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The Value of Contrast-Enhanced Ultrasound Features in Predicting HER2 Positive and Ki67 High Expression in Invasive Breast Cancer of No Special Type

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Abstract

Objective: To investigate the value of contrast-enhanced ultrasound features in predicting HER2 positive and Ki67 high expression in invasive breast cancer of no special type. ***Methods:*** Retrospective analysis was conducted on the correlation between contrast-enhanced ultrasound images of 92 patients with invasive breast cancer of no special type confirmed by pathology in our hospital and the expression of HER2 and Ki67, including 64 cases in the HER2 negative group and 28 cases in the positive group. Ki67 expression was low in 14 cases and high in 78 cases. The contrast-enhanced ultrasound indicators include: BI (Base Intensity), AT (Arrival Time), TTP (Time To Peak), PI (Peak Intensity), AS (Ascending Slope), DT/2 (Descending Time/2), DS (Descending Slope), AUC (Area Under Curve), maximum focal area ratio of before and after contrast-enhanced ultrasound (AR Area Ratio), Distance of anterior boundary of lesion to skin after contrast-enhanced ultrasound (DTS Distance to skin) and maximum diameter after contrast-enhanced ultrasound (D Diameter), and the above indexes were compared and analyzed in the negative and positive groups of HER2, and the low and high expression groups of Ki67. ***Results:*** Logistics regression analysis showed that DTS is an independent risk factor for HER2 positive ($P < 0.05$). AR was an independent risk factor for Ki67 high expression ($P < 0.05$). ***Conclusion:*** The combination of contra-enhanced ultrasound features has high diagnostic efficacy for HER2 positive and Ki67 high expression, which can provide an important reference.

Keywords: breast cancer, contrast-enhanced ultrasound, HER2, Ki67

1. Introduction

Ultrasonic imaging, which is grounded in gray-scale ultrasound, leverages contrast agents in the sound field to engender a contrast-enhanced image via the diffuse reflection interface. This imaging modality has enabled the monitoring of internal circulation and has been particularly useful in the

differential diagnosis of benign and malignant mammary gland tumors. As such, ultrasonic imaging has emerged as a valuable tool in current medical applications for mammary gland disease (Yuan Y, Xu M, Ren Y, et al, 2022; He H, Wu X, Jiang M, et al, 2023), and in the context of monitoring and treating breast cancer, the curative effect has become an essential factor of interest (Guo J, Wang BH, He M, et al, 2022). The relationship between imaging characteristics of breast cancer and HER2 positivity, as well as high Ki67 expression, has been the subject of limited research both domestically and internationally (Du R, Zhang H, Shu W, et al, 2018; Wen, B, Kong, W, Zhang, Y, et al, 2022). HER2 and Ki67 are critical biochemical markers that play a significant role in breast cancer, both in terms of treatment and prognosis (Weigelt B & Reis-Filho JS., 2010). This paper aims to investigate the role of ultrasonic imaging in predicting HER2 positive invasive cancer and the value of high Ki67 expression, with the ultimate goal of providing valuable information for clinical treatment decisions and prognosis assessments.

2. Materials and Methods

2.1 The General Information

We collected data on breast cancer patients from March 2022 to October 2022 in the breast surgery clinic at our hospital. The inclusion criteria for this study were as follows: (1) good-quality contrast-enhanced ultrasound images that clearly showed the lesions; (2) confirmation of invasive breast cancer via pathology; (3) breast ultrasound conducted within 1 week after biopsy or surgical therapy; (4) availability of complete immunohistochemical results; (5) presence of a single lesion for the breast cancer. Exclusion criteria: (1) a history of treatment for breast cancer before CEUS; (2) absence of further fluorescence in situ hybridization (FISH) dual probe testing for immunohistochemical HER2++; (3) presence of invasive special types of breast cancer; (4) presence of multiple lesions for the breast cancer. This study was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (Ethics number: 2022-185). This is a retrospective study, and the content of this article does not involve the privacy of patients, so no informed consent was signed.

2.2 Ultrasound Examination Methods

The study utilized the Mindray Resona 7 color ultrasonic diagnostic instrument with the conventional ultrasonic linear array probe (L14-5 WU) for conventional ultrasound imaging, while the wire array probe (L9-3U) was used for ultrasonic contrast-enhanced imaging with a low mechanical index (MI $<$ 0.1). SonoVue, a contrast medium, was utilized in the study and was added to the bottle before use. The microbubble suspension was prepared by shaking it with 5ml of 0.9% normal saline. The patient was placed in the supine position, lateral if necessary, with both arms in external rotation and in the abduction position to fully expose the breast. The 9L probe was selected to scan the section with the richest blood flow signal and switched to imaging mode. A contrast agent of 4.8 mL was injected through the anterior cubital vein followed by a quick injection of 5.0 mL of 0.9% normal saline to flush the tube. The timer button was pressed simultaneously, and the lesions were continuously observed in real-time for 180 seconds with image storage. The contrast-enhanced lesion range before and after contrast was outlined on the contrast section, DTS and D were recorded. Then the contrast-enhanced ultrasound quantitative analysis software of the machine was used to analyze the lesions and record the relevant contrast-enhanced ultrasound quantitative indicators: BI, AT, TTP, PI, AS, DT/2, DS and AUC were evaluated by two sonographers with more than 3 years of work experience. If the two sonographers had different opinions, the results were discussed together.

2.3 Immunohistochemical Staining Results

Coarse needle biopsy pathology after operation and (or) specimens of routine HE staining and immunohistochemical staining. The pathological type and histological grade of the lesions could be obtained by HE staining. Immunohistochemistry was performed by Roche automatic immunohistochemistry instrument. Brown particles appeared in tumor cell membrane as positive cells, and HER2 expression was divided into 0, +, ++, +++, among which 0 and + were negative, +++ was positive, and ++ was further tested by fluorescence in situ hybridization double probe; The presence of HER2 amplification was defined as positive, and the absence of amplification was defined as negative. The number of stained cells with brown particles in the nucleus ≥14% was defined as high Ki67 expression, and < 14% was defined

as low Ki67 expression (Nielsen TO, Leung SCY, Rimm DL, et al, 2021).

2.4 Statistical Methods

SPSS 26.0 software was used for statistical analysis. The measurement indicators were in accordance with normal distribution and homogeneity of variance, which were expressed as mean ± standard deviation. The t test was used to analyze and compare the differences of indicators between the groups. Indicators with $P < 0.1$ in univariate analysis were used as independent variables and included in multivariate logistic regression to screen out indicators that could predict Ki67 high expression and Her2 positive in invasive breast cancer. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Patient Characteristics and Pathological Findings

A total of 105 cases were collected in this study, 13 cases were excluded, and 92 cases were included. There were 92 lesions in 92 female patients, including 51 lesions in the left breast and 41 lesions in the right breast. The pathological types of all lesions were invasive breast cancer of no special type. According to the results of immunohistochemical staining, 64 cases (64/92, 69.57%) were HER2 negative and 28 cases (28/92, 30.43%) were Her2 positive. There were 14 cases (14/92, 15.22%) in the low Ki67 expression group and 78 cases (78/92, 84.78%) in the high Ki67 expression group.

3.2 Statistical Analysis Results

3.2.1 Results of Univariate Analysis

The BI, AT, TTP, PI, AS, DT/2, DS, AUC, AR, D and DTS of HER2 negative group and HER-2 positive group were respectively: (10.06±5.08)dB, (3.89±4.22)S, (20.15±6.81)S, (22.08±5.26)dB, (0.56±0.24)dB/S, (76.81±17.42)S, (-0.13±0.12)dB/S, (2341.97±838.85)dB·S, (124.70±12.52)%, (25.22±10.56)mm, (0.53±0.24)cm, (11.01±5.10)dB, (4.38±3.95)S, (21.37±7.94)S, (24.53±4.60)dB, (0.63±0.22)dB/S, (78.06±20.90)S, (-0.15±0.12)dB/S, (2637.62±843.27)dB·S, (124.71±15.84)%, (29.61±13.12)mm, (0.67±0.25)cm, The PI, D, and DTS indexes were $P < 0.1$, while the remaining indexes were $P > 0.1$, as shown in Table 1; The BI, AT, TTP, PI, AS, DT/2, DS, AUC, AR and DTS of the Ki67 low expression group and the Ki67 high expression group were respectively: (9.06±4.33)dB, (6.02±4.43)S, (21.45±6.75)S, (21.92±5.02)dB, (0.56±0.18)dB/S, (78.80±11.61)S, (-0.15±0.14)dB/S, (2340.55±757.47)dB·S, (116.43±9.19)%, (27.36±15.00)mm, (0.61±0.22)cm, (10.58±5.19)dB, (3.68±3.99)S, (20.35±7.25)S, (22.99±5.21)dB, (0.58±0.24)dB/S, (76.90±19.45)S, (-0.13±0.12)dB/S, (2448.36±865.18)dB·S, (126.19±13.68)%, (26.41±10.88)mm, (0.56±0.25) cm, compared with the two groups, the AR and AT indexes were $P < 0.1$, and the remaining indexes were $P > 0.1$, as shown in Table 2.

3.2.2 Results of Multivariate Logistic Regression Analysis

The CEUS indicators with $P < 0.1$ in Table 1 and Table 2 were used as independent variables, and whether HER2 was positive or not and whether Ki67 expression was high or not were used as dependent variables for multivariate Logistics regression analysis. The results showed that DTS is an independent risk factors for HER2 positive ($P < 0.05$), as shown in Table 3. AR was an independent risk factor for high Ki67 expression ($P < 0.05$), as shown in Table 4.

4. Discussion

Breast cancer is the leading cause of cancer death in women, and its incidence is increasing in young women (Ferlay J, Colombet M, Soerjomataram I, et al, 2019). HER-2, an important biomarker in breast cancer, is known to inhibit apoptosis and stimulate the production of vascular endothelial growth factor, leading to increased tumor blood supply, enhanced tumor invasion, and distant metastasis. The expression of HER2 has a significant impact on the prognosis of breast cancer patients. Ki67, another crucial marker, reflects the ability of tumors to proliferate. High expression of Ki67 often indicates poor tumor differentiation, easy metastasis, and a poor prognosis, making it a useful tool in evaluating breast cancer invasiveness. The expression levels of both HER2 and Ki67 are essential factors in predicting tumor behavior and prognosis in patients, and also play a key role in determining appropriate treatment options (Perou CM, Sørlie T, Eisen MB, et al, 2000). Assessing the expression of HER2 and Ki67 in breast cancer via ultrasound before surgery is crucial for clinical diagnosis and treatment. To explore the correlation between contrast-enhanced ultrasound (CEUS) characteristics of invasive breast cancer and HER2 positivity and high Ki67 expression, our study was conducted.

HER2 positive breast cancer tends to be more aggressive and rapidly growing than other types

of breast cancer. Logistic regression analysis showed that DTS is a significantly different between HER2 expression groups. In the univariate analysis, PI may be related to the overexpression of HER2 and the high expression of vascular endothelial growth factor, which can promote the formation of tumor nutrient vessels. With the increase of the number of tumor nutrient vessels, PI value increases (Shin I., 2021). The univarate analysis results of this investigation were the same as those reported in previous literature, and the PI of HER2 positive group was significantly higher than that of HER2 negative group (Jiang Haina, 2021). In the logistic regression result that PI is not statistically significant maybe because of less date. HER2 an lead to rapid tumor growth and increase the risk of lymph node metastasis. Lymphatic capillaries mainly mediate lymph node metastasis, and the distribution of lymphatic capillaries near and far from the skin is significantly different (Zhang YX, LI HJ & DU ZG., 2019). In this study, the lesions in HER-2 negative group were closer to the skin than those in HER2 positive group, which may be related to HER2 promoting tumor lymphangiogenesis.

Ki67 is a significant marker that evaluates the proliferation activity of tumor cells and has been useful in guiding treatment and predicting prognosis in breast cancer. In this study, we conducted univariate analysis to identify independent variables with statistically significant differences, which were subsequently included in binary logistics regression analysis. We identified AR as an independent risk factor for high Ki67 expression. Higher Ki67 expression reflects a greater invasive potential of the tumor and indicates a poor prognosis (Taneja, Jyoti, 2021; Velappan, Arumugam & Deepa Shunmugam, 2017). Univariate analysis of this study showed that there were significant differences in AR and AT among Ki-67 expression groups. The increased lesion area ratio of contrast-enhanced ultrasound to gray-scale ultrasound is an independent risk factor for high expression of Ki67. The results of this study are similar to those reported in the literature, but the evaluation of the expansion degree of the lesion after CEUS in this study is based on the area of the whole lesion, while the previous literature reports selected the longitudinal diameter and transverse diameter based on the diameter (Lv WH, Nie F, Zhang YX, et al, 2018). The indicators selected in this study can more comprehensively evaluate the scope of the lesion. According to DmytrenkoG, the higher the expression of Ki67, the more tumor cells in the lesion, and tumor cells have the effect of promoting angiogenesis (Dmytrenko G, Castro ME & Sales ME, 2017). CEUS can more clearly show the actual blood supply of the lesion, and the increase of the lesion area after CEUS can reflect the expression of Ki67 in the tumor to a certain extent. Previous studies have shown that antigen Ki67 expression is negatively correlated with AT value. In this study, the mean value of AT value in the Ki67 low expression group is higher than that in the high expression group, which is consistent with the results of previous studies (Du R, Zhang H, Shu W, et al, 2018). In the Ki67 high expression group, shorter AT means more abundant formation of abnormal blood vessels, which is associated with higher microvessel density within the lesion (Li J, Hou HF, Sun D, et al, 2019).

In conclusion, the logistic regression model of CEUS has certain characteristics for the judgment of HER2 positivity and high expression of Ki67. PI, D, and DTS can provide help for predicting HER2 positivity. DTS is of great significance for the judgment of HER2 positivity; AR and AT can provide help for the judgment of Ki67 high expression, and AR is of great significance for the judgment of Ki67 high expression. The limitation of this study is that only contrast-enhanced ultrasound was used to analyze the correlation between HER2 positive and Ki67 high expression. In the future, other ultrasound techniques can be combined, such as, firefly technology, elastography, and superb microvascular imaging were used to further analyze the relationship between molecular typing of breast cancer for further research; The sample size of this study is small, and further improvement is needed to expand the sample size in the future.

Table 1. Univariate analysis of Her2 expression in invasive breast cancer

	Her2-(64)	Her2+(28)	t	*P*
DTS (cm)	0.53±0.24	0. 67±0.25	-2.43	0.017

AR (%)	124.70±12. 52	124. 71±15.84	-.004	0.997
D (mm)	25.22±10.56	29.61±13.12	-1.701	0.092
BI	10.06±5.08	11.01±5.10	-0.836	0.405
AT	3.89±4.22	4.38±3.95	-0.518	0.606
TTP	20.15±6.81	21.37±7.94	-0.755	0.452
PI	22.08±5.26	24.53±4.60	-2.134	0.036
AS	0.56±0.24	0.63±0.22	-1.377	0.172
DT/2	76.81±17.42	78.06±20.90	-0.296	0.768
DS	-0.13±0.12	-0.15±0.12	0.936	0.352
AUC	2341.97±838.85	2637.62±843.27	-1.553	0.124

Table 2. Univariate analysis of Ki67 expression in invasive breast cancer

	Ki67 low expression (14)	Ki67 high expression (78)	t	*P*
DTS (cm)	0.61±0.22	0.56±0.25	0.661	0.510
AR (%)	116.43±9.19	126.19±13.68	-2.562	0.012
D (mm)	27.36±15.00	26.41±10.88	0.282	0.779
BI	9.06±4.33	10.58±5.19	-1.030	0.306
AT	6.02±4.43	3.68±3.99	1.989	0.050
TTP	21.45±6.75	20.35±7.25	0.527	0.599
PI	21.92±5.02	22.99±5.21	-0.706	0.578
AS	0.56±0.18	0.58±0.24	-0.299	0.766
DT/2	78.80±11.61	76.90±19.45	-0.353	0.725
DS	-0.15±0.14	-0.13±0.12	-0.352	0.726
AUC	2340.55±757.47	2448.36±865.18	-0.437	0.663

Table 3. Logistics regression model of HER2 positivity in invasive breast cancer

Factors	B	S.E.	Waldx^2	OR	*P*
DTS	2.024	1.017	3.961	7.570	0.047
D	0.029	0. 021	2.012	1.030	0.113
PI	0.082	0. 052	2.513	1.085	0.156
C	-4.737	1. 437	10.862	0.009	0.001

Table 4. Logistics regression model of high Ki67 expression in invasive breast cancer

Factors	B	S.E.	Waldx^2	OR	*P*
AR	0.080	0.034	5.578	1.084	0.018
AT	-0.131	0.074	3.124	0.877	0.077
C	-7.321	3.990	3.368	0.66	0.001

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