RESEARCH ARTICLE

Ultrasound Utility for Predicting Biological Behavior of Invasive Ductal Breast Cancers

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Abstract

Purpose: The aim of the study was to evaluate the correlation of ultrasound features with breast cancer molecular status. Materials and Methods: A retrospective review was performed of ultrasound findings in 263 patients diagnosed with breast invasive ductal carcinoma for comparison with immunohistochemistric results were obtained from each lesion. Relationships between ultrasound findings and molecular status were investigated by using multiple regression analysis by means of stepwise logistic regression. Differences in ultrasound criteria were assessed among women with different molecular status. Results: ER positivity was associated with small size, lobulate, angular or spiculated margin contours, absence of calcification, posterior tumor shadowing and low elasticity score; PR positivity was associated with small size, lobulate or angular or spiculated margin contours and absence of calcification; HER2 positivity was associated with presence of calcification and absence of any echogenic halo. The calculated models of predicted molecular status were accurate and discriminating with AUCs of 0.78, 0.74, and 0.74, respectively. Conclusions: Breast cnacer ultrasound features show some correlation with the molecular status. These models may help to expand the scope of ultrasound in predicting tumor biology.

Keywords: Ultrasound - breast cancer - molecular - biological behavior - logistic regression

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Introduction

Determination of the molecular status of invasive breast cancer is useful as a prognostic and predictive factor, and it has become standard practice in the management of breast cancer because estrogen receptor (ER) and human epidermal growth factor receptor2 (HER2) positivity predict response to endocrine therapy or targeted therapy with monoclonal antibodies directed against HER2 (Bauer et al., 2007; Doreen et al., 2011). If it is possible to predict molecular status on the basis of imaging characteristics, it could assist in both pretreatment planning and prognosis, as well as add to our understanding of the biologic behavior of this disease.

Breast ultrasound has gained widespread acceptance as an adjunct to mammography in diagnosis of evaluating clinical or radiological suspected abnormalities (Gordon et al., 1995; Rizzatto et al., 2001). Stavros et al. reported that it has high sensitivity (98.4%) and negative predictive (99.5%) value for diagnosing breast cancers (Stavros et al., 1995). Ultrasound (US), with its merits of safety and low cost, is becoming a preferred method for both physicians and patients. Hence, more attention is needed toward US imaging to determine whether certain type of tumor biologic factors can be predicted from imaging appearances.

A few studies have looked into correlation between ultrasound features and certain types of biologic behavior (Kim et al., 2008; Wang et al., 2008; Au et al., 2009; Ko et al., 2010; Irshad et al., 2013). Some studies also have discussed the features of triple-negative breast cancers (negativity of ER, PR, and HER2) (Dogan et al., 2010; Ko et al., 2010; Dogan et al., 2012; Krizmanich et al., 2012; Wojcinski et al., 2012; Li et al., 2014). However, correlations between ER, PR, HER2 and ultrasound findings in previous studies were varied and none had calculated the logistic regression model for reliable identification of molecular status (Kim et al., 2008; Wang et al., 2008; Au et al., 2009; Dogan et al., 2010; Ko et al., 2010a; 2010b; Dogan et al., 2012; Krizmanich et al., 2012; Wojcinski et al., 2012; Irshad et al., 2013). The aim of this retrospective study was thus to 1)evaluating the correlation of images findings with the corresponding molecular features, 2)characterizing the various ultrasound features in breast cancer by using logistic regression models, in order to identify the factors that might help in predicting the status of molecular.

Materials and Methods

Patients

Our study group consists of 357 consecutive patients with invasive breast cancer, performed surgery in the Second Affiliated Hospital of Harbin Medical University

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between Dec. 22, 2011 and Jan. 20, 2013, were initially detected by breast ultrasound examinations. 25 of the 347 patients were excluded because of treatment with neo-adjuvant, 48 were excluded due to the failure of surgery, 11 (have the lesions too deep) were excluded owing to indistinct ultrasound imaging and 10 were excluded on account of multiple lesions. Finally, 263 women with definite histological results were evaluated in the study.

Ultrasound examination

To keep the consistency of the imaging, all the real-time scanning was performed by one radiologist with 4 years experience in breast ultrasound, using HITACHI Vision 500 system (Hitachi Medical System, Tokyo, Japan) equipped with a linear probe of 5-13 MHz. The static images and cine clips of B-mode and elastograms, which contain transverse and a sagittal plane, were saved in the database for double-blind analysis. The ultrasound criteria were according to the Breast Imaging Reporting and Data System (BI-RADS) and the elasticity score criteria proposed by Itoh et al (Itoh et al., 2006). The characteristics considered were shown in Table 1. In this study, ''Not circumscribed'' margins were defined when the margin was indistinct, spiculated, angular, or microlobulated. Two breast radiologists with respectively 9, 13 years of clinical experience retrospectively and independently reviewed the ultrasound images. A consensus interpretation was reached in the cases of disagreement.

Histological examination

All resected tumors were stained with hematoxylin-eosin (HE) and performed in formalin-fixed, paraffin-embedded material for pathological diagnosis. Each sample was routinely tested for ER, PR, and HER2 with IHC. The cutoff point for ER- and PR-positive expression level was 1%. For HER2 expression, staining intensity was divided into four grades, with grades 0 and 1 considered as negative, grade 2 as determinate by fluorescence in situ hybridization, and grade 3 as positive.

Statistical analysis

To test the primary hypothesis that ultrasound features are associated with molecular status, univariate logistic regression models were performed and expressed as odds ratio (OR) with 95% confidence intervals (CI). In order to find the best combination of ultrasound-based indicators of molecular, multivariate model were built by means of logistic regression analysis. The stepwise regression method was used to select the parameters which were included in the final models. For a more rigorously screening, the selection of SLE and SLS were all 0.05. The parameters in the final model are independently associated with the molecular. The Hosmer-Lemeshow goodness-of-fit test was used to evaluate the overall fit of the final models. Discrimination and classification of each model or predictor were assessed using the concordance statistic (an approximation of the area under the receiver operating characteristic curve [AUC]). Inter-observer variability was assessed with the Cohen's kappa statistics (Svanholm et al., 1989). All *p*-values are two-tailed and *p* -Values lower than 0.05 were considered statistically significant. All statistical analysis was performed using the SAS System, version 9.2.

The model is as follows: $p=[1\div(1+e^{-z})]$

Where e is a base of natural logarithm, =2.71828..., and z is a linear combination of x_i variables and their estimators bi included in the model:

$z=b_0+b_1x_1+b_2x_2+\ldots+b_nx_n$

Results

Features

The study group comprised 263 patients for whom ultrasound images and molecular results were available and whose mean age at presentation was 50.63±9.90 years (range 22-76 years). The mean size of the lesions was 2.33mm (range 1-10.6 cm). The ultrasound findings are summarized in Table 1.

Observer agreement

Cohen's kappa statistics showed that the better inter-observer agreement was obtained. Level of inter-observer agreement were between 0.61~0.80, which means substantial agreement.

Univariate and multivariate analysis

Assessing the correlation between ultrasound features and biological markers, we found that differences in ultrasound criteria were most pronounced among women with different molecular status. Statistically significant results of the univariate and multivariate regression models, which was established using stepwise regression (SLE=0.05, SLS=0.05), comparing ultrasound parameters of 263 invasive cancers are given in Tables 3-5.

In details, the size of ER positive cancers was 27.7mm, whereas the size of ER negative cancers was 21.5mm (p=0.0012, AUC=0.68). A significantly higher percentage of lobulate (61.2%, p=0.48) and angular (31.9% p<0.0001) margin contour than smooth margin (6.9%) were noted in ER positive cancers (AUC=0.65). In ER positive cancer, posterior acoustic was less commonly enhancement (16.0%) and more commonly shadowing (27.7%, p=0.0005, AUC=0.66). ER positive cancers were less likely to be associated with calcification (pos versus neg, 47.9% versus 64%, p=0.0189, AUC=0.58). Comparing the ER negative cancers, lower elasticity score was common in ER positive cancers (p=0.013, AUC=0.51).

The ultrasound features of PR-positive breast cancers are similar with ER-positive breast cancers, except posterior tumor shadowing and low elasticity score, which had no statistical significance of PR positive cancers. The size of PR positive cancers was 21.2mm, whereas the size of PR negative cancers was 26.5mm (p=0.006, AUC=0.66). A higher percentage of lobulate (60.2%, p=0.0001) and angular (32.3%, p=0.007) margin contour than smooth margin (7.5%) were noted in PR positive cancers (AUC=0.61). PR positive cancers were less likely to be associated with calcification (pos versus neg, 41.6% versus69.6%, p=0.0189, AUC=0.58).

HER-2/neu positivity breast cancers were characterized

Table 1. Modeling Group Patients and Ultrasound Features

Variable	Features		Definition	Patients mm/N (%)
X1	Size			23.2586
X2	Shape	Oval, round	Oval, spherical or round	42 (15.97)
		irregular	Not round or oval	221 (84.03)
X3	Orientation	Parallel	Long axis of lesion parallels the skin line	186 (70.72)
		Not parallel	Long axis, not oriented along the skin line	77 (29.28)
X4	Margin	circumscribed	Sharp demarcation between tumor and surrounding tissue	19 (7.22)
		indistinct	Not circumscribed, blurry, exact position of the margin is hardly to define	244 (92.78)
X5	Margin contour	Smooth	Smooth, even margin without any irregular	27 (10.27)
		Lobulate	Short cycle undulations impart a scalloped appearance to the margin of the mass	169 (64.26)
		Angular, spiculate	Margin is formed or characterized by sharp lines projecting from the mass	67 (25.48)
X6	Post.acoustic	Indifferent	No shadowing or enhancement	142 (53.99)
		Enhancement	Increased posterior echo	61 (23.19)
		Shadowing	Decreased posterior echo, and combined pattern	60 (22.81)
X7	Calcification	Absent	No punctuated extensively hyperechoic foci	125 (47.53)
		Present	punctuated extensively hyperechoic foci	138 (52.47)
X8	Boundary	Abrupt interface	No thin capsule or echoic halo	205 (77.95)
		Echogenic Halo	Blurred, irregular hyperechoic rim around the lesion	58 (22.05)
X9	Echogenicity	Hyper-, isoechoic	Hyper or iso echogenicity than fat, e.g. fibroglandular tissue	253 (96.20)
		Complex, hypoechoic	Hypoechoic than fat tissue	10 (3.80)
X10	Elasticity score	2 and 3		29 (11.20)
		4		137 (52.09)
		5		97 (36.88)
X11	BI-RADS	III and IV		76 (28.90)
		V		187 (71.10)

Table 2. ER Status and Ultrasound Findings of the Breast Cancers

Variable	Feature	ER			β	S.E	Wald	*p*	OR(95%CI)	AUC
		Negative	Positive							
X1	size(cm)	27.67±13.85	21.50±11.08		-0.05	0.01	10.75	0.001	0.96(0.93-0.98)	0.68
X5	Margin contour									
	total						13.21	0.001		0.65
	Smooth	14	13							
	Lobulate	54	115	L *vs* S	1.46	0.52	7.77	0.005	4.29(1.54-11.92)	
	angular	7	60	A *vs* S	2.42	0.67	13.12	0.0003	11.27(3.04-41.81)	
X6	Post.acoustic									
	total						12.41	0.002		0.66
	Indifferent	36	106							
	Enhancement	31	30	E *vs* I	-1.07	0.35	9.16	0.003	0.34(0.17-0.68)	
	Shadowing	8	52	S *vs* I	0.55	0.48	1.32	0.25	1.73(0.68-4.41)	
X7	Calcification									
	Absent	27	98		-0.76	0.33	5.26	0.02	0.47(0.24-0.90)	0.58
	Present	48	90							
X10	Elasticity score									
	2 and 3	5	24		-0.62	0.24	6.56	0.01	0.54(0.34-0.87)	0.51
	4	43	94							
	5	27	70							
	constant					3.84	1.13	11.333	0.0008	

by presentation of calcifications (83.3% in HER-2 positive cancers versus 78.4% in negative cancers, p=0.0001, AUC=0.69). The echogenic halo was observed in 6.9% of HER-2 positive cancers, whereas 25.6% of HER-2 negative cancers (p= 0.0060; AUC=0.60).

AUC analysis

The final logistic models were established by the statistically significant results of the univariate and multivariate regression models. These models, which were established using stepwise regression (SLE=0.05, SLS=0.05), for predicting molecular status as follows:

$$Z\ (ER) = 3.84 - 0.05*X_1 + 2.42*X_{5A} + 1.46*X_{5L} + 0.54*X_{6E} - 1.07*X_{6A} - 0.76*X_7 - 0.62*X_{10}$$

$$Z\ (PR) = 0.73 - 0.04*X_1 + 2.05*X_{5A} + 1.30*X_{5L} - 1.30*X_7$$

$$Z\ (HER2) = -2.45 + 1.79*X_7 - 1.66*X_8$$

Table 3. PR Status and Ultrasound Findings of the Breast Cancers

Variable	Feature	ER			β	S.E	Wald	*p*	OR(95%CI)	AUC
		Negative	Positive							
X1	Size(cm)	26.45±12.90	21.24±11.37		-0.04	0.01	7.6	0.006	0.96(0.94-0.99)	0.66
X5	Margin contour									
	Total						14.51	0.0007		0.61
	Smooth	15	12							
	Lobulate	72	97	L *vs* S	1.3	0.48	7.41	0.007	3.68(1.44-9.41)	
	Angular	15	52	A *vs* S	2.05	0.54	14.42	0.0001	7.78(2.70-22.41)	
X7	Calcification									
	Absent	31	94		-1.3	0.3	18.78	<0.0001	0.27(0.15-0.49)	0.64
	Present	71	67							
	Constant				0.73	0.74	2.37	0.12		

Table 4. HER2 Status and Ultrasound Findings of the Breast Cancers

Variable	Feature	ER		β	S.E	Wald	*p*	OR(95%CI)	AUC
		Negative	Positive						
X7	Calcification								
	Absent	117	8	1.79	0.42	18.68	<0.0001	6.02(2.67-13.58)	0.69
	Present	98	40						
X8	Boundary								
	Abrupt interface	160	45	-1.66	0.63	6.97	0.008	0.19(0.06-0.0.65)	0.6
	Echogenic Halo	55	3						
	Constant			-2.45	0.37	44.03	<.0001		

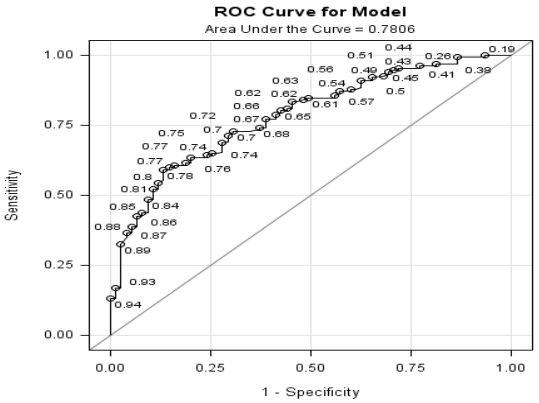

Figure 1. Graph Shows the Average ROC Curves of Predicted ER Status

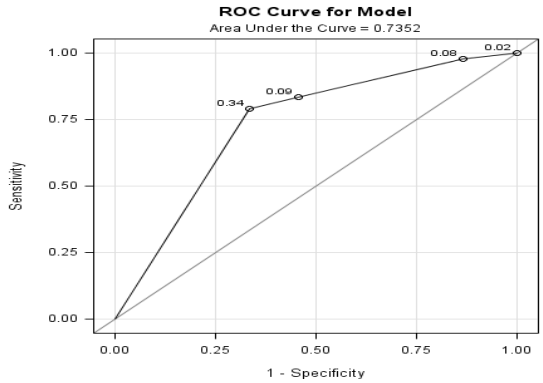

Figure 2. Graph Shows the Average ROC Curves of Predicted HER2 Status

The Hosmer-Lemeshow goodness-of-fit test showed value of 3.16 (p =0.92), 6.30 (p=0.61) and 0.72 (p=0.70), respectively, which mean these models fitted well. The results of receiver operating curves (AUC) are shown in Figure 1-3. The performances of the model that represent ER, PR, and HER2 status were good with AUC of 0.78, 0.74, and 0.74, respectively.

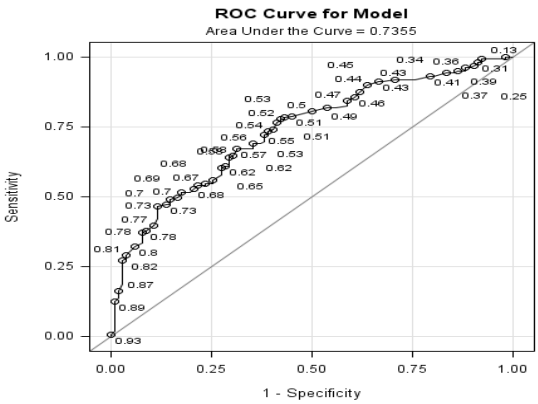

Figure 3. Graph Shows the Average ROC Curves of Predicted PR Status

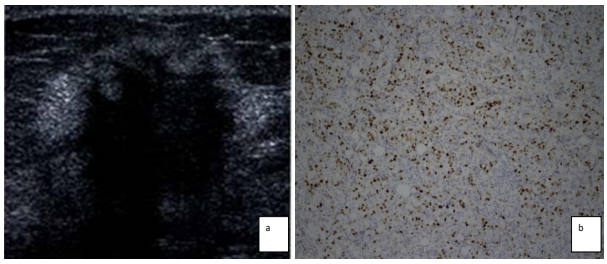

Figure 4. A) Ultrasound Imaging of ER/PR (+): Spiculated Margin Contour, Absence of Calcification, Posterior Tumor Shadowing; Presence of Echogenic Halo. B) Result of IHC: ER-Positive

Discussion

Clinically, breast cancer is a molecularly heterogeneous disease that has been categorized into three basic therapeutic groups. The ER positive group is the most numerous and diverse, with several genomic tests to assist in predicting outcomes for ER patients receiving endocrine therapy (Paik et al., 2004). The HER2 amplified group is

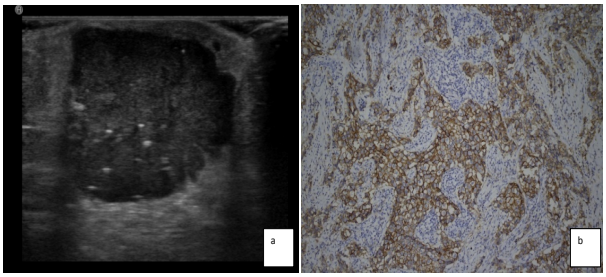

Figure 5. A) Ultrasound Imaging of HER2 (+): Presence of Calcification; Absence of Echogenic halo. B) Result of IHC: HER2-Positive

a great clinical success because of effective therapeutic targeting of HER2 (Slamon et al., 1987). Triple-negative breast cancers (TNBCs, lacking expression of ER, PR and HER2) are a group with only chemotherapy options (Perou, 2011). Hence, these biological markers (ER, PR, and HER2) can be applied to prediction of clinical response to medical treatment and prognosis. This retrospective study revealed that the three biological markers correlating with the ultrasound findings and established three logistic regression models on prediction of molecular status.

From this article, it was realized that the efficiency of single feature to estimate the molecular status was low. However, the efficiency was substantially improved by combination of the ultrasound features. Three models to predict the ER, PR, HER2 status based on patients' pathologic data were developed from the multivariate logistic regression models (SLE=0.05, SLS=0.05), which showed AUC of 0.78, 0.74, and 0.74 respectively. In our study, we found that ER positive was associated with small size, lobulate or angular or spiculated margin contour, absence of calcification, posterior tumor shadowing and low elasticity score; PR positive was associated with small size, lobulate or angular or spiculated margin contour and absence of calcification; HER2 positive was associated with presentation of calcifications and absence of echogenic halo.

Posterior shadowing is an important breast ultrasound criterion. Zonderland. (2000) reported that posterior shadowing is known as a parameter of a moderate power to differentiate between benign and malignant lesions. It has been known that shadowing is provided by a highly cellular fibroblastic proliferation, which more likely existed in low grade tumors. ER positive, which more likely be low grade, was associated with shadowing. This feature is in agreement with previous reports, Irshad. (2013) compared the ultrasound features of ER-negative/PR-negative and ER-positive/PR-positive cancers and concluded that the presence of the posterior shadowing was found to be a very strong predictor of a receptor ER-positive tumor (97% were receptor positive). Study of Ko ES, et al (Ko et al., 2010) showed that triple-negative breast cancer were less likely to be associated with posterior shadowing. Similar to other study, in our study posterior acoustic was more commonly posterior shadowing (27.7%) and less commonly posterior enhancement (16.0%) in ER positive cancers, which also demonstrated that a significant correlation between posterior tumor shadowing and ER status. The performances of posterior tumor shadowing predicted ER status with AUC of 0.66.

Margin contour is another important ultrasound criterion. Correlation was noted between the angular or spiculated and low tumor grade that may be explained by the greater desmoplastic reaction in these leading to noncircumscribe margins (Irshad et al., 2013). Wang. (2008) noted that while they found an association between spiculated margins and HER2 status among patients with ER-negative, ER-positive tumors can also manifest as spiculated masses. In addition, IIdefonso, et al (IIdefonso., 2008) showed that 63% of spiculated masses were ER positive. Similar as our results, circumscribe margin were more frequent in ER or PR negative cancers and lobulate or angular or spiculated margin contour were more frequent in ER or PR positive cancers (93.1%). The performances of margin contour predicted ER status with AUC of 0.66.

Our study results show that ER/PR positive cancers were much more likely to be associated with small size and low elasticity score. Marquet, et al (Marquet et al., 2002) found a statistically significant correlation between tumor size and posterior shadowing, and shadowing was more exited in ER positive breast cancer. Hence, similar to our result, ER/PR positive cancers were tended to be small. Some studies found that elastography was useful in diagnosing breast lesions in the clinical setting (Itoh et al., 2006; Parajuly et al., 2010; 2012). The higher elasticity score, the greater stiffness. This was also in agreement with our report that ER/PR positive cancers were tended to be low elasticity score. The performances of size and elasticity score predicted ER/PR status with AUC of 0.68, 0.51, respectively.

Assessment of HER2 positivity is important for the establishment of a treatment plan and the prediction of prognosis in patients with primary breast cancer (Taucher et al., 2003). Our results are consistent with prior studies (Kim et al., 2008; Ko et al., 2010) , which reported that expression of the HER2 oncogene strongly correlated with presence of calcification on ultrasound or mammography that may be related to prognosis. In addition, we found that the ultrasound findings among HER2 positive cancers were most commonly a mass without echogenic halo, which is defined as one of back scattering in US. The performances of calcification and echogenic halo predicted HER2 status with AUC of 0.74.

The results of our study might have some applications. Some sophisticated laboratory tests are not readily available or not cost-effective in many part of the world and biopsy is invasive and may cause physical and psychological discomfort in patients. On the other hand, ultrasound is a relatively inexpensive, easily operable, widely accessible tool, and recent advances in ultrasound technology, transducer design permit greater spatial, contrast resolution. It is now a key mode of imaging for the clinical diagnosis of breast cancer. If certain ultrasound features of breast cancer could predictive the biological behavior, it would lead to the recommendation to perform biopsies in the lesions of unknown character more frequently. Furthermore, our results could be useful for the implementation of a diagnostic ultrasound criterion

for the breast cancer to indicate the probability status of the molecular, which similar to BI-RADS category as proposed by the American College of Radiology. Such an instrument does not exist which need a long exploration process. It will be assisted in both pretreatment planning and prognosis, as well as add to our understanding of the biologic behavior of this disease.

Our study has the following limitations. First, our study is a retrospective study with small sample size, which needs a large population to confirm our results. The statistical significance of these findings may be insufficient. Second, we did not correlate with cancer stage at diagnosis and did not analyze the incidence of associated DCIS.

In conclusion, ultrasound pattern is correlated with biological markers in breast cancers. Ultrasound features of small size, lobulate or angular or spiculated margin contour, absence of calcification, posterior tumor shadowing and low elasticity score can be used to predict ER status; ultrasound features of small size, lobulate or angular or spiculated margin contour and absence of calcification can be used to predict PR status; ultrasound features of presentation of calcifications and absence of echogenic halo can be used to predict HER2 status. These results can assist in pretreatment planning and prognosis adding to a greater understanding of biological behavior.

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