The Breast 71 (2023) 69–73

Contents lists available at ScienceDirect

The Breast

journal homepage: www.journals.elsevier.com/the-breast

Differences between the efficacy of HER2(2+)/FISH-positive and HER2 (3+) in breast cancer during dual-target neoadjuvant therapy

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ARTICLE INFO

Keywords:
Human epidermal growth factor receptor 2 positive breast cancer
Pathological complete response
Neoadjuvant dual-targeted therapy
Clinicopathological features

ABSTRACT

Introduction: This study investigated the differences in efficacy between IHC(2+)/FISH-positive and IHC(3+) in HER2-positive breast cancer (BC) during neoadjuvant chemotherapy (NAC) combined with trastuzumab and pertuzumab. The research also aimed to provide insight into treatment strategies for clinical HER2(2+)/FISH-positive and HER2(3+) BC.

Materials and methods: A retrospective analysis was performed on the clinical and pathological data of patients with confirmed diagnoses of invasive BC treated via combined NAC and dual-target therapy who underwent surgery at the Breast Surgery Center of Sichuan Cancer Hospital between June 2019 and June 2022. The correlation between the clinicopathological characteristics and pathological complete response (pCR) was analyzed via the $\chi 2$ test, while logistic regression was performed using the SAS 9.4 statistical analysis software.

Results: This study examined 224 patients with an overall pCR rate of approximately 59.82%, which included 36 IHC(2+)/FISH-positive and 188 IHC(3+) cases with approximate pCR rates of 41.67% and 63.30%, respectively. Univariate and multifactorial analysis of the clinical and pathological data determined that age, menstrual status, family history, Ki67 expression, number of treatment cycles, and treatment regimen did not influence pCR. No statistical differences were evident between the univariate and multivariate models. However, the clinical stage, hormone receptor, and HER2 expression status significantly impacted pCR, with considerable consistent differences between the univariate and multifactor analyses.

Conclusions: HER2 IHC(3+) BC displays a higher pCR rate than HER2 IHC(2+)/FISH-positive BC ($p \leq 0.05$), with a positive correlation between the HER2 protein expression levels and the response to anti-HER2 therapy.

1. Background

In 2020, an estimated 19.3 million new cancer cases were reported worldwide. Female breast cancer (BC) has surpassed lung cancer to become the most common cancer, with about 2.3 million new cases, accounting for 11.7%. It is also the most common cause of global cancer-related death in women, presenting severe health risks [1]. Based on the criteria delineated by the 2013 St. Gallen International Breast Cancer Conference [2], BC can be divided into four categories according to the expression of the estrogen receptor (ER), progesterone receptor (RP), human epidermal growth factor receptor 2 (HER2), and proliferation cell nuclear antigen (Ki67), including Luminal A, Luminal B, HER2-positive , and triple-negative BC. HER2, a transmembrane protein displaying tyrosine kinase activity, binds to the cell membrane surface, where it is encoded by the HER2/neu proto-oncogene and participates in the cell proliferation and differentiation signaling pathway [3]. The HER2 expression status is vital for molecular typing and targeted HER2-positive BC therapy. HER2-positive BC can be divided into hormone receptor (HR)-negative (ER-negative/PR-negative) and HR-positive (ER-positive/PR-positive) BC based on the HR status, and HER2(2+)/fluorescence in-situ hybridization (FISH)-positive and HER2 (3+) BC according to the immunohistochemistry (IHC) and FISH results [4]. HER2-positive BC, accounting for about 20% of clinical cases, is highly malignant and prone to recurrence and metastasis [5].

Minimal research is available that compares the neoadjuvant chemotherapy (NAC) adjuvant dual-target therapy efficacy differences

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https://doi.org/10.1016/j.breast.2023.07.008
Received 7 June 2023; Received in revised form 13 July 2023; Accepted 14 July 2023
Available online 24 July 2023

between HER2(2+)/FISH-positive and HER2(3+) BC and analyzes the reasons for this variation. Furthermore, existing studies encountered challenges, such as inadequate sample size and insufficiently included standard and uniform objects.

This study carefully reviews and analyzes relevant case data from the Breast Surgery Center of the Sichuan Cancer Hospital to explore the differences between IHC(2+)/FISH-positive and IHC(3+) responses to treatment during dual-target therapy HER2-positive BC treatment. It also examines the clinicopathological factors that may lead to the differences and clarify the correlation between different HER2 protein overexpression levels and anti-HER2 therapy responses to provide evidence-based support for precise treatment.

2. Methods

2.1. Subjects

This study included complete case data of eligible patients diagnosed with HER2-positive invasive BC who received NAC adjuvant dual-target therapy and underwent surgery at the Breast Surgery Center of Sichuan Cancer Hospital from June 2019 to June 2022.

2.2. Exclusion criteria

The criteria excluded males, non-invasive BC, patients who did not receive standardized neoadjuvant adjuvant double-target therapy, incomplete case data, Stage I, and IV BC patients, occult BC, bilateral BC patients, midway treatment replacement programs, and prior cancer or concurrent diagnosis of other cancers at the time of admission.

2.3. Case parameters

General information including gender, age at diagnosis, case number, diagnosed menstrual status, family history of breast and ovarian cancer or other types of cancer.

The clinicopathologic data included clinical stage and tumor node metastasis (TNM) stage, neoadjuvant therapy (NAT) regimen and number of cycles, preoperative expression of ER, PR and Ki67 in the primary breast cancer foci, as well as IHC or FISH detection of HER2, postoperative lymph node metastasis, and Miller-Payne (MP) grading.

2.4. IHC interpretation criteria

ER/PR-positive [6] was defined as positive tumor nuclei staining of ≥1%. HR consists of ER and PR. HR-positive BC comprises positive ER and/or PR, while HR-negative BC includes negative ER and PR. HER2-positive [4] was defined as IHC(2+) and FISH-positive or IHC (3+). Based on the 2013 St. Gallen consensus [2], this study used 20% as the threshold value to determine the high or low Ki67 positivity index, with Ki67 ≥ 20% representing high expression and Ki67 < 20% denoting low expression.

2.5. NAT efficacy evaluation

The pathological complete response (pCR) was defined as a primary breast lesion with MP grading of grade 5 and no residual cancer cells in the regional lymph nodes (ypT0/isypN0) [7,8].Previous studies [9] have confirmed that HER2-positive BC patients who achieved pCR after receiving NAC combined with anti-HER2 therapy had longer event-free survival (EFS) and overall survival (OS) compared with non-pathological complete response (non-pCR) patients.Therefore, this study used pCR instead of EFS and OS as an efficacy assessment index.

2.6. Statistical methods

Clinical and pathological characteristics were compared and analyzed using the $\chi 2$ test, and all clinical and pathological characteristics were analyzed univariately and multivariately using logistic regression. Differences with p-values≤0.05 were considered statistically significant.

3. Research results

This study (Table 1) examined 224 HER2-positive patients with an overall pCR rate of approximately 59.82%, which included 36 IHC(2+)/FISH-positive and 188 IHC(3+) cases with approximate pCR rates of 41.67% and 63.30%, respectively, as determined via the statistical analysis of the clinicopathological data. The pCR rate was about 55.21% in 96 cases treated using an EC-THP regimen and approximately 63.28% in 128 cases exposed to a TCbHP regimen.

Univariate analysis (Table 2) showed that the clinical stage, HR, and HER2 expression status were significant factors affecting pCR ($p \leq 0.05$). A low clinical stage (pCR:65.19% VS 51.69%, 95% CI:0.33–0.99, $P = 0.045$) and negative HR (pCR:69.70% VS 52.00%, 95% CI:0.27–0.82, $P = 0.008$) and HER2(3+) states (pCR:63.30% VS 41.67%, 95%

Table 1
Baseline clinicopathological characteristics.

category Total (n = 224)	**pCR** (n = 134)	**non-pCR** (n = 90)	pCR (%)
Total	134	90	59.82
Age division			
≤35 years old	12	11	52.17
>35 years old and ≤55 years old	85	61	58.22
>55 years old	37	18	67.27
Menstrual state			
YES	70	40	63.64
NO	64	50	56.14
Family history of cancer			
No	124	82	60.19
Breast cancer	2	5	28.57
Ovarian cancer	1	1	50.00
Other tumors	7	2	77.78
cT stage			
cT1	6	1	85.71
cT2	103	68	60.23
cT3	15	10	60.00
cT4	10	11	47.62
cN stage			
cN0	29	13	69.05
cN1	69	42	62.16
cN2	14	14	50.00
cN3	22	21	51.16
Clinical stage			
II	88	47	65.19
III	46	43	51.69
ER (≥1% is positive)			
negative	78	34	69.64
positive	56	56	50.00
PR (≥1% is positive)			
negative	90	38	70.31
positive	44	52	45.83
HR status			
negative	69	30	69.70
positive	65	60	52.00
HER2 expression state			
HER2(3+)	119	69	63.30
HER2(2+) and FISH-positive	15	21	41.67
Ki67 expression			
<20%	9	8	52.94
≥20%	123	81	60.29
unknown	2	1	66.67
Treatment cycle			
six cycles	77	49	61.11
eight cycles	48	33	59.26
other	9	8	52.94
NAT regimen			
EC-THP	53	43	55.21
TCbHP	81	47	63.28

Table 2
Univariate analysis of the association between the NAT efficacy evaluation and clinicopathological variables.

	Univariate analysis model[a]		pCR (%)
	OR (95% CI)	*P*-value	
Age division		0.374	
≤35	Ref.		52.17
>35&≤55	1.28 (0.53-3.08)	0.586	58.22
>55	1.88 (0.70-5.09)	0.211	67.27
Menstrual state			
No	Ref.		56.14
Yes	1.37 (0.80-2.34)	0.253	63.64
Family history of cancer		0.296	
No	Ref.		60.19
Breast cancer	0.26 (0.05-1.40)	0.117	28.57
Ovarian cancer	0.66 (0.04-10.72)	0.771	50.00
Other tumors	2.31 (0.47-11.42)	0.303	77.78
Clinical stage			
II stage	Ref.		65.19
III stage	0.57 (0.33-0.99)	**0.045**	51.69
HR status			
Negative	Ref.		69.70
Positive	0.47(0.27-0.82)	**0.008**	52.00
HER2 expression state			
2+/FISH-positive	Ref.		41.67
3+	2.41 (1.17-4.99)	**0.017**	63.30
Ki67 expression			
<20%	Ref.		52.94
≥20%	1.35 (0.50-3.64)	0.554	60.39
Treatment cycle			
<8 cycles	Ref.		60.87
≥8 cycles	0.89 (0.52-1.54)	0.685	58.14
NAT regimen			
EC-THP	Ref.		55.21
TCbHP	1.40 (0.82-2.40)	0.223	63.28

Abbreviations: OR, odds ratio; CI, confidence interval
[a] Logistic regression was used for analysis. All statistical tests were two-sided.

Table 3
Multivariate analysis of the association between the NAT efficacy evaluation and clinicopathological variables.

	Multivariate analysis model[b]	
	OR (95% CI) for the pathological effect	*P*-value
Age division		0.8614
≤35	Ref.	
>35&≤55	1.27(0.48-3.34)	0.6320
>55	1.41(0.40-4.97)	0.5928
Menstrual state		0.3362
No	Ref.	
Yes	1.32(0.75-2.32)	
Family history of cancer		0.3333
No	Ref.	
Breast cancer	0.26(0.05-1.42)	0.1205
Ovarian cancer	0.55(0.03-9.38)	0.6804
Other tumors	0.55(0.03-9.38)	0.3856
Clinical stage		**0.0223**
II stage	Ref.	
III stage	0.52(0.29-0.91)	
HR status		**0.0150**
Negative		
Positive	0.49(0.28-0.87)	
HER2 expression state		**0.0467**
2+/FISH-positive	Ref.	
3+	2.15(1.01-4.56)	
Ki67 expression		0.3871
<20%	Ref.	
≥20%	1.62(0.54-4.87)	
Treatment cycle		0.6077
<8 cycles	Ref.	
≥8 cycles	1.17(0.64-2.16)	
NAT regimen		0.1642
EC-THP	Ref.	
TCbHP	2.03(0.75-5.47)	

Abbreviations: OR, odds ratio; CI, confidence interval
[b] Logistic regression was used for analysis. All statistical tests were two-sided. The OR was calculated with the non-pCR as the reference. Adjusted for age division, menstrual state, family history of cancer, clinical stage, HR status, Ki67 expression, treatment cycle, and NAT

CI:1.17–4.99, $P = 0.017$) displayed higher pCR rates. Age, menstrual status, family history, Ki67 expression status, number of treatment cycles, and treatment regimen did not affect pCR ($p > 0.05$). All clinicopathological data were included in the multivariate analysis (Table 3). A multivariate analysis model was constructed, which indicated that the clinical stage (95% CI:0.29–0.91, $P = 0.0223$), HR expression status (95% CI:0.28–0.87, $P = 0.0150$), and HER2 expression status (95% CI:1.01–4.56, $P = 0.0467$) were statistically significant for pCR occurrence.

The results showed that the HER2 expression level, HR expression status, and clinical stage were significant independent influencing factors of pCR.

4. Discussion

4.1. Ki67

The failure of Ki67 to translate into pCR ($p > 0.05$) may be due to the fact that HER2-positive BC tend to show a higher Ki67 index [[10,11]]. As in the case sample shown in this study, the sample as a whole was biased towards having a higher Ki67 index and no effective stratification was achieved when using 20% as the threshold for Ki67, thus failing to show a statistical difference.However, the baseline clinicopathological characteristics in this study showed a positive correlation between a high Ki67 index (Ki67 ≥ 20% vs. Ki67 < 20%) and a higher pCR rate (60.39% vs. 52.94%), possibly because cancer cells displaying high Ki67 expression were more sensitive to chemotherapy [12]. Therefore, Ki67 may be a potential pCR predictor.

4.2. Clinical stage

Consistent, statistically significant differences ($P \leq 0.05$) were found in both the univariate and multivariate analysis models. The clinical stage was confirmed as an independent predictor of pCR, that is, the clinical stage level was negatively correlated with pCR.

4.3. HR

HR showed consistent, statistically significant differences ($p \leq 0.05$) during the univariate and multivariate analyses. This showed that HR is a significant, independent pCR predictor, that is, a pCR rate is more likely influenced by an HR-negative state than in a positive state, which is consistent with previous research findings that the pCR rate of HER-2-positive/HR-positive BC is lower than HER2-positive/HR-negative BC [[13–15]]. This may be related to the complex interaction between ER and PR and the HER2 signaling pathway [[16,17]].

4.4. HER2

Statistically significant differences ($p \leq 0.05$) were evident in the univariate and multivariate analysis models due to different HER2 expression levels. This confirmed that the HER2 expression level was an independent pCR predictor, which was consistent with several previous studies [18]. Although both HER2 IHC(2+)/FISH-positive BC and HER2 IHC(3+) BC are HER2-positive BC, they display significant differences in their response to treatment. It is speculated that the action mechanism [19] of trastuzumab and pertuzumab against HER2 depends on the drug, acting by binding directly to the HER2 protein on the cell surface instead of at the HER2 gene level. Therefore, the HER2 protein expression level is a key factor determining the response to anti-HER2 therapy.

4.5. Limitations

Since this is a single-center, large-sample, retrospective study, more multi-center, prospective, basic research is necessary to provide more solid evidence-based medical evidence.

5. Conclusion

In summary, the Ki67 expression level, menstrual status, age, and NAT regimen (EC-THP vs. TCbHP) are not independent factors influencing the pCR rate during NAC adjuvant to dual-targeted trastuzumab and pertuzumab ($p > 0.05$). The clinical stage, HR, and HER2 expression status are significant, independent influencers of pCR ($p \leq 0.05$).The HER2 expression level affects and determines the efficacy response to NAT with adjuvant trastuzumab and dual-target pertuzumab.

Author contributions

JjL was responsible for the concept and design of the research. All authors contributed to the acquisition, analysis, and interpretation of the data. The draft was produced by WC. All authors made critical revisions to the manuscript. The statistical analysis was conducted by WC, FxL, and DlL, while JJ provided administrative, technical, and material support. All authors read and approved the final manuscript.

Availability of data and materials

The published article [and its supplementary information files] includes all data generated and analyzed during this study.

Statement of ethics

This study was approved by the Ethics Committee of Medical Research and Medical New Technology of Sichuan Cancer Hospital. The procedures used are consistent with the principles of the Helsinki Declaration.

Funding sources

This study was supported by the Natural Science Foundation of Sichuan Province (funding number: 2023NSFSC0548) and the National Natural Science Foundation of China (funding number: 82003522). Support has been provided in research design and communication, literature download, and article publication.

Declaration of competing interest

All authors disclosed no relevant relationships.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.breast.2023.07.008.

Abbreviations

SAS	Statistical analysis
BC	Breast cancer
ER	Estrogen receptor
RP	Progesterone receptor
HER2	Human epidermal growth factor receptor 2
Ki67	Proliferation cell nuclear antigen
HR	Hormone receptor
FISH	Fluorescence in-situ hybridization
IHC	Immunohistochemistry
NAC	Neoadjuvant chemotherapy
TNM	Tumor node metastasis
NAT	Neoadjuvant therapy
MP	Miller-Payne grade
pCR	Pathologic complete response
EFS	Event-free survival
OS	Overall survival
non-pCR	Non-pathological complete remission
OR	Odds ratio
CI	Confidence interval

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