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Original Research

Analysis of the Clinical Molecular Characteristics and Neoadjuvant Chemotherapy Response in Patients with Human Epidermal Growth Factor Receptor 2-Negative Breast Cancer and Axillary Lymph Node Metastasis

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Abstract

Objective: This study aimed to investigate the clinical molecular characteristics in patients with human epidermal growth factor receptor 2 (HER2)-negative breast cancer and axillary lymph node metastasis and explored the related factors of the neoadjuvant chemotherapy (NAC) response. **Methods**: The data of 185 patients with HER2-negative breast cancer and axillary lymph node metastasis who were treated in the Department of Breast Center of the Affiliated Hospital of Qingdao University from July 2017 to July 2021 were retrospectively analyzed. The clinical features and the related factors for the responses of the primary tumor and axillary lymph node metastasis to NAC were analyzed. Statistical analysis was conducted using the SPSS 26.0 statistical software. Univariate analysis was conducted using the χ^2 test, and multivariate analysis was conducted using logistic regression analysis. **Results**: The differences in estrogen receptor (ER), progesterone receptor (PR), and Ki67 among the three HER2-negative subgroups (the immunohistochemistry (IHC)0 group, IHC1+ group, and IHC2+/*in situ* hybridization– group) were statistically significant ($p < 0.05$). Univariate analysis revealed that the differences in the tumor stage, ER, PR, and Ki67 among the groups based on the response of the primary tumor to NAC were statistically significant ($p < 0.05$), and the differences in ER, PR, and Ki67 among the groups based on the response of axillary lymph node metastasis to NAC were statistically significant ($p < 0.05$). Multivariate analysis revealed that the difference in Ki67 among the groups based on the response of axillary lymph node metastasis to NAC was statistically significant ($p < 0.05$). **Conclusions**: When the expression level of HER2-negative IHC increases, the positive rates of ER and PR increase. A smaller tumor, negative ER, negative PR, and a Ki67 level >30% indicate a good effect of NAC for primary tumors. Negative ER, negative PR, and a Ki67 level >30% indicate a good effect of NAC for axillary lymph node metastasis. Therefore, Ki67 may be an independent factor affecting the efficacy of NAC for axillary lymph node metastasis.

Keywords: HER2-negative; breast cancer; axillary lymph nodes; neoadjuvant chemotherapy

1. Introduction

Breast cancer is the most commonly occurring cancer among women worldwide. As the incidence continues to increase, this disease seriously endangers the physical and mental health of women [1].

Since the last century, neoadjuvant therapy has been clinically applied. Neoadjuvant therapy includes neoadjuvant chemotherapy (NAC), neoadjuvant targeted therapy, and neoadjuvant endocrine therapy. Guidelines at home and abroad reflect neoadjuvant therapy as a standard treatment for locally advanced breast cancer. The application of targeted therapy, especially a double target combination, shows a higher pathological complete remission (pCR) rate in neoadjuvant therapy for human epidermal growth factor receptor 2 (HER2)-positive breast cancer, and the long-term survival rate of patients with pCR rates is higher than that of those who did not receive the treatment [2]. The overall effective rate of NAC in patients with breast cancer is 60%–90%, but there are still 10%–35% of patients with poor efficacy and even poor progression during chemotherapy [3]. Patients with hormone receptor-positive breast cancer are not sensitive to NAC. These patients have a pCR rate of 6%–12%, while patients that are HER2-negative have a pCR rate of approximately 7% [4,5]. The complete remission (CR) rate of NAC based on paclitaxel and anthracycline administration in triple negative breast cancer is 30%–40% [6].

The HER2 protein expression level is assessed by immunohistochemistry (IHC) and *in situ* hybridization (ISH) [7]. A HER2-negative result includes a low HER2 expression (IHC1+ or IHC2+ and fluorescence *in situ* hybridization-negative) and a zero HER2 expression

(IHC0). Antibody-coupled drugs provide new therapeutic options for patients with breast cancer, including those with a low expression of HER2. The latest study published in *The Lancet* [8] revealed that the hormone receptor-positive rate, pCR rate, disease-free survival rate, and overall survival rate were different between low HER2 expression tumors and zero HER2 expression tumors. Low HER2 expression tumors have unique biological characteristics, different therapeutic effects, and survival results, especially for drug-resistant and hormone receptor-negative tumors.

In the present study, the investigators studied the factors related to NAC for HER2-negative breast cancer, primary breast cancer, and axillary lymph node double metastasis breast cancer. Furthermore, HER2-negative cancers were divided into three subgroups to study their clinical molecular characteristics; this is helpful for subtype refinement and precise treatment of HER2-negative breast cancer. For patients with poor efficacy of NAC, preliminary screening can be performed based on clinical molecular characteristics, and patients with low HER2 expressions can be treated with antibody–drug conjugate (ADC) drugs.

2. Information and Methods

2.1 Clinical Data Acquisition

The data of patients with breast cancer who were treated in the Department of Breast Center of the Affiliated Hospital of Qingdao University from July 2017 to July 2021 were retrospectively analyzed. Neoadjuvant chemotherapy and surgery were performed in the Department of Breast Center.

2.2 Inclusion Criteria

The inclusion criteria were as follows: if the primary tumors and axillary lymph node tumors were diagnosed as invasive carcinoma by a core needle biopsy; patients who were female; if the immunohistochemical information included estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki67; if there was no presence of distant metastasis; patients who had not received malignant tumor-related treatment before the treatment administered in this study; if the first-line regimen of NAC was anthracycline combined with paclitaxel or anthracycline sequential paclitaxel; patients who had undergone a modified radical mastectomy.

2.3 Exclusion Criteria

The exclusion criteria were as follows: if the primary tumor had been diagnosed as a carcinoma *in situ* or a specific type of cancer; women who were pregnant or lactating; if the immunohistochemical information was uncertain or insufficient; patients with distant metastasis; patients who had received malignant tumor-related treatment before the treatment administered in this study; if there had been a change of NAC regimen; if a mastectomy had not been performed or an axillary lymph node dissection had not been completed.

2.4 Data Acquisition and Database Establishment

Patients who were HER2-negative were divided into three groups: an IHC0, IHC1+, and IHC2+/ISH– group. The NAC responses of the primary tumors were divided into the G1–G3 and G4–G5 groups according to the Miller–Payne grades. The treatment responses of the axillary lymph node tumors were divided into the CR of axillary lymph nodes (apCR) group and the non-CR of axillary lymph nodes (non-apCR) group according to the remission rates. Age, body mass index (BMI), menarche age, menstrual status, clinical tumor stage (cT), ER, PR, HER2, Ki67, and pCR were collected.

2.5 Statistical Analysis

Data were statistically analyzed using the SPSS 26.0 statistical software (IBM Corp, Armonk, NY, USA). Clinical molecular characteristics and related factors of the treatment response of the primary tumor and axillary lymph node metastasis were compared among the HER2-negative breast cancer subgroups using the χ^2 test or Fisher's exact probability method. Univariate analysis was conducted using the χ^2 test or Fisher's exact probability method to screen out significant variables, which were then included in the multivariate logistic analysis. A p value of <0.05 was considered statistically significant.

3. Results

3.1 Basic Data of the Patients

In this study, 185 patients with HER2-negative breast cancer and axillary lymph node metastasis were enrolled. In 23 patients, primary tumors and axillary lymph node metastasis achieved CR. In 13 patients, only primary tumors achieved CR, and in 24 patients, only axillary lymph node metastasis achieved CR (Table 1).

3.2 Clinical Molecular Characteristics in the Different Subgroups of HER2-Negative Breast Cancer

There were 66 cases of IHC0, 87 cases of IHC1+, and 32 cases of IHC2+/ISH–. There were no significant differences in age, BMI, menarche age, menstrual status, cT, primary tumor treatment response, or axillary lymph node metastasis treatment response among the HER2-negative subgroups. The differences in ER, PR, and Ki67 among the three HER2-negative subgroups were statistically significant ($p < 0.05$, Table 2).

3.3 Analysis of Related Factors for the Response of Primary Tumors to NAC

Among the patients, there were 129 cases with a Miller–Payne grade of G1–G3 and 56 cases with a Miller–Payne grade of G4–G5.

Table 1. Patient demographic and clinical characteristics at baseline.

Characteristic		Total population, n (%)
Age	<40	29 (15.68%)
	40–60	117 (63.24%)
	>60	39 (21.08%)
BMI (Kg/m^2)	≤25	89 (48.11%)
	>25	96 (51.89%)
Menarche age	<14	42 (22.70%)
	14–16	112 (60.54%)
	>16	31 (16.76%)
Menstrual status	Premenopausal	91 (49.19%)
	Postmenopausal	94 (50.81%)
cT	T1	24 (12.97%)
	T2	88 (47.57%)
	T3	40 (21.62%)
	T4	33 (17.84%)
ER status (biopsy)	Negative	41 (22.16%)
	Low-Positive (1–10%)	11 (5.95%)
	High-Positive (>10%)	133 (71.89%)
PR status (biopsy)*	Negative	64 (34.59%)
	Low-Positive (1–20%)	43 (23.24%)
	High-Positive (>20%)	78 (42.16%)
HER2 (biopsy)	IHC0	66 (35.68%)
	IHC1+	87 (47.03%)
	IHC2+/ISH–	32 (17.30%)
Ki67 expression (biopsy)	<15%	12 (6.49%)
	15–30%	67 (36.22%)
	>30%	106 (57.30%)
PCR	aPCR	24 (40.00%)
	bPCR	13 (21.67%)
	PCR	23 (38.33%)

Note: *According to CSCO guidelines, PR20% serves as the threshold for Luminal A and Luminal B.

3.3.1 Univariate Analysis

There were no significant differences in age, BMI, menarche age, menstrual status, cT, HER2, or ER among the groups based on the response of the primary tumor to NAC. The differences in ER, PR, and Ki67 among the groups based on the response of axillary lymph node metastasis to NAC were statistically significant ($p < 0.05$, Table 3).

3.3.2 Multivariate Analysis

Estrogen receptor, PR, Ki67, and the response of axillary lymph node metastasis to NAC were included in the binary logistic regression for multivariate analysis. The results revealed that there was a significant difference in the response of axillary lymph node metastasis to NAC ($p < 0.05$, Table 4).

3.4 Analysis of Related Factors for the Response of Axillary Lymph Node Metastasis to NAC

There were 47 cases of apCR and 138 cases of non-apCR.

3.4.1 Univariate Analysis

There were no significant differences in age, BMI, menarche age, menstrual status, cT, and HER2 among the groups based on the response of axillary lymph node metastasis to NAC, and the differences in ER, PR, and Ki67 among the groups based on the response of the primary tumor to NAC were statistically significant ($p < 0.05$, Table 5).

3.4.2 Multivariate Analysis

Estrogen receptor, PR, Ki67, and the response of the primary tumor to NAC were included in the binary logistic regression for multivariate analysis. The results revealed that there were significant differences in Ki67 between the responses of axillary lymph node metastasis to NAC and the responses of primary tumors to NAC ($p < 0.05$). These results indicate that Ki67 (OR = 3.571, 95% CI: 1.386–9.201, $p = 0.008$) may be an independent factor affecting the response of axillary lymph node metastasis to NAC (Table 6).

In this study, ER, PR, and Ki67 were expressed differently in the three HER2-negative subgroups, and the negative-to-positive ratio of ER and PR decreased gradually in the IHC0, IHC1+, and IHC2+/ISH– subgroups. The negative-to-positive ratio of ER was 50.00%, 20.83%, and 14.29%, respectively, and the negative-to-positive ratio of PR was 88.57%, 40.32%, and 28.00%, respectively. In HER2-negative breast cancer, when the expression level of IHC increases, the positive rates of ER and PR increase.

Univariate analysis revealed that the efficacy of NAC for primary tumors might be related to the tumor stage, ER, PR, Ki67, and the response of axillary lymph node metastasis to NAC. The efficacy of NAC for axillary lymph node metastasis may be related to ER, PR, Ki67, and the response of primary tumors to NAC. Multivariate analysis revealed that Ki67 might be an independent factor affecting the efficacy of NAC for axillary lymph node metastasis. A smaller tumor, negative ER, negative PR, and a Ki67 level >30% indicate a good effect of NAC on the primary tumor. Negative ER, negative PR, and a Ki67 level >30% indicate a good effect of NAC for axillary lymph node metastasis. Therefore, Ki67 may be an independent factor affecting the efficacy of NAC for axillary lymph node metastasis.

4. Discussion

The number of patients with breast cancer who receive neoadjuvant therapy is increasing [9]. Compared with adju-

Table 2. Association between clinicopathological factors and HER2-negative subtypes.

Characteristics		n	IHC0	IHC1+	IHC2+/ISH–	χ^2	p
age	<40	29	11	12	6		
	40–60	117	40	59	18		
	>60	39	15	16	8	1.655	0.799
BMI (Kg/m^2)	≤25	89	29	43	17		
	>25	96	37	44	15	0.843	0.656
Menarche age	<14	42	19	16	7		
	14–16	112	39	55	18		
	>16	31	8	16	7	3.507	0.477
Menstrual status	Premenopause	91	35	46	10		
	Postmenopausa	94	31	41	22	4.982	0.083
cT	T1	24	12	7	5		
	T2	88	27	49	12		
	T3	40	12	21	7		
	T4	33	15	10	8	10.265	0.114
ER	Negative	41	22	15	4		
	Positive	144	44	72	28	7.728	0.021
PR	Negative	63	31	25	7		
	Positive	122	35	62	25	8.112	0.017
Ki67	<15%	19	5	5	9		
	15–30%	60	18	36	6		
	>30%	106	43	46	17	17.774	0.001
Response of primary tumor	G1–G3	129	44	61	24		
	G4–G5	56	22	26	8	0.721	0.697
Response of axillary lymph nodes	apCR	47	18	21	8		
	Non-apCR	138	48	66	24	0.198	0.906

Table 3. Association between clinicopathological factors and primary tumors response.

Characteristics		n	G1–G3	G4–G5	χ^2	p
age	<40	30	18	12		
	40–60	116	83	33		
	>60	39	28	11	1.607	0.448
BMI (Kg/m^2)	≤25	89	56	33		
	>25	96	73	23	3.767	0.052
Menarche age	<14	42	29	13		
	14–16	112	74	38		
	>16	31	26	5	3.656	0.161
Menstrual status	Premenopause	76	55	21		
	Postmenopausa	109	74	35	0.426	0.514
cT	T1	24	12	12		
	T2	88	61	27		
	T3	40	28	12		
	T4	33	28	5	8.008	0.046
HER2	IHC0	66	44	22		
	IHC1+	87	61	26		
	IHC2+/ISH–	32	24	8	0.721	0.697
ER	Negative	40	22	18		
	Positive	145	107	38	5.246	0.022
PR	Negative	62	33	29		
	Positive	123	96	27	12.034	0.001
Ki67	≤30%	79	66	13		
	>30%	106	63	43	12.466	0.000
Response of axillary lymph nodes	apCR	47	18	29		
	Non-apCR	138	111	27	29.491	0.000

Table 4. Multivariate logistic regression analysis for primary tumors response.

Clinicopathological factors	B	SE	Wald	p	OR (95% CI)
ER	–0.45	0.524	0.737	0.391	0.638 (0.228–1.781)
PR	0.802	0.454	3.127	0.077	2.230 (0.917–5.425)
Ki67	–0.731	0.422	2.996	0.083	0.482 (0.211–1.102)
Response of axillary lymph nodes	1.58	0.409	14.946	0.000	4.854 (2.179–10.814)

Table 5. Association between clinicopathological factors and axilla response.

Characteristics		n	apCR	Non-apCR	χ^2	p
age	<40	29	9	20		
	40–60	117	26	91		
	>60	39	12	27	1.703	0.427
BMI(Kg/m^2)	≤25	89	23	66		
	>25	96	24	72	0.017	0.895
Menarche age	<14	42	7	35		
	14–16	112	35	77		
	>16	31	5	26	5.119	0.077
Menstrual status	Premenopause	91	25	66		
	Postmenopausa	94	22	72	0.404	0.525
cT	T1	24	8	16		
	T2	88	26	62		
	T3	40	7	33		
	T4	33	6	27	3.820	0.282
HER2	IHC0	66	18	48		
	IHC1+	87	21	66		
	IHC2+/ISH–	32	8	24	0.198	0.906
ER	Negative	41	20	21		
	Positive	144	27	117	15.187	0.000
PR	Negative	63	27	36		
	Positive	122	20	102	15.353	0.000
Ki67	≤30%	79	7	72		
	>30%	106	40	66	19.915	0.000
Response of primary tumor	G1–G3	129	18	111		
	G4–G5	56	29	27	29.491	0.000

Table 6. Multivariate logistic regression analysis for axilla response.

Clinicopathological factors	B	SE	Wald	p	OR (95% CI)
ER	–0.655	0.492	1.773	0.183	0.520 (0.198–1.362)
PR	–0.446	0.463	0.930	0.375	0.640 (0.258–1.585)
Ki67	1.273	0.483	6.950	0.008	3.571 (1.386–9.201)
Response of primary tumor	1.551	0.396	15.324	0.000	4.715 (2.169–10.250)

vant chemotherapy, NAC can dynamically monitor the drug sensitivity of tumors during chemotherapy, facilitate timely adjustment of drug dosage, and ensure the best curative effect of chemotherapy [10,11]. In addition, NAC can reduce the clinical stage, increase the surgical resection and breast preservation rates, and greatly improve the quality of life and prognosis of patients. The five-year survival rate also significantly improves for patients who achieve pCR after NAC [12].

Breast cancer therapy enters a new level with ADC intervention. This treatment produces exciting results not only in patients with HER2-positive breast cancer but also in patients with low HER2 expression breast cancer [13–16]. In this study, gene expression analysis revealed that a low expression of HER2 exists in luminal and non-luminal types of breast cancer, but ER is usually positive, especially in the luminal B type [17]. According to immunohistochemical expression levels, HER2-negative breast cancer can be subdivided into the IHC0, IHC1+, and IHC2+/ISH– groups, while low HER2 expression groups include IHC1+ and IHC2+/ISH– levels. We discovered differences in the clinical molecular characteristics among the three sub-

groups that showed that the conditions of a positive ER, positive PR, a Ki67 level <15%, and IHC2+/ISH– expression increased, which suggested that an IHC2+/ISH– expression is more common in the luminal A type. Luminal A is known to be insensitive to chemotherapy, and ADC agents are expected to provide a more feasible and effective treatment for these patients.

A previous study revealed that compared with zero HER2 expression, a low expression of HER2 was usually related to a higher histological grade and proliferation rate, and the prognosis was poorer [18–21]. Recent data has revealed that the effective treatment rate of patients with HER2+ breast cancer was higher than that of patients with HER1+ breast cancer. Gentile *et al*. [22] established that there were no significant differences in age, histological grade, tumor size, lymph node status, and chemotherapy regimen between an effective and ineffective group. In unscreened invasive breast cancer, determining mass size under an ultrasound was a strong prognostic factor [23,24]. In a study of women receiving NAC, determining tumor size under an ultrasound was not associated with metastasis-free survival, although there is evidence claiming that small tumors are more likely to achieve CR [25]. The Ki67 protein affects cell cycles and DNA synthesis, reflects the proliferation of tumor cells, and is also associated with the development and prognosis of breast cancer [26].

In this study, a smaller tumor, negative ER, negative PR, and a Ki67 level >30% indicated a good effect of NAC for primary tumors. Negative ER, negative PR, and a Ki67 level >30% indicated a good effect of NAC for axillary lymph node metastasis. Therefore, Ki67 may be an independent factor in the response of axillary lymph node metastasis. Based on existing data and experimental results, experts suggest that HER2 can be subdivided into the positive HER2, negative HER2 (zero expression of HER2), and low HER2 expression types to formulate different treatment regimens. Therefore, about 55% of breast cancers will be classified as a low expression of HER2. HER2 low expression breast cancer may be heterogeneous in the microenvironment of tumor-infiltrating lymphocyte enrichment, which may be related to antibody-dependent cytotoxicity [18]. However, there are different types of low HER2 expression heterogeneity, and whether it will lead to different degrees of treatment response remains to be determined. The dichotomous definitions of positive HER2 and negative HER2 are currently undergoing a series of changes. By identifying the low HER2 expression types and studying ADC drugs, HER2-negative breast cancer may be subdivided into low expression and zero expression of HER2 groups to formulate different treatment options.

5. Conclusions

HER2-negative breast cancer cannot be treated with targeted therapy, and the PCR rate of NAC is low, especially for hormone receptor-positive breast cancer. It is expected that early surgical or ADC intervention can help to achieve long-term benefits by screening patients with poor efficacy and poor prognosis through clinical analysis. However, this will require further study using a larger sample size.

Author Contributions

Conception and design of the research—YZ, JPM. Acquisition of data—JPM, JHZ, TW, YM. Analysis and interpretation of the data—JPM, JZ. Statistical analysis—JPM. Obtaining financing—YZ. Writing of the manuscript—JPM, SF. Critical revision of the manuscript for intellectual content—HBW. All authors read and approved the final draft.

Ethics Approval and Consent to Participate

This study was conducted with approval from the Ethics Committee of Yantai Penglai People's Hospital (202013). This study was conducted in accordance with the declaration of Helsinki. Written informed consent was obtained from all participants.

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Conflict of Interest

The authors declare no conflict of interest.

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