ORIGINAL RESEARCH

Association Between the HER2 Protein Expression Level and the Efficacy of Neoadjuvant Chemotherapy in HER2-Positive Breast Cancer

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Objective: This study aimed to assess the relationship between human epidermal growth factor receptor-2 (HER2) protein expression level and clinicopathological features of HER2-positive breast cancer, and to analyze whether the expression level of HER2 protein could predict the response to anti-HER2 therapy.

Methods: The present study included 296 patients with HER2-positive breast cancer receiving neoadjuvant chemotherapy (NAC) containing trastuzumab between January 2014 and November 2019. The univariate comparisons of the differences in clinicopathological parameters between different HER2 protein expression groups, and the association between HER2 protein expression level and efficacy of NAC, were made using a X^2 test or Mann–Whitney U-test. Multivariate analyses of the differences in clinicopathological parameters between different HER2 protein expression groups, and the association between HER2 protein expression level and efficacy of NAC, were performed using logistic regression analysis.

Results: A total of 110 patients achieved a pathological complete response (pCR) after NAC. The pCR rate was 37.2%. The study showed that patients who were HR-negative, AR-positive, and CK5/6-negative had significantly higher expression level of HER2 protein [odds ratio (OR) = 0.183, $P < 0.001$; OR = 6.414, $P = 0.004$; OR = 0.261, $P = 0.004$, respectively]. Patients with HER2 3+ detected by immunohistochemistry (IHC) had significantly higher pCR rates compared with patients with HER2 2+. The HER2 protein expression level might effectively predict the efficacy of NAC in patients with HER2-positive breast cancer (OR = 3.520, $P = 0.003$).

Conclusion: The HER2 protein expression level was related to multiple clinical features in patients with HER2-positive breast cancer. For example, hormone receptor, androgen receptor, cytokeratin5/6, and HER2 protein expression level may be used to predict the response to NAC in patients with HER2-positive breast cancer and may serve as a predictive factor for NAC efficacy.

Keywords: breast neoplasm, human epidermal growth factor receptor-2, neoadjuvant chemotherapy, pathologic complete response

Introduction

Breast cancer is the most common malignant tumor among woman in China. Its incidence has been on the rise, and the onset age tends to be young.[1] Breast cancer with human epidermal growth factor receptor-2 (HER2) overexpression is an aggressive subtype of breast cancer, promoting cancer cell growth. HER2-positive breast cancer accounts for about 15%–20% of patients with breast cancer.[2]

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Neoadjuvant chemotherapy (NAC) largely increases the chances of operative and the chances of breast-conserving surgery. NAC combined with preoperative anti-HER2 therapy is the standard treatment for HER2-positive breast cancer, which can improve the pathologic complete response (pCR) rate. The overall survival (OS) and disease-free survival (DFS) of patients who had achieved pCR after receiving NAC significantly improved compared with the OS and DFS of patients without pCR.[3–6] Therefore, developing predictive factors for NAC efficacy was important to select candidates with an improved predicted prognosis. Studies have found that high HER2 protein expression in patients receiving anti-HER2 treatment may indicate a better prognosis. For example, the analysis of the CLEOPATRA Phase III trial showed that HER2 3+ by immunohistochemistry (IHC) was associated with an improvement in progression-free survival (PFS) and OS ($P = 0.05$) in both the trastuzumab plus docetaxel groups dividing by pertuzumab or placebo.[7] A previous study explored the response to NAC depending on the HER2 protein expression level. It showed that the expression level of HER2 protein was related to the clinical response rate following NAC ($P = 0.01$), but not related to the pCR rate ($P = 0.05$); however, the sample size is small ($n=40$).[8] The studies on the association between HER2 protein expression and NAC efficacy are relatively few. In the present study, the data of 296 patients with HER2-positive breast cancer, who were treated at the Breast Cancer Center of Henan Cancer Hospital from January 2014 to November 2019, were retrospectively collected. The HER2 protein expression level and the correlation between clinicopathological features were analyzed, and the predictive value of the pCR rate after NAC was further discussed.

Patients and Methods
Patients and Samples
This retrospective study included 296 female patients with breast cancer, who were treated at the Breast Cancer Center of Henan Cancer Hospital from January 2014 to November 2019. The inclusion criteria were as follows: (1) ultrasound-guided core needle biopsy performed in patients with invasive breast cancer prior to chemotherapy; (2) date on estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR), HER2, Ki-67, cytokeratin5/6 (CK5/6), and epidermal growth factor receptor (EGFR) available for HER2-positive breast cancer; and (3) the cT stage determined by the tumor size during the clinical physical examination according to the seventh edition of American Joint Committee on Cancer (AJCC) clinical staging of patients with breast cancer; the status confirmed by core needle biopsy If axillary lymph nodes were palpated positively or imaging studies were suspicious for metastasis; (4) no history of radiotherapy, chemotherapy, endocrine therapy, or other antitumor treatments; (5) no contraindications of chemotherapy and NAC; and (6) breast-conserving surgery or modified radical mastectomy after receiving four to eight cycles of standard regimen containing trastuzumab, and dissection of the axillary lymph nodes. The exclusion criteria were as follows: (1) male sex; (2) HER2-negative or inflammatory breast cancer; (3) distant metastases detected via the imaging examination; (4) surgery not conducted, resulting in failure to assess response to chemotherapy; (5) incomplete medical records; (6) patients with severe cardiopulmonary dysfunction; and (7) patients with another malignant tumors (Figure 1).

Neoadjuvant Chemotherapy
Of the 296 patients, 175 received 6 cycles of the TCH regimen (docetaxel + carboplatin + herceptin), 28 received 6 cycles of the PH regimen (paclitaxel + herceptin), and 93 received 4 cycles of the EC regimen (epirubicin + cyclophosphamide) following 4 cycles of the TH regimen (docetaxel + herceptin).

Immunostaining
The tissue samples were fixed with 10% neutral formaldehyde, embedded in paraffin, and cut into 4-µm-thick
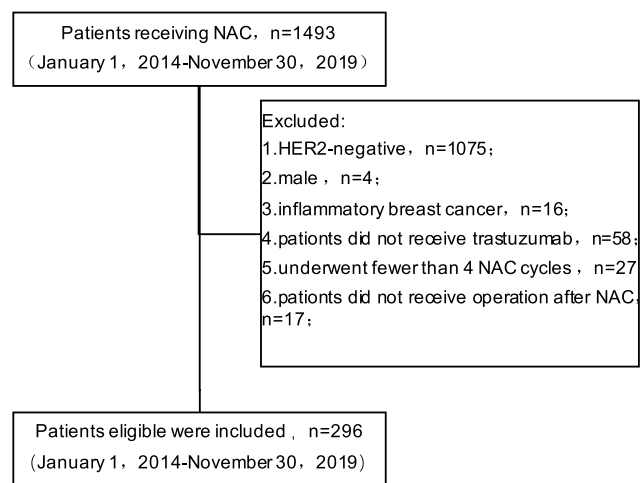

Figure 1 Flow chart of patients screening.

continuous slices for IHC (to determine ER, PR, AR, HER2, Ki-67, CK5/6, and EGFR statuses). The criteria were as follows: (1) The criterion for determining ER and PR positivity was that 1% or more of tumor cells exhibited positive nuclear staining by IHC, positivity for either One or both was defined as hormone receptor (HR) positive, and both were negative at the same time as HR-negative. (2) The criterion for determining AR positivity was that 10% of tumor cells exhibited positive nuclear staining by IHC. (3) According to the recommendations of the American Society of Clinical Oncology-College of American Pathologists (ASCO-CAP) in 2013, HER2 positivity was defined as 3+ by IHC or amplification by fluorescence in situ hybridization (FISH). (4) The average ratio of Ki-67-positive cells to all tumor cells was used as a quantitative indicator after selecting 10 high-power visual fields for each section. The Ki67 expression threshold was 30% (high expression, ≥30%; low expression, <30%). (5) Complete pathological response was defined as the absence of invasive breast cancer cells in the breast and lymph nodes after NAC (ypT0ypN0).[9]

Statistical Analysis

All statistical analyses were performed using SPSS 22.0 (SPSS, Inc.). Patients were divided into HER2 2+ and HER2 3+ groups by IHC. Univariate comparisons of the difference in clinicopathological parameters (age, menopausal status, HR status, AR status, Ki67 index, CK5/6 status, and EGFR status) between the two groups, and the association between HER2 protein expression level and efficacy of NAC was analyzed using the X^2 test, and the difference in clinicopathological parameters (cT stage and cN stage) between the two groups was determined using the Mann–Whitney U-test. Multivariate analyses of the difference in clinicopathological parameters between the two groups, and the association between HER2 protein expression level and efficacy of NAC, were performed using logistic regression analysis. Factors with $P<0.2$ in the univariate comparisons were included in the multivariate analysis model. A P value of<0.05 indicated a statistically significant difference.

Result
Patient Characteristics

A total of 296 patients with HER2-positive breast cancer were recruited, all of whom were woman. The median age was 47.5 years (range, 23–72 years). Further, 189 (63.9%) patients were premenopausal.

Also, 218 (73.6%) patients were cT1/2 and 184 (62.2%) patients were HR-positive. Of the 296 patients, 57 (19.3%) were assorted into the HER2 2+ group and 239 (80.7%) into the HER2 3+ group. A pCR was noted in 110 (37.2%) patients but not in 186 (62.8%) patients.

Associations Between HER2 Protein Expression Level and Clinicopathological Parameters

HR-positive patients were more likely to be HER2 2+ compared with HR-negative patients (24.7% vs 10.0%; P = 0.002). The HER2 2+ rate was lower in the AR-positive group (17.4%) than in the AR-negative group (57.1%; P = 0.001). The CK5/6-positive group exhibited a higher HER2 3 + rate compared with the CK5/6-negetive group (60.0% vs 83.5%; P = 0.001). The Clinicopathological features, such as age, menopausal status, cT stage, cN stage, Ki67 index, and EGFR status, were not significantly different in patients with different HER2 protein expression levels (P > 0.05; Table 1). The results of multivariate analysis showed that HR status (P < 0.001), AR status (P = 0.004), and CK5/6 status (P = 0.004) were all related to the HER2 protein expression level. The results of multivariate analysis showed that patients who were HR-negative, AR-positive, and CK5/6-negative had a significantly higher expression level of HER2 protein (OR = 0.183, P < 0.001; OR = 6.414, P = 0.004; OR = 0.261, P = 0.004, respectively; Table 2).

Pathological Response After NAC

The pCR rate was 37.2%. pCR was achieved in 11 of 57 patients in the HER2 2+ group, with a pCR rate of 19.3%. Also, pCR was achieved in 99 of 139 patients in the HER2 3+group, with a pCR rate of 41.4%. Significant differences in the pCR rate depending on the HER2 protein expression level are shown in Table 3. The univariate regression analysis in both groups revealed that the pCR rate in patients with high HER2 protein expression and those with a low cT stage was significantly higher (P = 0.002; P < 0.001, respectively). No significant association was found between pCR and other clinicopathological features including age, menstrual status, cN stage, HR status, AR status, Ki67 index, CK5/6 status, and EGFR status (Table 3). Factors with $P<0.2$ in univariate comparisons were included in the multivariate logistic regression analysis. The multivariate analysis revealed that the HER2 protein expression level was an independent predictive

Table 1 Univariate Analyses for the Associations Between HER2 Protein Expression Level and Clinicopathological Characteristics

Characteristics	N ((%)	HER2 2+ (%)	HER2 3+ (%)	χ^2/Z value	P value
Age (years)≤50	185(63)	33(18)	152(82)	0.936	0.424
Age (years)>50	111(37)	24(22)	87(78)		
Premenopausal	189(64)	34(18)	155(82)	0.540	0.462
Postmenopausal	107(36)	23(21)	84(78)		
cT				−0.142	0.887
T1	27(9)	5(19)	22(81)		
T2	191(65)	37(19)	159(81)		
T3	51(17)	8(16)	43(84)		
T4	27(9)	7(26)	20(74)		
Cn				−0.335	0.737
N0	45(15)	9(20)	36(80)		
N1	126(43)	21(17)	105(83)		
N2	61(21)	15(25)	46(75)		
N3	64(22)	12(19)	52(81)		
HR-negative	110(37)	11(10)	99(90)	8.456	0.002
HR-positive	186(63)	46(25)	140(75)		
AR-negative	14(5)	8(57)	6(43)	11.129	0.001
AR-positive	282(95)	49(17)	233(83)		
Ki-67 low expression	30(10)	5(17)	25(83)	4.358	0.704
Ki-67 high expression	266(90)	52(20)	214(80)		
CK5/6-negative	261(88)	43(16)	218(84)	10.985	0.001
CK5/6-positive	35(11)	14(40)	21(60)		
EGFR-negative	175(59)	33(19)	142(81)	0.044	0.834
EGFR-positive	121(41)	24(20)	97(80)		

Abbreviations: HER2, human epidermal growth factor receptor-2; HR, hormone receptor; EGFR, epidermal growth factor; cT, clinical tumor size; cN, clinical nodal status.

factor for pCR (OR = 3.520, P = 0.003). Additionally, The cT stage was also an independent predictive factor for pCR (P = 0.003; Table 4).

Discussion

The overexpression and/or amplification of HER2 is not only correlated with disease progression and poor prognosis but also the only biomarker to decide anti-HER2

Table 2 Multivariate Analyses for the Associations Between HER2 Protein Expression Level and Clinicopathological Characteristics

Characteristics	OR	95% CI	P value
HR status	0.183	0.076~0.439	<0.001
AR status	6.413	1.789~22.987	0.004
CK5/6 status	0.261	0.104~0.655	0.004

Abbreviation: HR, hormone receptor.

therapy.[10] The emergence of trastuzumab has significantly improved the prognosis of patients with HER2-positive breast cancer,[11] However, no apparent clinical benefit has been achieved in a considerable proportion of patients. The heterogeneity of HER2 protein expression and its predictive value for prognosis and neoadjuvant efficacy have received attention.[7,12] The results of this study revealed that HR-negative, AR-positive, and CK5/6-negative patients had a higher expression level of HER2 protein. Patients with HER2 3+ detected by IHC had a higher pCR rate compared with patients with HER2 2+. Higher HER2 protein expression was predictive of pCR to NAC.

In this study, the expression level of HER2 protein was significantly higher in the HR-negative group than in the HR-positive group. Previous reports suggested some correlation between ER and HER2 signaling pathways. ER inhibited HER2 expression under the action of estrogen, and HER2 expression was enhanced when ER expression

Table 3 Univariate Analyses for the Predictive Factors of Pathological Complete Response

Variables	n	pCR (%)	Non-pCR (%)	χ^2/Z value	P value
Age (years)≤50	185	61(33)	124(67)	2.616	0.054
Age (years)>50	111	49(44)	62(56)		
Premenopausal	187	64(34)	123(66)	0.553	0.290
Postmenopausal	109	44(40)	65(60)		
cT				−3.952	<0.001
T1	27	18(67)	9(33)		
T2	191	74(39)	117(61)		
T3	51	13(25)	38(75)		
T4	27	5(19)	22(81)		
cN				−1.596	0.110
N0	45	14(31)	31(69)		
N1	126	46(37)	80(63)		
N2	61	18(30)	43(70)		
N3	64	32(50)	32(50)		
HER2 2+	57	11(19)	46(81)	8.475	0.002
HER2 3+	239	99(41)	140(59)		
HR-negative	110	40(36)	70(68)	0.080	0.777
HR-positive	186	70(38)	116(62)		
AR-negative	14	3(21)	11(79)	1.374	0.212
AR-positive	282	107(38)	175(62)		
Ki-67 low expression	30	12(40)	18(60)	0.125	0.734
Ki-67 high expression	266	98(37)	168(63)		
CK5/6-negative	261	97(37)	164(63)	0.130	0.998
CK5/6-positive	35	13(37)	22(63)		
EGFR-negative	175	57(33)	118(67)	1.740	0.123
EGFR-positive	121	50(41)	71(59)		

Abbreviations: HER2, human epidermal growth factor receptor-2; HR, hormone receptor; EGFR, epidermal growth factor; pCR, complete pathological response; cT, clinical tumor size; cN, clinical nodal status.

Table 4 Multivariate Analysis of Factors Affecting the Efficacy of Neoadjuvant Chemotherapy for HER2-Positive Breast Cancer

Variables	OR	95% CI	P value
Age (years)≤50	1		
Age (years)>50	1.819	1.046~3.164	0.034
cT			0.003
T1	1		
T2	0.077	0.017~0.345	0.001
T3	0.295	0.090~0.964	0.043
T4	0.494	0.132~1.845	0.294
cN			0.327
N0	1		
N1	1.839	0.768~4.403	0.171
N2	1.733	0.874~3.434	0.115
N3	1.935	0.842~4.444	0.120
HER2			
2+	1		
3+	3.520	1.533~8.079	0.003
EGFR-negative	1		
EGFR-positive	1.340	0.783~2.293	0.285

Abbreviations: HER2, human epidermal growth factor receptor-2; cT, clinical tumor size; cN, clinical nodal status; EGFR, epidermal growth factor.

negative group. These differences in results might be due in large part to the different molecular subtype composition ratios of the study population and the different distribution of clinicopathological characteristics. In addition, the assessment methods for AR, such as IHC, TMA, RIA, RPPA, were not uniform. And IHC also had different criteria for the definition of AR-positivity, including positive cells ≥10%, ≥5% and ≥1%.[16] In this study, AR-positivity was defined as ≥10%.

In this study, pCR rates of patients with HER2 3+ detected by IHC was significantly higher. The data showed that the HER2 protein expression level in patients with HER2-positive breast cancer might effectively predict response to trastuzumab therapy. Three mechanisms were proposed for trastuzumab antitumor activity: (1) antibody-dependent cytotoxicity (ADCC); (2) cell cycle arrest caused by cascade inhibition of MAPK and PI3K signals; and (3) increased production of anti-angiogenic factors.[18,19] In the whole-body context, natural killer cell–mediated ADCC is stimulated by the binding of trastuzumab on the surface of HER2-overexpressing cells, which binds to the extracellular domain IV of HER2.[20] Laboratory studies have also indicated that the degree of the ADCC response may be proportional to the HER2 protein expression level.[21,22] Several basic studies

decreased.[13] The study by Liu et al showed that HR expression negatively correlated with HER2 expression ($r = -0.338$, $p = 0.000$).[14] The result of this study was consistent with previous findings. Some previous studies on the relationship between AR and HER2 expression showed no correlation between the expression of AR and HER2,[15,16] some other studies revealed that the HER2 protein expression level was lower in the AR-positive group than in the AR-negative group.[17] However, The present study showed that the HER protein expression level was higher in the AR-positive group than in the AR-

theoretically supported the present study. However, no evidence is available to show that the other two mechanisms are related to the HER2 protein expression level. According to the ASCO-CAP guidelines in 2013, tumors with the HER2/CEP17 ratio ≥2.0 were considered HER2-positive when using FISH. Patients in the BCIRG-006 trial, whose breast cancers had an HER2/CEP17 ratio ≥2.0 but HER2 copy number >4 signals per cell, could not benefit from trastuzumab therapy (HR, 1.10; 95% CI, 0.31–3.89; $P = 0.886$).[23] Patients of this group might be a reason why patients with HER2 2+ had a lower pCR rate compared with patients with HER2 3+.

In a study from Burstein at al., IHC was used to detect the expression level of HER2 protein and analyze its value in predicting the efficacy of NAC.[8] The results showed that the clinical response rate of HER2 2+ breast cancer was significantly lower than that of HER2 3+ breast cancer (38% vs 84%), but no difference in pCR rate was observed ($P = 1.0$), which might be because of the small sample size ($n = 40$). Another study showed that pCR occurred significantly more frequently in HER2 3+ tumors (P = 0.009).[24] In a study from Scaltriti et al.[12] HER2 protein expression strongly correlated with pCR in both HR-negative (ratio of 2.5; 95% CI, 1.44–4.20; $P = 0.001$) and HR-positive (ratio of 3.1; 95% CI, 1.61–5.81 $P = 0.001$) groups of patients treated with the combination of Lapatinib and Trastuzumab. This study used HERmark analysis to quantify the expression level of HER2 protein, which was more accurate and sensitive compared with IHC. The results of the present study were consistent with previous findings. In previous studies, HR-negative patients had more chances to achieve pCR compared with HR-positive patients.[25,26] The present study showed that the HR status was not related to pCR because of the small sample size.

This study showed that the HER2 protein expression level in patients with HER2-positive breast cancer might effectively predict the response to NAC. Interestingly, the predictive value of HER2 expression for the efficacy of NAC has been described at several levels. A study indicated that the expression level of HER2 mRNA significantly correlated with pCR in HER2-positive and ER-positive breast cancer ($P = 0.004$), but had no correlation in ER-negative and HER2-positive breast cancer.[27] A study including 14,597 patients demonstrated that the association between HER2/CEP17 ratio and pCR to NAC was linear ($P < 0.001$).[28] Patients with HER2 heterogeneity seemed to be less responsive to anti-HER2 targeted therapy, as

demonstrated by a lower achievement of pCR following NAC containing trastuzumab.[29,30] Therefore, it is important to increase the accuracy of HER2 testing and explore the intrinsic subtypes of HER2-positive breast cancer to suggest rational therapeutic strategies and predict the efficacy of anti-HER2 treatment.

This study, using IHC to determine the expression level of HER2 protein and analyze its value in predicting the efficacy of NAC, had the largest sample size to date, thus providing an important basis for the exploration of more accurate HER2 testing, intrinsic subtype of HER2-positive breast cancer, and individualized treatment. However, the study had several limitations: it was retrospective; the analysis could only be performed in a subpopulation; it was subject to the misclassification bias or errors in record keeping; and it did not demonstrate whether OS and DFS were improved after achieving this increase in pCR rates. The results of this study showed the clinical utility of the HER2 protein expression level as a predictive factor for the prognosis of patients receiving NAC, which can be further validated by increasing the sample size and involving multicenter collaboration.

In summary, the present study showed that the HER2 protein expression level was related to multiple clinical features, such as HR, AR, and CK5/6, in patients with HER2-positive breast cancer. Also, the HER2 protein expression level might serve as an independent predictor of pCR to NAC.

Abbreviations

ADCC, antibody-dependent cellular cytotoxicity; AJCC, American Joint Committee on Cancer; AR, androgen receptor; ASCO-CAP, American Society of Clinical Oncology-College of American Pathologists; CK5/6, cytokeratin5/6; DFS, disease-free survival; EGFR, epidermal growth factor receptor; ER, estrogen receptor; FISH, fluorescence in situ hybridization; HER2, epidermal growth factor receptor-2; HR, hormone receptor; IHC, immunohistochemistry; NAC, neoadjuvant chemotherapy; OS, overall survival; pCR, pathological complete response; PFS, progression-free survival; PR, progesterone receptor.

Ethics Approval and Consent to Participate

This study was complied with the Declaration of Helsinki and approved by the Ethics Committee of the Affiliated

Cancer Hospital of Zhengzhou University. The Ethics Committee of the Affiliated Cancer Hospital of Zhengzhou University did not require patients to agree to review their medical records (medical records were written by doctors and belonged to the medical records room. On the premise of not disclosing the privacy of patients, doctors can use it for clinical research). All data analyzed is anonymous, patient data are kept strictly confidential.

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Disclosure

The authors have no competing interests to declare.

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