-negative.

Statistical Analyses

Color and power Doppler findings, including RI and the average vascular diameter, were compared statistically among the different receptor groups using an analysis of variance (ANOVA). Similarly, t-tests were used to compare the mean and the Chi-square test was used to compare the distribution of the above-defined Doppler patterns in various receptor groups in malignant breast cancer. Data were presented as the mean ± standard deviation (SD). In all cases, $P<0.05$ was considered to be statistically significant. Data analysis was performed by SPSS (version 16.0) software [Statistical Procedures for Social Sciences; Chicago, Illinois, USA].

Results

There were 43 breast cancer patients whose data were analyzed. The mean diameter of their breast cancer tumors was 20.24 mm (range, 5–45 mm).

The patients' mean age was 50.79±10.03 years (range, 23–72 years). IHC profiles showed that 36 patients (83.72%) were ER-positive, 30 patients (69.76%) were PR-positive, and 12 (27.90%) patients were Her-2 positive.

The prevalence of biomarker groups in this study was as follows: luminal A, 21 patients (48.83%); luminal B, 15 patients (34.88%); Her-2 amplifier, two patients (4.65%), and triple-negative, five patients (11.62%). Thirty-seven patients (86.04%) with malignant masses had detectable flow and six patients (13.95%) did not have detectable flow. The ER-positive and PR-positive types of breast cancers had the highest rate of vascular presence in color Doppler US, and 30 out of 36 (83.33%) ER-positive tumors and 25 out of 30 (83.33%) PR-positive tumors had the detectable flow, but there was no statistically significant difference (P=0.567 and P=0.649). The same was true for Her-2 receptor and no significant relationship was found between vascular presence and Her-2 positivity (P=1.000).

Table1. The relation of different flow patterns and biomarker groups

		Biomarker groups				Total
		a	b	Herceptin	triple-negative	
Flow type in sonography	No flow	3 14.3%	3 20.0%	0 0.0%	0 0.0%	6 14.0%
	Feeder	5 23.8%	2 13.3%	0 0.0%	0 0.0%	7 16.3%
	Peripheral-capsular hyperemia	9 42.9%	6 40.0%	2 100.0%	2 40.0%	19 44.2%
	Whole	4 19.0%	4 26.7%	0 0.0%	+3 60.0%	11 25.6%

Maximum vessel diameter in color Doppler was measured for all masses with detectable arterial flow (in millimeters). The maximum vessel diameter in different biomarker groups was not significantly different (ER, P=0.385; PR, P=0.252; HER-2, P=0.811).

In the lesions that had detectable flow, spectral waveform Doppler was obtained, the maximum RI value was recorded in each mass, and the mean of each biomarker group was measured. There were no significant differences between the RI values for these groups (ER, P=0.599; PR, P=0.861; HER-2, P=0.802).

The malignant masses were classified based on different color mass Doppler patterns (no flow, feeder, capsular peripheral flow, and entire mass flow patterns). There was no significant relationship among these groups and with various biomarkers (ER, P=0.28; PR, P=0.58; HER-2, P=0.76). These defined groups and their relationship to the biomarker groups of breast cancers are summarized in Table 1.

There was no significant difference among

various biomarker groups in luminal A, luminal B, Her2 amplifiers, and triple-negative tumors for these kinds of flow patterns (P=0.53).

Discussion

Currently, breast cancer treatment depends on tumor IHC characteristics.[9, 19] These tests are not available everywhere and they are invasive and expensive. Therefore, researchers have investigated non-invasive methods such as imaging findings that may predict tumor IHC preoperatively to help with the patient's treatment plan. In the literature, some studies have evaluated the correlations between various imaging methods, such as mammography, US, MRI, and PET, with histopathologic markers.[10,11, 20-24] Additionally, newer research also investigated the functional imaging characteristics to determine IHC biomarkers[25], which are promising but not practical to use in clinical centers worldwide.

Angiogenesis via secretion of angiogenic factors plays an important role in the growth, extension, and formation of metastasis in breast cancer.[26, 27] Thus, Doppler sonography may allow evaluation of vascularization to differentiate between benign and malignant breast lesions[16,28,29,] and it may even predict tumor IHC.

Ultrasonography is the most important adjunct modality to mammography that is used to screen and diagnose breast cancer, and it is available and inexpensive. It is also used to guide biopsy and breast interventions.[30] Doppler US can be performed in the same session as the gray scale US and it is used in addition to B-mode US to differentiate between benign and malignant lesions.[31] Breast cancer is a tumor that is usually located superficially, which is suited for Doppler sonography and easy penetrability with the linear transducer. US intravenous contrast agents were not available at our center for use in this setting, and using contrast agents is more expensive, time consuming, and slightly invasive compared to simple color Doppler sonography.

Jose *et al*. showed that, although malignant tumors have significantly more vascularization than benign tumors, malignancy in lesions without flow in color Doppler cannot be eliminated because vessels were unable to be detected in 32% of breast cancers.[16]

There was no similar previous study that explored color Doppler in the different IHC breast cancer groups, so the development of a reliable decision-making non-invasive imaging method, which is a part of usual diagnostic process for patients with breast cancer and which predicts tumor molecular subtypes, is valuable.

Other imaging methods such as grayscale sonography, breast MRI, and PET were used by various researchers to differentiate between various biomarkers. Koo *et al*. showed that triple-negative tumors have a higher 18F fluorodeoxyglucose (FDG) uptake in FDG PET (1.67-fold) than luminal A tumors.[11] Youk *et al*. reported that triple-negative, especially androgen receptor-positive triple-negative cancers, have more necrotic tissue than other cancers, which yields a high signal intensity in T2-weighted MRI images and a high apparent diffusion coefficient value.[10] Yu-Sohn showed that the triple-negative subtype showed a higher incidence of masses on mammography compared to the other subtypes.[24] Zhang proposed a team decision approach that integrated multiple decision trees based on special gray scale features of each subtype and they obtained high accuracy using their models.[13]

Various Doppler sonography parameters have been analyzed to determine their usefulness in differentiating between breast cancer biomarker groups. Our analysis did not show significant differences in the RI, vessel diameter, and flow patterns with different biomarkers.

There were several advantages to this study. First, all patients had T1 (tumor ≤20 mm for the largest diameter) or T2 (tumor >20 mm but ≤50 mm for the largest diameter) masses based on the TNM staging of breast cancer. Second, all patients underwent surgery and IHC was performed on the surgical samples. Third, in contrast to previous studies where biomarkers were used to predict breast cancers, we did not only compare triple-negative tumors with other IHC groups. We included and compared all of the following biomarker groups: luminal A, Her-2 enriched, and triple-negative tumors. Fourth, a useful model of color Doppler pattern classification was introduced for breast cancers, which may be beneficial in future research.

The main limitation of the present study was missing some patients and arbitrarily excluding some patients, so that the number of patients was fairly small. For stronger conclusions, a future study with a larger sample size is required.

In conclusion, There was no significant association between tumor's IHC and different color and spectral parameters of Doppler sonography.

Conflict of Interests

The authors declare that there is no conflict of interest.

Financial Disclosure

None of the authors have any financial interests relevant to the manuscript.

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