Analysis of Breast Cancer: HER2 Expression and Imaging Findings on Mammography and Ultrasound*

JIANG Zhen-ni，LIN Qing△ , QI Chun-hua, CUI Chun-xiao, FEI Jie, BIAN Tian-tian

(Department radiology, The Affiliated Hospital Of Medical College ,Qingdao University, Qingdao, Shandong, 266100, China)

ABSTRACT **Objective:** To comparatively analyze the correlation between positive and negative expressions of human epidermal growth receptor 2 (HER2) and mammography and ultrasound imaging manifestations. **Methods:** 139 breast cancer patients with expressions of oestrogen receptor (ER) and progesterone receptor (PR) were analyzed through mammography and ultrasound morphology before surgery, and expression of HER2 were detected after surgery. The characteristic imaging manifestations were also compared and analyzed between HER2+/ER-/PR- breast cancer group and HER2-/ER-/PR- breast cancer group. The main aspects on mammography contain gland density and lesion type (mass, calcification or structure disturbance). Mass was analyzed by margin and size. And calcification was analyzed by morphology and distribution. Main analyses on ultrasound were margin, with or without hyperecho focus and axillary lymph nodes metastasis, blood flow signal. **Results:** The characteristic manifestations of breast cancer with HER2+/ER-/PR- on mammography were simple mass（x^2=8.067, P=0.005）or mass with calcification（x^2=4.384, P=0.036）, calcification usually show rodlike or branching calcification （x^2=5.723,, P=0.017）, with clustered distribution （x^2=12.110, P=0.007）. Most diameters of mass were >2cm （x^2=4.933, P=0.026）. The characteristic manifestations of ultrasound were mass with calcification （x^2=16.134, P<0.001）, unclear margin （x^2=9.188, P=0.010）, combined with axillary lymph nodes metastasis （x^2=4.069, P=0.044）. **Conclusion:** In hormone receptor negative cases, Breast cancer of HER2 positive was relatively more characteristic than HER2 negative on imaging findings.

Key words: Mammography; Ultrasonography; Breast neoplasm; Receptor, erbB-2

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Introduction

HER2(human epidermal growth factor receptor 2, HER2), located at the long arm of human chromosome 17 (17q12)[1] is over-expressed in about 18% to 20% cells of breast cancer and is known as an important prognostic and predict factor of breast cancer [2]. Over expression of HER2 indicates vigorous cell proliferation and strong ability of invasion and is positively associated with high malignancy of tumor, lymph node metastasis, and advanced stage, recurrence of tumor, short disease-free and overall survival, and poor prognosis but is negatively associated with expression oestrogen receptor (ER) and progesterone receptor (PR)[3]. So what is the difference of imaging findings between the two subtypes of expression and non-expression of HER2 of breast cancer when the hormonal receptors are negative? We retrospectively analyzed clinical pathologic records of 139 patients of breast cancer and confirmed different subtypes of expression of HER2 of breast cancer. And we tried to investigate and compare the characteristic imaging findings of 2 group subtypes of breast cancer and 2 kinds of imaging methods. This paper has certain help for early breast cancer and provides basis for clinical treatment of Positive HER2

breast cancer. All these were very important to diagnosis of breast cancer.

1 Material and materials

1.1 Baseline material

Eight hundred and forty-eight female patients aged 27 to 81 (median age 54 years old) who underwent surgery and had a pathologically confirmed diagnosis of primary breast cancer between April of 2012 and April of 2013 in our hospital were included. mammography?and breast ultrasonograpy were performed before surgery and expression of ER, PR and HER2 of breast cancer tissues was detected by immunohistochemical staining after surgery. 138 patients with both negative expression of ER and PR were divided into two groups according to the different expression of HER2: 40 patients with expression of HER2+/ER-/PR- and 99 patients with expression of HER2-/ER-/PR-.

1.2 Methods

1.2.1 Mammography

The examination was performed with Hologic Selenia Dimensions Mammography system. Imaging condition was: the voltage ranged from 20 to 40 kV, the current ranged from 200 to 400

mAs, the distance between the x ray tube and imaging detector was 70 mm. The patients had routinely imaged in mediolateral oblique and craniocaudal view for each breast. The evaluation of breast density was according to the ACR Classification criterion formulated by American college of radiology [4,5]. The observed contents of the two subtypes were according with the criterion of breast imaging reporting and data system which is formulated and supported by American College of Radiology [4], including the breast density and the lesion type (mass, calcification, structure disorder). To the mass, the analysis was mainly about its edge and size, while to the calcification was form and distribution. 2 radiologists specialty in Breast imaging diagnosis were together reading the mammography films under the condition that the clinical and pathologic diagnoses were unknown.

1.2.2 Breast Ultrasonography

The examination was performed with Acuson S2000 Color Ultrasound System (made in Germany). The Transducer Frequency was 9 MHz. The patients had routine ultrasound examination for each breast. When abnormal echo was found, scanning criss-cross on the abnormal parts. Observed the shape, edge, internal echo of the mass, detected whether there was strong echo points or axillary lymph nodes metastasis. Used Doppler color flow imaging to observe the shape and distribution of Blood flow signal in and around the mass, recorded the value and employed Adler [6] Semi-quantitative method in classification. Pulse Doppler sampling was used to detect the characteristics of the blood flow. The angle between the ultrasound beam and blood flow was adjusted to < 60°. Measured the arterial blood flow resistance index and recorded the data.

1.2.3 Pathological examination

Specimens were detected with immunohistochemistry after surgery. ER(-) and PR (-) were defined as positive immunohistochemical cells<1% or no positive cells. HER2 immunohistochemical staining defined as: 0 or + was negative, +++ was positive. Chromogenic in situ hybridization was performed when ++ present. Reference criterion: (1) Amplification (positive), cellular mean HER2 copies>6.0; (2) No amplification (negative), cellular mean HER2 copies<4.0.

1.3 Statistical analysis

Analysis was undertaken using SPSS for Windows (17th ed, IBM). The comparisons between characteristics of mammography and ultrasonography of breast cancer with HER2+/ER-/PR- and HER2-/ER-/PR- were analyzed using chi-square test. P<0.05 was considered significant.

2 Results

2.1 Pathology and immunohistochemistry

Twenty patients (50%)who diagnosed as HER2+/ER-/PR- breast cancer group were aged between 45 and 60. And nine (22%)of them were over 60 years old. Forty patients (40%)with

HER2-/ER-/PR- breast cancer group were aged between 45 and 60. And 45 patients (46%)were over 60 years old. There was statistically significant difference between the two groups(x^2=7.298，P=0.026). 14 patients (35%)had a tumor with a diameter ≤ 2cm and 26 patients (65%)had a tumor with a diameter >2cm in the HER2+/ER-/PR- group. 59 patients (60%)had a tumor with a diameter ≤ 2cm and 40 patients (40%)had a tumor with a diameter >2cm in the HER2-/ER-/PR- group. There was statistically significant difference between the two groups(x^2=6.911,P=0.009. 19 patients (48%)with HER2+/ER-/PR- group were combined with lymphatic metastasis. 28 patients (28%)with HER2-/ER-/PR- group were combined with lymphatic metastasis. A statistically significant difference was found between the two groups (x^2=4.701,P=0.030). Most of the pathological types in the two groups with no statistically significant difference were invasive ductal carcinoma with higher histological classification. See to table 1.

Table 1 HER-2 pathological and clinical characteristics of different breast cancer expressions [n(%)]

	HER2+/ER-/PR-(n=40)	HER2-/ER-/PR- (n=99)	x^2value	Pvalue
Age			7.298	0.026
≤ 45	11(28)	14(14)		
45-60	20(50)	40(40)		
>60	9(22)	45(46)		
Tumor size (cm)			6.911	0.009
≤ 2cm	14(35)	59(60)		
>2cm	26(65)	40(40)		
Lymph node metastasis			4.701	0.030
With	19(48)	28(28)		
Without	21(52)	71(72)		
Pathological type				
DCIS	4	9[a]		
IDC	35	88[b]		
IMPC	1	0		
Leaf malignancies	0	1		
Mucinous adenocarcinoma	0	1		

superscript：DCIS：Ductal carcinoma in situ
　　　IDC：Invasive ductal carcinoma
　　　a：Nine DCIS cases include seven simple DCIS cases ，one DCIS with early micro infiltration case and one DCIS with Paget's case
　　　b：Only one IDC case was with some parts of DCIS

2.2 Findings of mammography

2.2.1 Comparisons of density Of the gland density classification, There were 11(%)was nonpyknotic gland and 29 (%) were pyknotic gland in the HER2+/ER-/PR- grou. There were 39(39%) were nonpyknotic gland and 60 (61%)were pyknotic gland in the

HER2-/ER-/PR- group. There was statistically significant difference between the two groups(x^2=1.750,P=0.186).

2.2.2　Comparisons of lesion type

In mammography, 8 （20% ）of lesions in HER2+/ER-/PR- group and 45 (46%) of lesions in HER2-/ER-/PR- group were simple tumors, respectively （x^2=8.067,P=0.005）. 14 （35%) and 24 (24%) of lesions in each group were simple calcification, respectively（x^2=1.573,P=0.210）.15 (38%) and 20 (20%) of lesions in each group were tumor with calcification （x^2=4.384,P=0.036）. 1 （3%)and 7 （7%)of lesion manifestations was architectural distortion respectively in each group(x^2=1.121,P=0.290）. 24 (60%) and 68 (69%) of lesion manifestations were present as mass respectively in each group（x^2=1.127,P=0.289）. 29 (73%)and 44 (45%) of lesion manifestations were present as calcification in each group (x^2=8.686, P=0.003).

2.2.3　Comparisons of mass margin

Of the mass margin, 3 （13%)and 10 （15%)were clear, 8 （33%)and 32(47%)were obscured, 13（54%)and 26（38%)were burr, respectively in the two groups （x^2=1.892,P=0.388）. 3 patients （13%)had a tumor with a diameter ≤ 2cm and 21 patients （87%)had a tumor with a diameter >2cm in the first group. 25 patients （37%)had a tumor with a diameter ≤ 2cm and 43 patients （63%)had a tumor with a diameter >2cm in the other group. There was statistically significant difference between the two groups(x^2=4.933,P=0.026）.

2.2.4　Comparisons of calcification distribution and morphology

Of the calcification in HER2+/ER-/PR- group, 17（59%)were clustered, 10（34%)were regional, 2（7%)were segmental, no one was diffuse distribution. Of the calcification in HER2-/ER-/PR- group, 9（20%)were clustered, 24（55%)were regional, 9（20%) were segmental, 2（5%)were diffuse distribution. There was statistically significant difference between the two groups（x^2=12.110, P=0.007). Of the calcification shape, 19 （66%)were punctate or amorphous, 10（34%)were claviform or dendroid in the first group and 39（89%)were punctate or amorphous, 5（11%)were claviform or dendroid in the other group. There was statistically significant difference between the two groups （x^2=5.723,P=0.017). (See Table 2).

Table 2 The relationship between manifestations of HER-2 and characteristics of mammography [n(%)]

	HER2+/ER-/PR-(n=40)	HER2-/ER-/PR-(n=99)	x^2Value	P Value
Nonpyknotic[a]	11(28)	39(39)	1.750	0.186
Pyknotic[b]	29(72)	60(61)		
Lesion manifestations				
Simple mass	8 (20)	45(46)	8.067	0.005
Simple calcification	14(35)	24(24)	1.573	0.210
Mass with calcification	15(38)	20(20)	4.384	0.036
Architectural distortion	1(3)	7(7)	1.121	0.290
All mass[c]	24(60)	68(69)	1.127	0.289
All calcification[d]	29(73)	44(45)	8.686	0.003
Mass margin			1.892	0.388
Clear	3(13)	10(15)		
Obscured	8(33)	32(47)		
Burr	13(54)	26(38)		
Mass size			4.933	0.026
≤ 2cm	3(13)	25(37)		
>2cm	21(87)	43(63)		
Calcification distribution			12.110	0.007
Cluster	17(59)	9(20)		
Regional	10(34)	24(55)		
Segmental	2(7)	9(20)		
Diffuse	0(0)	2(5)		
Calcification morphology			5.723	0.017
Punctate or amorphous	19(66)	39(89)		
Claviform or dendroid	10(34)	5(11)		

Notes: a Nonpyknotic gland includes ACR GradeⅠand ACR GradeⅡ; Glandular tissues occupy by less than 50% of whole breast size.

　　　b Pyknotic gland includes ACR GradeⅢand ACR Grade IV; Glandular tissues take up more than 50% of whole breast size

　　　c All mass includes simple mass and mass with calcification

　　　d All calcification includes Simple calcification, mass with calcification and structure distortion

2.3 Findings of ultrasound scan

3 (See Table 4h nodes metastasis Of the lesion type on ultrasound scan, 38 (95%)and 88 (89%)were masses in the HER2+/ER-/PR- group and HER2-/ER-/PR- group respectively (x²=1.255,P=0.263). 18 (45%)and 20 (20%) were structural changed with calcification respectively (x²=8.819,P=0.003). 19 (48%)and 15 (15%)were masses with calcification respectively (x²=16.134,P<0.001). 2(5%)and 9(9%)were confused areas respectively(x²=0.654,P=0.419). Of the mass margin , 2 (5%)and 26 (30%)were clear, 33 (87%)and 58 (66%)were obscured, 3 (8%)and 4 (4%)were crab-like, respectively in the two groups (x²=9.188,P=0.010). Of blood flow signals, 10 (28%)and 33 (33%)were in zero grade, 6(15%)and 15(15%)were in grade I, 4 (10%)and 5(5%)were in grade II, 19(47%)and 46(47%)were in grade III, respectively in the two groups (x²=1.391,P=0.708). There were 23(58%)and 36(36%) patients who were suspected to have axillary lymph nodes metastasis respectively in the two groups (x²=0.260,P=0.610). (See Table 3).

Table 3 The relationship between manifestations of HER-2 and characteristics of ultrasonics [n(%)]

	HER2+/ER-/PR- (n=40)	HER2-/ER-/PR- (n=99)	x² Value	P Value
Mass	38(95)	88(89)	1.255	0.263
Structural changed with calcification a	18(45)	20(20)	8.819	0.003
Mass with calcification a	19(48)	15(15)	16.134	<0.001
Structure of the clutter	2 (5%)	9(9%)	0.654	0.419
Margin			9.188	0.010
Clear	2(5)	26(30)		
Obscured	33(87)	58(66)		
Crab-like	3(8)	4(4)		
Blood flow signals			1.793	0.616
Zero grade	10(28)	33(33)		
Grade I	6(15)	15(15)		
Grade II	4(10)	5(5)		
GradeIII	20(47)	46(47)		
Axillary lymph nodes metastasis			4.069	0.044
With	22(58)	36(36)		
Without	18(42)	63(64)		

Notes: a Mass with calcification includes mass with calcification and structure distortion with calcification, which has been verified as calcification in mammography

3 Discussions

To some extent, the expression of ER, PR and HER2 reflected biological behavior of breast cancer and patients prognosis. Theoretically, histological and pathologic changes caused by different gene expression could be imaged through iconography techniques.

Our analyses found that most onset ages of patients with HER2+/ER-/PR- breast cancer were between 45 and 60 years old. And the diameters of mass were mostly over 2 cm. Invasive ductal carcinoma with higher axillary lymph nodes metastasis rate and higher histological grade was common pathologically. In contrast, most onset ages of patients with HER2-/ER-/PR- breast cancer was over 60 years old. And the diameters of mass were mostly less than 2cm. Invasive ductal carcinoma with higher histological grade and fewer chance of lymph nodes metastasis was also common pathologically. Positive expression of HER2 was positively associated with axillary lymph nodes metastasis. But in the group with lymph nodes metastasis, expression rate of HER2 was higher than the group without lymph nodes metastasis.

3.1 Findings of mammography

The formation of calcification internal lesion of breast cancer may be caused by malnutrition and necrosis of regional lesion whose cells dissolve and a number of phosphate radicals were freed by nucleic acid and combine with regionally increased calcium ions so that phosphate calcium salts are deposited. Pathology found that tumor poor in differentiation and high metabolism tended to be necrosis and resulted in deposition of calcium salt. Evans et al [7] found that clustered calcification of 126 patients with ductal carcinoma in situ were common when HER2 oncogene was over-expressed whereas without the expression of HER2, no calcification was present. So the study suggested that the existence of internal mass was associated with the expression of HER2. Seo et al [8] reported in the study of relation between calcification lesion in mammography of breast cancer and over-expression of tumor HER2 that malignant calcification were significantly associated

with the over-expression of HER2. Our study found that HER2+/ER-/PR- breast cancers were mostly pyknotic gland and usually were in rodlike shape, branching calcification and clustered distribution, which was consistent with foreign studies. When mass with calcification was present, the diameter of mass was longer than that in HER2-/ER-/PR- breast cancer group. This result was not consistent with results that mass of HER2- breast cancer was bigger than that of other type reported by Rakha etal [9].

The inconsistency may be caused by difference of measure methods and needs to be further confirmed by other studies. HER2-/ER-/PR- breast cancers were mostly simple mass, round in shape, clear in margin, not easy to identify from benign lesions and usually not combined with calcification. These results were consistent with that Yang [10] et al reported and there were no significant difference compared with typical breast cancer.
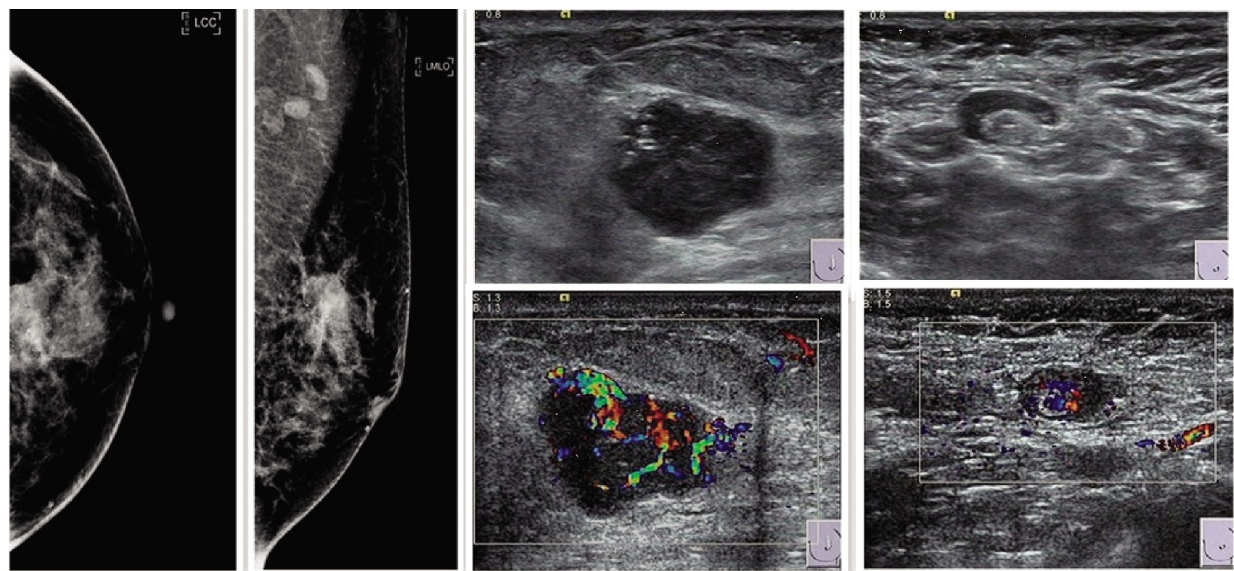

Fig. 1 F/49Y: Clinical history: The left breast lump has been last more than three months since it was found. On mammography: The stellate high density shadows are found on the upper quadrant of left breast (see arrow). There are short rodlike, branching calcification shadows and gland structural disorder inside. On ultrasound scan: hypoechoic mass lesions are found at 12 o 'clock of graph. The size is about 2.5× 1.6cm with irregular shape, unclear margin and punctate hyperecho inside. CDFI：A rich blood flow signal is visible inside RI：0.72. enlargement of lymph nodes are found In the left armpit, with cortical thickening, rich blood flow signal. The immune receptors inspection:HER2+\ER-\PR-. Pathological examination: Invasive ductal carcinoma

3.2 Findings of ultrasound scan

Foreign studies[11,12] confirmed that the presence of microcalcification was associated with positive expression of HER2 which suggested that breast cancer with sign of microcalcification was related to higher advanced malignancy. Zhang Jing et al [13] also found that the low expression level of HER2 was associated with microcalcification of breast cancer tissue. Our study showed that HER2+/ER-/PR- breast cancer on ultrasound was present with hypoechoic mass, irregular shape, obscured margin and hyperechoic sign inside. This conclusion was consistent with studies at home and abroad[11-13]. The over-expression of HER2 gene resulted in increased mitosis and made some abnormal cells lose control of organism and acquired independent growth so that the possible metastasis of tumor was increased [14]. Our study also showed that patients with HER2+/ER-/PR- breast cancer mostly combined with enlargement of axillary lymph nodes which were spherical with obscured and thickening cortex and medulla, reduce or disappearance of mild hyperecho of medulla. But other studies reported that the positive expression of ER, PR and HER2 was not consistent with lymphatic metastasis[15]. The possible explanations for the dis-

crepancy might be that the factors influencing clinical prognosis were complicated and were numerous. Still, there are many mechanisms undiscovered and needed to investigate. Qian Chaowen et al found that positive rate of HER2- with grade 2-3 of classification was higher than of grade 0-1 which indicated that high expression rate of HER2 was associated with malignancy of breast cancer [16]. But we found that no statically significance in difference of blood flow. The reasons might be that expression of ER and PR was significantly and negatively associated with expression of HER2 oncogene protein [2] and number of patients with HER2+/ER-/PR- breast cancer was relatively small. HER2-/ER-/PR- breast cancer on ultrasound was present with low echo mass, clear margin and regular morphology.

The characteristic manifestations of HER2+/ER-/PR- breast cancer on mammography was present with simple mass or mass with calcification, rodlike or dendroid calcification, clustered distribution and mass with diameter >2 cm. The manifestations of ultrasonography were hypoechoic mass, unclear margin, with punctate hyperecho inside and usually combined with axillary lymph nodes metastasis. In contrast, HER2-/ER-/PR- breast cancer on
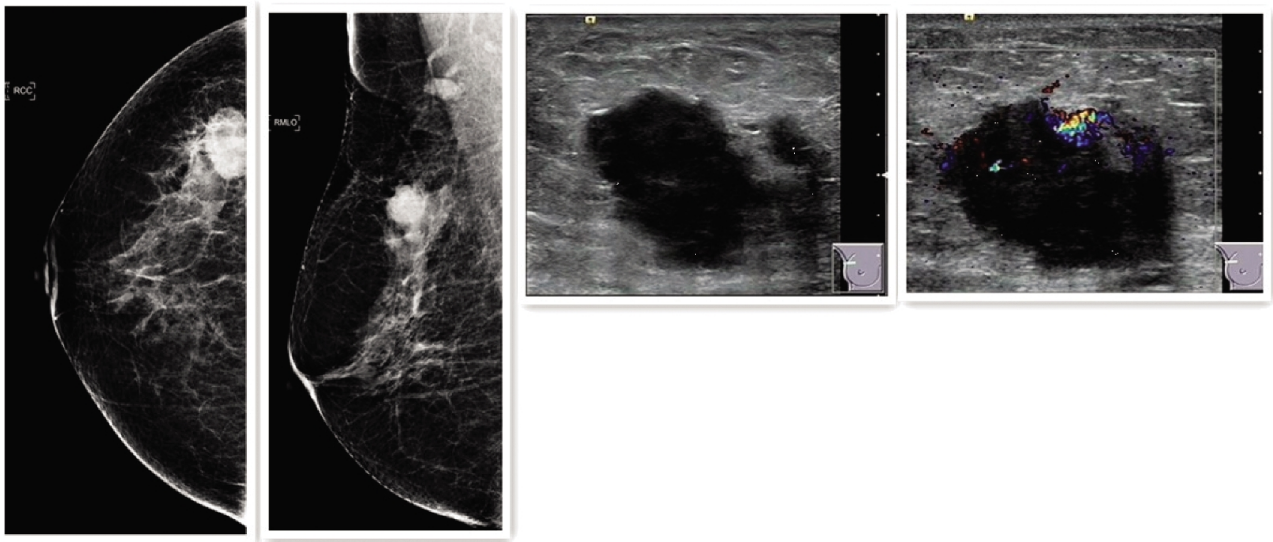
Fig. 2 F/53Y: clinical history: The left breast lump has been last a month since it was found. On mammography: The irregular slightly high density shadows are found on the upper quadrant of left breast, with partly clear margin. On ultrasound scan: hypoechoic mass lesions are found at 10 o 'clock of graph. The Size is about 2.4 x 1.2 cm , with irregular shape, obscured margin and malignant areola. CDFI：A rich blood flow signal is visible inside and around RI：0.72. The immune receptors inspection：HER2-\ER-\PR- . Pathological examination: Invasive ductal carcinoma.

mammography was present with simple mass, round shape, clear margin and little calcification. The manifestations of ultrasonography were hypoechoic mass, regular shape, clear margin, usually not combined with axillary lymph nodes metastasis.

In conclusion, Mammographic and Ultrasound are the preferred examination methods for early breast cancer. Both two methods have their own advantages, and to combine with them can effectively reduce the missed diagnosis of breast cancer . Our experiment has initially tried to investigate the association between iconography of breast cancer and molecular biology and further studies are needed.

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乳腺癌 HER2 表达与乳腺 X 线及超声影像表现的相关性分析 *

蒋珍妮　　林　青△　　齐春华　　崔春晓　　费　杰　　边甜甜

（青岛大学医学院附属医院放射科 山东 青岛 266100）

摘要 目的：对比分析乳腺癌人类表皮生长因子受体 2(human epidermal growth factor receptor 2，HER2)表达阳性和阴性与乳腺癌的 X 线及超声影像表现的相关性。方法：对 139 例雌激素受体（oestrogen receptor ER）、孕激素受体（progesterone receptor PR）表达均阴性的乳腺癌患者术前行 X 线及超声形态学分析，术后标本测定癌细胞的人类表皮生长因子受体 2（HER2）表达情况，比较并分析 HER2+/ER-/PR- 乳腺癌和 HER2-/ER-/PR- 乳腺癌影像学的特征性表现，在 X 线上主要分析:腺体致密度、及病变类型（肿块、钙化、结构紊乱）。其中肿块主要分析形状、边缘、大小；钙化主要分析形态、分布，在超声上主要分析:肿块边缘、有无强回声点、有无腋淋巴结转移，血流情况。结果：HER2+/ER-/PR- 乳腺癌在 X 线上的特征性表现为单纯肿块（x^2=8.067，P=0.005）或肿块伴钙化（x^2=4.384，P=0.036），棒状、分枝状钙化（x^2=5.723，P=0.017），簇状分布（x^2=12.110，P=0.007），肿块直径多 ＞2cm（x^2=4.933，P=0.026），而在腺体致密度、肿块边缘方面差异无统计学意义。超声的特征性表现为肿块伴钙化(x^2=16.134，P<0.001)、边界不清(x^2=9.188，P=0.010)、伴腋下淋巴结转移(x^2=5.210，P=0.022)，血流方面差异无统计学意义。结论：HER2+/ER-/PR- 乳腺癌影像学有特征性表现，X 线常表现为单纯肿块或肿块伴钙化，棒状、分枝状钙化，簇状分布，肿块直径多 ＞2cm;超声常表现为低回声肿块、边界不清、其内可见点状强回声、常伴腋下淋巴结转移。
关键词：乳房 X 线摄影术；超生；乳腺肿瘤；受体, erbB-2
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