

# Correlation of the ultrasound imaging of breast cancer and the expression of molecular biological indexes

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**Abstract:** This paper aims to 137 cases of breast malignant tumors confirmed by surgery and pathology were collected. All patients received ultrasound examination and were not treated by radiotherapy or chemotherapy before operation. The preoperative ultrasound images of breast cancer patients and postoperative pathological characteristics of paraffin sections were retrospectively observed. Ultrasound indicators included tumor size, shape, ratio of the longest/ shortest dimension, edge of the spiculation and peripheral hyperechoic halo sign, internal echo types, micro calcification, posterior echo types, blood flow and signs of lymph node metastasis. The pathological indexes included histological type, infiltrating ductal carcinoma (IDC), histological grading, cancer tissue in the interior and edge of the lesion and stroma and expression of molecular biology indexes (ER, PR and C-erbB-2). The correlation of ultrasound imaging of breast cancer and his to pathological type and grading were analyzed and the ultrasonic characteristics and the his to pathological features was compared; then to explore the correlation of ultrasonic imaging of breast cancer and the expression of ER, PR and C-erbB-2. the tumor size of IDC (>2cm) was lager than that of the ductal carcinoma in situ (DCIS) and the early-stage IDC, with statistical significance ( $P<0.05$ ), but compared with the specific carcinoma of breast ( $P>0.05$ ). The ratio of irregular mass of IDC was more than that of the specific carcinoma of breast ( $P<0.05$ ), but without statistical significance compared with the DCIS and early-stage IDC ( $P>0.05$ ). The spiculate margin in the IDC was more than that of the early-stage IDC, DCIS and specific carcinoma of breast ( $P>0.05$ ). The ratio of the longest/ shortest dimension, peripheral hyperechoic halo sign, internal echo, micro calcification, posterior echo types were not related to the types of breast cancer ( $P>0.05$ ). The irregular mass rate in the III IDC were much higher than that in the I and II level IDC ( $P>0.05$ ). The posterior echo attenuation in the I IDC was much higher than the II and III IDC ( $P>0.05$ ). The tumor size, spiculate margin, peripheral hyperechoic halo, internal echo types, micro calcification were not significantly related to the histological grading of IDC. The tumor with larger size (>2cm), enhanced posterior echo, or lower internal echo will have a high histological classification and high rate of cancer tissue ( $P<0.05$ ). The tumor with enhanced peripheral hyperechoic halo sign will have a higher histological classification and higher rate of cancer tissue in the peripheral tissues, compared with the tumors without halo ( $P<0.05$ ). The tumor shape, ratio of the longest/ shortest dimension, spiculate margin were not significantly related to the internal tissues ( $P>0.05$ ). The ratio of the longest/ shortest dimension (>1), spiculate margin and halo signs were related to the positive expression of ER and PR ( $P<0.05$ ). The internal necrosis was related to the negative expression of PR, ( $P<0.05$ ). The tumor size, shape, posterior echo types and blood flow were not significantly related to the expression of ER, PR and C-erbB-2 ( $P>0.05$ ).

**Keywords:** Breast cancer; Ultrasound; Molecular biology.

## INTRODUCTION

In recent years, with the rapid development and wide application of the X ray, CT, ultrasound, Magnetic Resonance Imaging (MRI), the detection rate and accuracy of diagnosis of carcinoma in situ, T<sub>0</sub> cancer and early-stage lesion are increasing (Kasami *et al*, 2008). At present, the study of breast cancer imaging has already reached the molecular biological level, combining with the biomarkers, it is possible to diagnose and predict diseases on the molecular and cellular level (Kim *et al*, 2008). ER, PR and C-erbB-2 are the important prognostic factors, also commonly used in recent studies (Mankotf, 2008). The biological characteristics of tumor determine the development of the morphology of the breast cancer, infecting its ultrasonic imaging. This study combined the

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ultrasonic imaging of breast cancer and molecular biological indexes, aiming to analyze the ultrasonic features on the pathological and molecular level, improving the accuracy of the diagnosis of the ultrasonic clinicians, providing comprehensive and reliable imaging information for its treatment and prognosis evolution. Thus the ultrasonic examination will play a important role in the clinical treatment. Ethics Committee of Xinxiang Central Hospital has approved this research.

## MATERIALS AND METHODS

### General information

137 cases of breast malignant tumors confirmed by surgery and pathology were selected from our hospital during December 2012 to November 2014. All patients were female, aging from 26-81 years, with an average of

49.90±9.90 years. And all of them received preoperative high-frequency ultrasound examination and were not treated by radiotherapy or chemotherapy before operation. Their pathological characteristics of paraffin sections were retrospectively observed and taken as the normal control.

#### **Ultrasonic diagnostic apparatus and detection method**

GE Vivid 7 Dimension, simens Acuson Sequois 512 color Doppler echocardiograph were applied in this study, with the probe of 7-12MHz. The patients were laying on the back, exposing their breast detected by the probe. Firstly, wheel-shaped scan the breast around the nipple, spin 360° along the clockwise over lappingly, then scan the central zone of the breast, then the axilla. When the patients were detected the axilla, with hands down the head, extending two arms to expose the axilla, the size, shape and blood flow of the lymph node should be recorded. Correspondingly, the imaging characteristics of the tumor size(<1cm/1-2cm/>2cm), shape(regular/irregular), ratio of the longest/ shortest dimension (>1/<1), edge of the spiculation (Yes/No) and peripheral hyperechoic halo sign (Yes/No), internal echo types (non-echo zone/ no non-echo zone), micro calcification (Yes/No), blood flow (0/1/2/3 levels) and signs of lymph node metastasis (Yes/No). The criteria of the blood flow and signs of lymph node metastasis were shown in the following: (1) According to the Adler grading standards the blood flow could be divided into four grades including (Tafreshi *et al*, 2010): 0 level, no blood stream signs in the diseased region; 1 level, a few blood flow, one or two spotted blood flow (the diameter<1mm); 2 level, middle blood flow, 3-4 spotted blood vessel/one main blood vessel, with the vascular length close to or more than half of the diseased region; 3 level, rich blood flow, more than 5 spotted blood vessels or 2 longer blood vessels. The 0-1 levels were defined as the hypovascular tumor while the 2-3 levels as the hypervascular tumor. (2) The axillary lymph node could be divided into the benign and malignant type according to the previous literature (Ferrara *et al*, 2007). The benign one involved the three standards: ①Oval shape, ratio of longer diameter/shorter diameter ≥1.5; ②normal hilus structure, homogeneous “C” hypo-echoic signs on the cortex; ③ few evenly-distributed blood signs. The other lymph node types were classified as metastatic of metastatic lymph nodes.

#### **Pathological methods**

Two experienced pathology physician made a diagnosis of the breast disease with double-blind method. The regular tumor sections were fixed by 10% formalin, embedded by paraffin, dyed by HE. Then they were dealt with immunohistochemical method which was assisted by the department and dyed by the Elivision™ plus method, the dying process was as followings:

1) Dewax and hydrate the paraffin sections, wash three times with PBS (PH 7.4), each time lasting for 3 min;

- 2) Repair the tissue antigens accordingly;
- 3) Add one drop or 50 μ solution (3% hydrogen peroxide) if necessary, incubate 10 min at room temperature with the purpose to block the endogenous peroxidase, then wash three times with PBS (PH 7.4), each 3min;
- 4) Clear up the PBS solution, add one or 50μ primary antigen (selected by users), incubate 60 min at room temperature or 4°C overnight;
- 5) Wash with PBS for three times each time lasts 5 min;
- 6) Clear up the PBS, add one drop or 5 0μ Polymer enhancer (Solution A), incubate at room temperature for 20min;
- 7) Wash with PBS for three times each time lasts 3min;
- 8) Clear up the PBS, add one drop or 50 μ enzyme-labeled antibody against mouse/ rabbit Polymer (Solution B), incubate at room temperature for 30 min;
- 9) Wash with PBS for three times each 3 min;
- 10) Clear up the PBS, add two drops or 100 μ fresh DAB or AEC liquid, then observe under the microscope for 3-10min, with the positive expression dyed with brown or red;
- 11) wash with distilled water or tap-water, redye with hematoxylin, differentiate with 0.1% hydrochloric acid, wash up with tap-water, PBS wash up to present with blue;
- 12) If use DAB to develop, dry with gradient alcohol, then kept in the neutral gum (xylene); if use AEC, they can not be dehydrated with gradient alcohol, but sealed directly in the mounting medium.

The criteria of ER and PR: The claybank particle in the nucleus reflects the positive expression of ER and PR, with 1-9% referring to low expression, ≥10% high expression. While the criteria of C-erbB-2: The claybank particle in the diolame reflects its positive expression, with <10% meaning negative, 10-30% weak positiveness, >30% positive. All the regents in this trial were bought from Fuzhou Maixin Biotechnology Co., Ltd.

#### **STATISTICAL ANALYSIS**

SPSS 13.0 was used to analyze the data in this study,  $\chi^2$  was used to check the measurement data, P<0.05 was statistically significant.

#### **RESULTS**

##### **Pathological results**

The Pathological results of the 137 breast cancer patients showed that the tumor diameter was among 0.5-6.1 cm, the biological indicator expression was shown in table 1. The positive expression rate of ER, PR and C-erbB-2 were 56.2%, 45.3% and 75.2% respectively.

##### **Ultrasonic imaging results**

As shown in table 2, among the 137 patients with breast cancer, there were 64 cases (46.7%) with tumor size >2cm,

**Table 1:** expression of molecular biological indicators in 137 breast cancer cases (N, %)

Molecular biological indicators	ER	PR	C-erbB-2
Cases (N)	77	62	103
Positive expression percent (%)	56.2	45.3	75.2

**Table 2:** Ultrasonic imaging of breast cancer in 137 cases (N, %)

Ultrasound imaging of breast cancer		Cases (N)	Occurrence rate (%)
Tumor size	<1cm	17	12.4
	1-2cm	56	40.9
	>2cm	64	46.7
Tumor shape	Regular shape	24	17.5
	Irregular shape	113	82.5
Ratio of the longest/ shortest dimension	>1	49	35.8
	<1	88	64.2
Spiculated margin	Yes	80	58.4
	No	57	41.6
Hyperechoic signs	Yes	40	29.2
	No	97	70.8
Interior anechoic area	No	113	82.5
	Yes	24	17.5
Microcalcification	Yes	82	59.9
	No	55	40.1
Posterior echo signs	Attenuation	43	31.4
	Enhance	26	19.0
	No changes	68	49.6
Lymphatic metastasis	Yes	61	44.5
	No	76	55.5
Blood flow	0	5	3.6
	1	54	39.4
	2	50	36.5
	3	28	20.4

56 cases with 1-2cm tumor (40.9%), and 17 cases <1cm (12.4%); 113 cases with irregular-shape tumor (82.5%); 49 cases with the ratio of the longest/shortest dimension>1 (35.8%); 80 cases with spiculate margin (58.4%); 40 cases with peripheral hyperechoic halo sign (29.2%); 24 cases with internal non-echo signs (17.5%); 87 cases with micro calcification (63.5%); 43 cases with internal non-echo attenuation (31.4%), 26 cases with internal non-echo enhancement (19.0%), 68 cases without internal non-echo changes (49.6%); 61 cases with lymph node metastasis (44.5%); 28 cases with blood flow 3 level (20.4%), 54 cases with 1 level (39.4%), 50 cases with 2 level (36.5%), and 5 cases with 0 level (3.6%).

#### **Correlation of ultrasonic imaging features and the expression of molecular biological indicators**

From table 3 we can see that in the spiculate margin group, the expression rate of ER and PR are 66.3% and 53.8% respectively, both higher than those of the burr-free group ( $\chi^2=7.883, 5.600, P<0.05$ ). The expression rate of ER and PR in the peripheral hyperechoic halo signs group were 75.0% and 60.0% respectively, higher than those of the group without peripheral hyperechoic halo signs

( $\chi^2=8.108, 4.957, P<0.05$ ), indicating that their expression is associated with the peripheral hyperechoic halo signs. The negative expression rate of PR in the group with internal non-echo signs were 75.0%, higher than that of the group without internal non-echo signs ( $\chi^2=4.819, P<0.05$ ), indicating that the negative expression of PR was influenced by the internal non-echo signs; the positive expression of C-erbB-2 in the micro calcification group is 81.7%, higher than that of the group without micro calcification ( $\chi^2=4.661, P<0.05$ ).

The tumor size is not significantly related to the expression of ER, PR and C-erbB-2 ( $\chi^2=0.302, 1.248, 3.817, P>0.05$ ). Neither the Posterior echo signs ( $\chi^2=0.029, 0.884, 3.792; P>0.05$ ), nor the Lymphatic metastasis had strong relation with the expression of ER, PR and C-erbB-2 ( $\chi^2=1.295, 2.530, 0.117; P>0.05$ ). The blood slow inside the tumor did not significantly influenced the expression of ER, PR and C-erbB-2 ( $\chi^2=1.247, 2.065, 2.511; P>0.05$ ).

#### **DISCUSSION**

Molecular biological behavior determines the

morphological changes of breast cancer, and then affects the performance of the ultrasound imaging. ER, PR and C-erbB-2 are three common prognostic factors in breast cancer. ER, PR are located in the epithelial nucleus, involving in a variety of diseases, especially in breast cancer, with the positive expression rates as high as 50%-80%, 60-70% respectively, and their expression level in elder women and postmenopausal women are higher than those in the younger women. Therefore, they are two important prognostic factors in breast cancer (Tafreshi et al, 2010). Their expression determine whether the endocrine therapy can be used. A number of studies have confirmed that there would be a weak endocrine therapy effect in treating the tumor with negative expression of ER, PR, because of the high malignant, and the disease-free survival and overall survival time were very short. C-erbB-2 is the homologous genes of Neu gene in human beings, located in the 17 chromosome, also called HER-2, belonging to the EGFR family, presenting a over-expression state in 20-30% breast cancer (Ferrara et al, 2007). It will encode 185KD-molecular-weight trans membrane tyrosine kinase growth factor receptor which will express about 25-60% breast cancer (McCavert et al, 2009). A large number of research had showed that C-erbB-2 and its receptor in breast cancer had close relation with tumor grading, distant metastasis and high mortality, it was another prognostic factors secondary to estrogen receptor, playing an important role in recurrence. At present, the tumors with positive expression of C-erbB-2 are treated by Herceptin molecular targeted therapy. Herceptin therapy can improve the disease-free survival and overall survival and life quality in patients with early and advanced metastatic breast cancer, with a significant effect of improving prognosis. Therefore, the expression of molecular biological indicators can guide clinical drug selection and prognosis of tumor. In this study, the ultrasound imaging features of breast cancer were combined with molecular biological indicators and they were related to each other to some degree.

Tumor shape: the malignant invasion will cause irregular shape. Stavros et al hold the idea that the ratio of the longest/ shortest dimension  $>1$  was because the malignant tumor grew out of the normal tissue leading to the enlargement of the front and post diameter, it was an important index to identify the malignant degree of the tumor. In this study, the positive rate of ER and PR were 75.5% and 59.2% respectively in the tumor with the ratio of the longest/ shortest dimension  $>1$ , significantly higher than the tumor with the ratio  $<1$  of 45.5% and 37.5%. While low positive rate of ER and PR predicted a low invisibility, indicating there was a higher malignant degree in the ratio  $>1$  than the ratio  $<1$ . Though some studies showed that the ratio of the longest/shortest dimension  $>1$  was one of the ultrasonic imaging features of breast cancer (Tsunoda, 2008). However, it did not show a high positive rate of the ratio of the longest/

shortest dimension  $>1$  in this study of 35.8% (49/137), indicating that more and more malignant tumor performed characteristics similar to that of the benign tumor. Therefore, the ratio of the longest/ shortest dimension  $<1$  should be carefully noticed in the ultrasonic diagnosis, combining with other malignant characteristics. Since there are not similar literature at present, this conclusion should be further proved.

## CONCLUSION

The ultrasonic imaging features had relation with the expression of ER, PR and C-erbB-2. The positive expression rates of ER and PR in the tumor with the ratio of the longest/ shortest dimension  $>1$  are higher; the tumor with spiculate margin and periperal high echo signs will perform a high ER and PR positive expression rate; the tumor with internal anechoic area presents with a high negative expression rate of PR; there will be a high positive expression rate of C-erbB-2 in the tumor with calcification. The tumor size, posterior echo type, blood flow and signs of lymphatic metastasis have no significant relation to the expression of ER, PR and C-erbB-2.

The results in this study showed that the ultrasonic imaging of breast cancer is related to the histological classification. Different histological types will reflect different growth and characteristics of tumor. The occurrence rate of the tumor  $>2$ cm in the IDC was higher than the DCIS and early-stage IDC, without significant difference with the specific tumor. The occurrence rate of irregular shape and spiculate margin in the IDC were higher than in the specific type of breast cancer. Peripheral hyperechoic halo sign, micro calcification, internal echo and posterior echo signs were not obviously related to the histological types. Therefore, the irregular shape and spiculate margin are the typical characteristics in the IDC region different from other types of breast cancer. The tumor size in the IDC was larger than that of the DCIS and IDC, with the potential reason that the infiltration lead to the rapid growth of the tumor.

Ultrasonic imaging of breast cancer was related to the histological differentiation in the IDC. The occurrence rate of irregular tumor in the III IDC was much higher than I and II level; the occurrence rate of the posterior echo attenuation in the I level was much higher than the II and III IDC. The tumor size, boundary characteristics, internal echo and microcalcification were not significantly related to the IDC levels. III IDC had a bad differentiation, high malignant degree, enhanced invasion and an irregular shape. The high rate of the posterior echo attenuation in the I IDC indicated that the the tumor was well differentiated with low malignancy.

There were more cancerous tissues in the heterogeneous and low-echo tumor than in the heterogeneous and iso-

**Table 3:** Relationship between ultrasound imaging of breast cancer and molecular biological indicators (N)

Breast cancer ultrasound imaging	Cases (N)	ER			PR			C-erbB-2			
		+	$\chi^2$	P	+	$\chi^2$	P	+	$\chi^2$	P	
Tumor size	<1cm	17	9			6			10		
	1-2cm	56	33	0.302	>0.05	28	1.248	>0.05	41	3.817	>0.05
	>2cm	64	35			28			52		
Tumor shape	Regular shape	24	12	0.455	>0.05	8	1.669	>0.05	16	1.131	>0.05
	Irregular shape	113	65			54			87		
Ratio of the longest/ shortest dimension	>1	49	37	11.551	<0.05	29	5.973	<0.05	35	0.576	>0.05
	<1	88	40			33			68		
Spiculated margin	Yes	80	53	7.883	<0.05	43	5.600	<0.05	56	2.768	>0.05
	No	57	24			19			47		
Hyperechoic signs	Yes	40	30	8.108	<0.05	24	4.957	<0.05	28	0.813	>0.05
	No	97	47	0.049	>0.05	38	4.819	<0.05	75	1.131	>0.05
Interior anechoic area	No	113	64			56			87		
	Yes	24	13			6			16		
Micro calcification	Yes	82	51	2.978	>0.05	42	2.933	>0.05	67	4.661	<0.05
	No	55	26			20			36		
Posterior echo signs	Attenuation	43	24			22			32		
	Enhance	26	15	0.029	>0.05	11	0.884	>0.05	16	3.792	>0.05
	No changes	68	38			29			55		
Lymphatic metastasis	Yes	61	31	1.295	>0.05	23	2.530	>0.05	45	0.117	>0.05
	No	76	46			39			58		
Blood flow	0	5	3			2			5		
	1	54	32	1.247	>0.05	28	2.065	>0.05	38	2.511	>0.05
	2	50	25			22			38		
	3	28	17			10			22		

echoic or mixed echo; a higher rate of cancerous tissue will be found in the enhanced posterior echo tumor than in the attenuated tumor or non-echo changes; the cancerous tissues in the hyperechoic halo tumor were more than the tumor without hyperechoic halo. The morphology and size of breast cancer had no significant relation to the inner structures. The significant relation could not be seen between the spiculate margin and the peripheral tissue. This results reflected that the ultrasonic imaging of breast cancer was closely related to its histopathological characteristics. Since ultrasound imaging was based on pathology, the inner echo and rear echo signs reflected the inner structure of breast cancer. The tumor with more cancerous tissues inside will have rather unique component, less acoustical interfaces, better acoustic transmissibility, lower inner echo and enhanced rear echo signs.

The tumor (the ratio of the longest/shortest dimension >1) has a higher positive expression of ER and PR, so do the tumors with spiculate margin or peripheral hyperechoic halo signs. The tumor with no echo inside will show a

high positive PR rate. The positive expression rate of C-erbB-2 in the tumor with calcification is higher. The tumor size, posterior echo signs, blood flow and lymph node metastasis have no significant correlation to the expression of the three molecular biological indexes. Therefore, the expression of molecular biological indexes is something related to the ultrasonic imaging of breast cancer whose features reflect the biological characteristics.

The study of the correlation of ultrasonic imaging of breast cancer and biological markers will contribute to the understanding of the breast cancer characteristics of the ultrasonic clinicians, improving the accuracy of ultrasonic diagnosis. The ultrasonic imaging of breast cancer can help predict the molecular biological expression of the tumor, providing a comprehensive imaging information for the choice of tumor treatment and prognosis evolution.

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