

Correlations between the expression of C-erbB-2, CD34 and ER in breast cancer patients and the signs of conventional ultrasonography and ultrasound elastography

Y. LIU¹, W. XIONG², J.-M. XU¹, Y.-X. LIU¹, J. ZHANG³

¹Department of Ultrasound Diagnosis, China Meitan General Hospital, Chaoyang District, Beijing, P.R. China

²Department of Radiochemical, Tangshan People's Hospital, Lunan District, Tangshan, Hebei, P.R. China

³Department of Ultrasound Diagnosis, Dongzhimen Hospital, Beijing University of Chinese Medicine, Dongcheng District, Beijing, P.R. China

Yan Liu and Wei Xiong contributed equally to this study

Abstract. – OBJECTIVE: This study aims to investigate the correlations between the expression of human epidermal growth factor receptor 2 (C-erbB-2), CD34, and estrogen receptor (ER) in breast cancer (BC) patients, and the signs of conventional ultrasonography and ultrasound elastography.

PATIENTS AND METHODS: Clinical data of 88 patients who were pathologically diagnosed as breast cancer were retrospectively analyzed. HI VISION Avius color ultrasound device was used to perform ultrasound and related ultrasound parameters were analyzed. Expressions of C-erbB-2, CD34, and ER were detected by immunohistochemistry.

RESULTS: There was a correlation between positive expression of C-erbB-2 and the blood supply, lymph node metastasis and microcalcification in patients ($p < 0.05$). Patients were divided into high expression group and low expression group according to the median expression level of CD34. Expression of CD34 was correlated with the ultrasound sign of lymph node metastasis ($p < 0.05$). Positive expression of ER was correlated with the morphology, margin, and perimeter of the tumor ($p < 0.05$). Expression levels of C-erbB-2, CD34, and ER were found to be increased in BC patients.

CONCLUSIONS: Combination of upregulated expression of C-erbB-2, CD34, and ER and signs of ultrasound can improve the diagnosis of BC.

Key Words:

C-erbB-2, CD34, ER, Ultrasound, Breast cancer.

Introduction

Breast cancer (BC) is one of the most common cancers in females worldwide. BC accounts for more than 20% of the female malignant tumor and brings a serious threat to women's life as well as physical and mental health^{1,2}. In the United States, BC ranks first among female malignant cancers, and the mortality rate is among the top three³. In China, the incidence of BC is low, but is gradually rising every year, and the onset age is becoming younger and younger⁴. Studies⁵ have shown that the cure rate of BC through early treatment can reach 90%, but cure rate can only reach 40% for advanced stages. Therefore, early diagnosis and treatment are critical for the survival of BC patients.

The pathogenesis of BC is still unclear, which leads to poor outcomes of prevention and treatment of this disease⁶. Development of imaging techniques has enabled the early diagnosis of BC, which is beneficial for prognosis⁷. Ultrasound elastography has been widely used in clinical practices, including the diagnosis of BC. Studies⁸ have shown that human epidermal growth factor receptor-2 (C-erbB-2), CD34, and estrogen receptor (ER) are indicators in the diagnosis of BC.

In this study, we investigated the correlations between the expression of C-erbB-2, CD34, and ER in breast cancer patients and the signs of conventional ultrasonography and ultrasound elastography. Our study provided references for the diagnosis of BC.

Patients and Methods

Patients

A total of 88 BC patients who underwent surgery in China Meitan General Hospital and Tangshan People's Hospital & Tangshan Tumor Hospital from May 2014 to September 2015 were selected. Age of patients ranged from 29 to 70 years, with a mean age of (55.1 ± 11.5) years. Those patients included 71 cases of infiltrating ductal carcinoma, 9 cases of ductal carcinoma *in situ*, 4 cases of invasive lobular carcinoma, 3 cases of mucinous carcinoma, and 1 case of phyllode tumor (Table I for details).

Inclusion and Exclusion Criteria

Inclusion criteria: BC patients, patients' age > 18 years; duration of the disease $\geq$ six months; patients who did not receive drug treatment recently; patients who did not receive radiotherapy and chemotherapy before surgery; patients without inherited diseases and congenital defects.

Exclusion criteria: patients who have blood relationship with other patients; patients with respiratory disease; patients with depression, patients with hypertension, patients with diabetes mellitus; patients who do not cooperate with treatment and follow-up.

Detection Method

HI VISION Avius (Hitachi Color Doppler Ultrasound Scanner, Tokyo, Japan) was used to perform ultrasonography for 88 patients. A Real-time array of high-frequency probes was used to perform ultrasound elastography. Probe frequency was 6-13 MHz. Two-dimensional

ultrasonography image signs were observed. Those signs included lesion size, shape, margin, burr, peripheral and posterior echo attenuation, and axillary lymph nodes. Color Doppler ultrasound was used to detect the condition of blood flow in patients. Blood supply was assessed by using the Adler semi-quantitative grading method. To evaluate the patient's lesion stiffness in Real-time elastic ultrasound mode and elasticity assessment was performed according to Itoh 5 points scoring method, the score was greater than three.

Immunohistochemical Detection

Tissues were embedded in paraffin and tissue sections were prepared. Dewaxing was performed by passing a series of graded concentrations of ethanol. Antigen retrieval was performed and tissue sections were incubated with H_2O_2 (3%) at room temperature for 10-15 min. After washing with phosphate-buffered saline (PBS), blocking was performed by using the bovine serum. After that, slides were incubated with primary antibodies of C-erbB-2, CD34 and ER (Doko) at room temperature for 2 h. After washing with PBS, polymer enhancer was added and incubated at room temperature for 30 min. After washing with PBS, enzyme-conjugated anti-mouse/rabbit polymer was added and incubated at room temperature for 30 min. After washing with PBS, diaminobenzidine (DAB) solution was added. After hematoxylin counterstain, slides were dehydrated and sealed.

Detection and Assessment Standards of Related Indicators⁹

C-erbB-2 positive expression: cells with brownish brown cell membrane or brown particles were treated as positive cells. 0 and 1+ point was negative. Tissues with 2+ points were further subjected to fluorescence *in situ* hybridization (FISH) to further confirm the expression of C-erbB-2. 3+ was positive.

CD34 microvessel count: according to the method of microvascular counting, vascular endothelial cells with brownish yellow staining on cytoplasm or membrane were treated as positive. Endothelial cells that can be isolated from peripheral blood vessels and tumor cells as well as connective tissue were all treated as single countable microvessels. Three most densely colored visual fields were selected under low magnification (100 $\times$), and microvessel count was performed under high magnification

Table I. Patients' clinical data.

Groups		Cases [no. (%)]
Age	> 50	43 (48.86)
	$\leq$ 50	45 (51.14)
Residence	Urban area	62 (70.45)
	Rural area	26 (29.55)
Education level	< College	32 (36.36)
	$\geq$ College	56 (63.64)
Cancer type	Infiltrating ductal carcinoma	71 (80.68)
	Ductal carcinoma <i>in situ</i>	9 (10.23)
	Invasive lobular carcinoma	4 (4.54)
	Mucinous carcinoma	3 (3.41)
	Phyllode tumor	1 (1.14)

(400 ×). Median volume diameter (MVD) was calculated and tissues were divided into high expression and low expression groups according to the median expression level.

ER-positive expression: cells with brown-yellow particles in nucleus and non-specifically stained cells in the high-intensity background were treated as positive cells. A total of 4 visual fields were observed, and positive cells accounted for < 1% indicated ER-positive.

Statistical Analysis

SPSS20.0 software (Beka, Shanghai, China) was used to analyze all the collected data. Count data were expressed as rate (%) and compared by chi-square test. $p < 0.05$ was considered to be statistically significant.

Results

Correlations Between Positive Expression of C-erB-2 and Ultrasound Signs

Positive expression of C-erB-2 was not significantly correlated with tumor size, tumor shape,

tumor edge (smooth or not), perimeter (clear or not), and echo attenuation (negative or positive) ($p > 0.05$). However, positive expression of C-erB-2 was associated with blood supply, lymph node metastasis, and microcalcification ($p < 0.05$) (Table II for details).

Correlations Between Positive Expression of CD34 and Ultrasound Signs

Patients were divided into high expression group and low expression group according to the median expression level of CD34. Correlation analysis between positive expression of CD34 and ultrasound signs showed that expression of CD34 was not significantly correlated with tumor size, tumor morphology, margins, perimeter, echo attenuation, and microcalcification of blood supply ($p > 0.05$), but was significantly correlated with lymph node metastasis ($p < 0.05$) (Table III for details).

Correlations Between Positive Expression of ER and Ultrasound Signs

Correlation analysis between positive expression of ER and ultrasound signs showed that

Table II. Correlations between positive expression of C-erB-2 and ultrasound signs.

Ultrasound signs	C-erB-2		X^2	p
	Positive (no. = 53)	Negative (no. = 35)		
Tumor size				
> 2 cm	33	15	3.202	0.074
≤ 2 cm	20	20		
Tumor Morphology				
Regular	21	15	0.091	0.763
Irregular	32	20		
Edge				
Smooth	21	16	0.321	0.571
Not smooth	32	19		
Perimeter				
Clear	22	14	0.019	0.88
Not clear	31	21		
Echo attenuation				
Yes	28	23	1.436	0.231
No	25	12		
Blood supply				
Rich blood supply	40	12	14.792	0.001
Lack of blood supply	13	23		
Microcalcification				
Yes	45	17	13.369	0.001
No	8	18		
Lymph node metastasis				
Yes	44	18	10.106	0.001
No	9	17		

Table III. Correlations between positive expression of ER and ultrasound signs.

Ultrasound signs	CD34		χ^2	<i>p</i>
	High expression (no. = 52)	Low expression (no. = 36)		
Tumor size				
> 2 cm	32	16	2.507	0.113
≤ 2 cm	20	20		
Tumor Morphology				
Regular	18	18	2.083	0.149
Irregular	34	18		
Edge				
Smooth	20	17	0.670	0.413
Not smooth	32	19		
Perimeter				
Clear	17	19	3.550	0.060
Not clear	35	17		
Echo attenuation				
Yes	33	18	1.582	0.208
No	19	18		
Blood supply				
Rich blood supply	35	17	3.550	0.060
Lack of blood supply	17	19		
Microcalcification				
Yes	40	22	2.555	0.110
No	12	14		
Lymph node metastasis				
Yes	42	20	6.497	0.011
No	10	16		

expression of ER was not significantly correlated with tumor size, echo attenuation, microcalcification, and blood supply ($p > 0.05$), but was significantly correlated with tumor morphology, margin, and perimeter ($p < 0.05$) (Table IV for details).

Discussion

Malignant tumors are one of the serious health problems faced by the whole world. BC, as one of the most common cancers in females, results in more than one million new cases and 500,000 deaths every year¹⁰. Fan et al¹¹ showed that incidence of BC ranks first among all malignancies in female urban residents in China, and the mortality rate has reached 38.00%, indicating BC has a serious impact on the life safety and life quality of women, which will continue to draw people's attention. As a heterogeneous disease, BC shares similar pathological processes with many types of tumors, leading to poor treatment outcomes and prognosis¹². Development of cancers is accompanied with morphological changes of the tumor caused by changes in gene expression.

Morphological changes can be reflected by ultrasound signs. Therefore, there should be a link between ultrasound signs and related biological indicators¹³.

With the development of diagnostic techniques, early diagnosis rate of BC has gradually increased¹⁴. Ultrasound elastography was first proposed by Ophir et al¹⁵. Its principle is imaging on different tissue hardness, and its first clinical application has shown its advantages in many aspects, which also has a promising diagnostic value in the detection of superficial organ diseases. C-erbB-2, CD34, and ER are the most representative biological indicators for clinical diagnosis of BC. Expression of C-erbB-2 is closely correlated with the occurrence and development of BC. The measurement of the expression level of C-erbB-2 provides references for the determination of the degree of malignancy and tumor metastasis¹⁶. Studies have shown that C-erbB-2 differential expression is correlated with the prognosis of patients with BC, and it can be used as an independent factor for the diagnosis and evaluation of BC¹⁷. CD34 is a highly glycosylated transmembrane protein molecule. As a member of the cadherin family, CD34 is specifically ex-

Table IV. Correlations between positive expression of ER and ultrasound signs.

Ultrasound signs	ER		X ²	p
	Positive (no. = 55)	Negative (no. = 33)		
Tumor size				
> 2 cm	26	22	3.129	0.077
≤ 2 cm	29	11		
Tumor Morphology				
Regular	30	6	11.282	0.001
Irregular	25	27		
Edge				
Smooth	29	8	6.868	0.009
Not smooth	26	25		
Perimeter				
Clear	31	5	14.491	0.001
Not clear	24	28		
Echo attenuation				
Yes	28	23	2.988	0.084
No	27	10		
Blood supply				
Rich blood supply	33	19	0.05	0.823
Lack of blood supply	22	14		
Microcalcification				
Yes	39	23	0.015	0.903
No	16	10		
Lymph node metastasis				
Yes	41	21	1.179	0.278
No	14	12		

pressed in vascular endothelial cells. Expression of CD34 reflects the density of blood vessels in tumors¹⁸. Positive expression of ER is considered to be an ideal biomarker for BC differentiation and prognosis of BC patients¹⁹.

Lymph node metastasis was presented as a conical and round hypoechoic nodule in ultrasound imaging. Patients with metastases were more likely to have increased malignancy. In this research, we found that positive expression of C-erB-2 was positively correlated with lymph node metastasis, indicating the high degree of malignancy of those tumor tissues, and the efficacy of endocrine therapy may be poor. Tumor blood supply, as an important sign of tumor morphological changes, is important for the development of tumors, so detection of blood supply is conducive to the judgment of tumor invasion and prognosis²⁰. In this work, positive expression of C-erB-2 was significantly correlated with blood supply. Calcification of BC is usually microcalcification and no acoustic shadow at the back, which is mainly caused by tissue degeneration and necrosis of malignant tumors and precipitation of calcium salts²¹. In this report, we found that positive C-erB-2 expression was significantly correlated with microcalcification,

indicating that degree of malignancy is increased with calcification, the tumor will invade and the prognosis of patients is poor. It has been observed²² that the morphological margins and perimeters of tumors are related to the positive rate of ER. In this investigation, it was found that the positive expression of ER was significantly correlated with regular morphology, smooth tissue and unclear perimeter. This data suggest that ER-positive patients have a higher degree of tumor differentiation, lower degree of malignancy, and better results in endocrine therapy. Therefore, we can preliminarily conclude that the combination of ultrasound signs and C-erB-2, CD34, and ER expression may improve the diagnosis of BC.

However, there are still some shortcomings in this research. Firstly, the low sample size is our main problem, although whether it affects our outcome is unclear. In addition, the time point of detection is single; we did not use multi-time points detection, which cannot understand the related conditions of tumor timely. So, we will increase the number of our research samples in future studies, as well as the number of time points to improve our results, further to ensure the accuracy of our results.

Conclusions

We showed that the combination of elevated levels of C-erbB-2, CD34, and ER in breast cancer patients and ultrasound signs may improve the diagnosis of BC.

Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of China Meitan General Hospital and Tangshan People's Hospital & Tangshan Tumor Hospital. Patients who participated in this research, signed the informed consent and had complete clinical data. Signed written informed consents were obtained from the patients and/or guardians.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

- 1) RHEINBAY E, PARASURAMAN P, GRIMSBY J, TIAO G, ENGRETZ JM, KIM J, LAWRENCE MS, TAYLOR-WEINER A, RODRIGUEZ-CUEVAS S, ROSENBERG M, HESS J, STEWART C, MARUVKA YE, STOJANOV P, CORTES ML, SEPO S, CIBULSKIS C, TRACY A, PUGH TJ, LEE J, ZHENG Z, ELLISEN LW, IAFRATE AJ, BOEHM JS, GABRIEL SB, MEYERSON M, GOLUB TR, BASELGA J, HIDALGO-MIRANDA A, SHIODA T, BERNARDS A, LANDER ES, GETZ G. Recurrent and functional regulatory mutations in breast cancer. *Nature* 2017; 547: 55-60.
- 2) ZHOU L, ZHAO LC, JIANG N, WANG XL, ZHOU XN, LUO XL, REN J. MicroRNA miR-590-5p inhibits breast cancer cell stemness and metastasis by targeting SOX2. *Eur Rev Med Pharmacol Sci* 2017; 21: 87-94.
- 3) DESANTIS CE, MA J, GODING SAUER A, NEWMAN LA, JEMAL A. Breast cancer statistics, 2017, racial disparity in mortality by state. *CA Cancer J Clin* 2017; 67: 439-448.
- 4) ZAKI SM, ABDEL-AZEEZ HA, EL NAGAR MR, METWALLY KA, S AHMED MM. Analysis of FHIT gene methylation in egyptian breast cancer women: association with clinicopathological features. *Asian Pac J Cancer Prev* 2015; 16: 1235-1239.
- 5) DACOSTA BYFIELD S, ABUSHAMAA A, BECKER L, SHEPHERD S, RICKER J, BONNET P. Abstract P5-08-15: Real-world treatment patterns and survival among triple negative breast cancer patients versus patients with other breast cancer subtypes in early stage breast cancer. *Cancer Res* 2017; 77: P5-08-15-P05-08-15.
- 6) MAFU TS, SEPTEMBER AV, SHAMLEY D. The potential role of angiogenesis in the development of shoulder pain, shoulder dysfunction, and lymphedema after breast cancer treatment. *Cancer Manag Res* 2018; 10: 81-90.
- 7) MORI N, MUGIKURA S, TAKAHASHI S, ITO K, TAKASAWA C, LI L, MIYASHITA M, KASAJIMA A, MORI Y, ISHIDA T, KODAMA T, TAKASE K. Quantitative analysis of contrast-enhanced ultrasound imaging in invasive breast cancer: a novel technique to obtain histopathologic information of microvessel density. *Ultrasound Med Biol* 2017; 43: 607-614.
- 8) BEWICK M, CHADDERTON T, CONLON M, LAFRENIE R, MORRIS D, STEWART D, GLUCK S. Expression of C-erbB-2/HER-2 in patients with metastatic breast cancer undergoing high-dose chemotherapy and autologous blood stem cell support. *Bone Marrow Transplant* 1999; 24: 377-384.
- 9) BIDARD FC, PEETERS DJ, FEHM T, NOLE F, GISBERT-CRIADO R, MAVROUDIS D, GRISANTI S, GENERALI D, GARCIA-SAENZ JA, STEBBING J, CALDAS C, GAZZANIGA P, MANSO L, ZAMARCHI R, DE LASCOITI AF, DE MATTOS-ARRUDA L, IGNATIADIS M, LEBOSKY R, VAN LAERE SJ, MEIER-STIEGEN F, SANDRI MT, VIDAL-MARTINEZ J, POLITAKI E, CONSOLI F, BOTTINI A, DIAZ-RUBIO E, KRELL J, DAWSON SJ, RAIMONDI C, RUTTEN A, JANNI W, MUNZONE E, CARAÑANA V, AGELAKI S, ALMICI C, DIRIX L, SOLOMAYER EF, ZORZINO L, JOHANNES H, REIS-FILHO JS, PANTEL K, PIERGA JY, MICHIELS S. Clinical validity of circulating tumour cells in patients with metastatic breast cancer: a pooled analysis of individual patient data. *Lancet Oncol* 2014; 15: 406-414.
- 10) BENSON AB 3RD, BEKALI-SABA T, BEN-JOSEF E, BLUMGART L, CLARY BM, CURLEY SA, DAVILA R, EARLE CC, ENSMINGER WD, GIBBS JF, LAHERU D, MALAFA MP, MARRERO J, MERANZE SG, MULVIHILL SJ, PARK JO, POSEY JA, SACHDEV J, SALEM R, SIGURDSON ER, SOFOCLEOUS C, VAUTHEY JN, VENOOK AP, GOFF LW, YEN Y, ZHU AX. Hepatobiliary cancers. Clinical practice guidelines in oncology. *J Natl Compr Canc Netw* 2006; 4: 728-750.
- 11) FAN L, STRASSER-WEIPPL K, LI JJ, ST LOUIS J, FINKELSTEIN DM, YU KD, CHEN WQ, SHAO ZM, GOSS PE. Breast cancer in China. *Lancet Oncol* 2014; 15: e279-289.
- 12) VELIKYAN I, WENNBORG A, FELDWISCH J, LINDMAN H, CARLSSON J, SÖRENSEN J. Good manufacturing practice production of [(68)Ga]Ga-ABY-025 for HER2 specific breast cancer imaging. *Am J Nucl Med Mol Imaging* 2016; 6: 135-153.
- 13) PAIS A, BITON IE, MARGALIT R, DEGANI H. Characterization of estrogen-receptor-targeted contrast agents in solution, breast cancer cells, and tumors in vivo. *Magn Reson Med* 2013; 70: 193-206.
- 14) GARCIA-MURILLAS I, SCHIAVON G, WEIGELT B, NG C, HREBIEN S, CUTTS RJ, CHEANG M, OSIN P, NERURKAR A, KOZAREWA I, GARRIDO JA, DOWSETT M, REIS-FILHO JS, SMITH IE, TURNER NC. Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer. *Sci Transl Med* 2015; 7: 302ra133.
- 15) OPHIR J, CESPEDES I, PONNEKANTI H, YAZDI Y, LI X. Elastography: a quantitative method for imaging the elasticity of biological tissues. *Ultrason Imaging* 1991; 13: 111-134.
- 16) BURRIS HA, BARVE MA, HAMILTON EP, BARDIA A, SOLIMAN HH, JARLENSKI D, MOSHER R, BERGSTROM DA. A phase Ib, first-in-human, dose escalation and expansion

- study of XMT-1522, a novel antibody-drug conjugate (ADC) directed against HER2, in patients with advanced breast cancer and other advanced tumors expressing HER2. *J Clin Oncol* 2017; 35: TPS2606.
- 17) VEERARAGHAVAN J, DE ANGELIS C, REIS-FILHO JS, PASCUAL T, PRAT A, RIMAWI MF, OSBORNE CK, SCHIFF R. De-escalation of treatment in HER2-positive breast cancer: determinants of response and mechanisms of resistance. *Breast* 2017; 34: S19-s26.
 - 18) NASIR A, HOLZER TR, CHEN M, MAN MZ, SCHADE AE. Differential expression of VEGFR2 protein in HER2 positive primary human breast cancer: potential relevance to anti-angiogenic therapies. *Cancer Cell Int* 2017; 17: 56.
 - 19) McDERMOTT MS, CHUMANEVICH AA, LIM CU, LIANG J, CHEN M, ALTILIA S, OLIVER D, RAE JM, SHTUTMAN M, KIARIS H, GYDRFFY B, RONINSON IB, BROUDE EV. Inhibition of CDK8 mediator kinase suppresses estrogen dependent transcription and the growth of estrogen receptor positive breast cancer. *Oncotarget* 2017; 8: 12558-12575.
 - 20) LV YG, BAO JH, XU DU, YAN OH, LI YJ, YUAN DL, MA JH. Characteristic analysis of pulmonary ground-glass lesions with the help of 64-slice CT technology. *Eur Rev Med Pharmacol Sci* 2017; 21: 3212-3217.
 - 21) COŞAR ZS1, CETIN M, TEPE TK, CETIN R, ZARALI AC. Concordance of mammographic classifications of microcalcifications in breast cancer diagnosis: Utility of the Breast Imaging Reporting and Data System (fourth edition). *Clin Imaging* 2005; 29: 389-395.
 - 22) GADALLA SE, ALEXANDRAKI A, LINDSTRÖM MS, NISTÉR M, ERICSSON C. Uncoupling of the ERα regulated morphological phenotype from the cancer stem cell phenotype in human breast cancer cell lines. *Biochem Biophys Res Commun* 2011; 405: 581-587.