Assessment of the predictive role of oestrogen and progesterone receptor status in breast cancer patients treated with neoadjuvant chemotherapy: A retrospective analysis for 689 patients

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Research

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Abstract

Background : To explore the predictive indicators in hormone receptor (HR)-positive breast cancer (BC) patients receiving neoadjuvant chemotherapy (NACT) and to evaluate the value of quantitative oestrogen receptor (ER) and progesterone receptor (PR) in predicting tumour response. **Methods :** Six hundred eighty-nine BC patients with HR-positive status who were treated with anthracycline, epirubicin and taxane NACT treatment were retrospectively analysed. Clinical and pathological features of the patients were used to evaluate the response to NACT. **Results :** Patients with larger tumour sizes (OR 1.657 CI 1.186-2.313 $p=0.003$), those who were in a premenopausal status (OR 1.458 CI 1.039-2.045 $p=0.029$) and those with higher Ki67 levels (OR 1.735 CI 1.231-2.444 $p=0.002$) exhibited a better therapy response. Among the patients in the postmenopausal subgroup, a lower pretreatment ER or PR expression were associated with a reduction in tumour size, and the cut-off values for ER and PR were 87.5% and 65%, respectively ($p=0.006$ and $p=0.05$). Decreased expression of ER and PR was also observed after NACT treatment ($p=0.028$ and $p<0.001$, respectively) but played only a predictive role in the Her-2-negative subgroup; the cut-off values for decreased ER and PR were 17.5% and 26.5%, respectively ($p=0.044$ and $p<0.001$). **Conclusions :** Semiquantified pretreatment HR expression can be used to predict the response of NACT in postmenopausal BC patients. Decreased ER and PR expression is also associated with a reduction in tumour size in Her-2-negative subtypes treated with NACT.

Introduction

Breast cancer (BC) is the leading cause of cancer-related death among women worldwide, and approximately 75% of all BCs are hormone receptor (HR) positive [1]. Neoadjuvant chemotherapy (NACT) has become a well-established approach to treat large or locally advanced BCs [2, 3, 4], which shrinks the tumour to improve the chances of surgical resection and to evaluate the tumour response to chemotherapeutic agents [5, 6, 7]. Although HR-positive BC patients are sensitive to endocrine therapy, they have an undesirable response to NACT treatment compared to the HR-negative subtypes. However, NACT is necessary for a large number of HR + BC patients. Unfortunately, studies investigating the prediction of tumour response after NACT treatment among HR-positive BC patients are scarce [7, 8].

Oestrogen receptor (ER)/progesterone receptor (PR) status has been reported to be a predictive marker of tumour response in endocrine therapy [8], but few studies have assessed the quantitative status of ER and PR as predictive indicators in NACT. In our study, a semiquantitative method was used to evaluate the predictive effect of pretreatment ER and PR expression. In addition, the change in the semiquantitative expression of ER and PR was also evaluated as a possible predictor for NACT treatment.

Methods

Patients and treatments

A total of 689 BC patients characterized as ER and/or PR positive who had undergone NACT and surgery at the Department of Endocrine Breast Surgery in the First Affiliated Hospital of Chongqing Medical University between January 2012 and November 2017 were recruited. All patients received at least 3 cycles of treatment with the TEC regimen: cyclophosphamide (500 mg/m²), epirubicin (75 mg/m²), and docetaxel (75 mg/m²) every 21 days. Patients with bilateral breast cancer, male breast cancer, and a history of contralateral breast cancer were excluded. In our study, Herceptin was not used in patients with Her-2-positive status before the operation.

Pathology assessment

ER and PR expression were evaluated by the Pathology Department of Chongqing Medical University, and the results were evaluated as the percentage of breast cancer cells with ER/PR staining confirmed via immunohistochemistry (IHC). Samples were defined as ER (+) or PR (+) when at least 1% of nuclei were stained [9]. Her-2 status was considered to be positive when more than 10% of the tumour cells showed a staining intensity of 3+ via IHC or showed a > 2.2-fold increase via fluorescence in situ hybridization (FISH) [10]. Regarding Ki67, a range of 500 to 1000 cells were counted to calculate the percentage of positive tumour cell nuclei, including hot spot areas, and 14% was defined as the best cut-off value [11].

Evaluation of treatment efficacy

Ultrasonography and magnetic resonance imaging (MRI) were used to evaluate the response to NACT. We compared the primary imaging materials to the latest materials before the operation to evaluate tumour shrinkage. The Response Evaluation Criteria in Solid Tumours (RECIST) guidelines version 1.1 were used to evaluate the treatment response as follows: CR (complete remission) was considered if all the target lesions had disappeared, and the short axis of any pathological lymph nodes was less than 10 mm; PR (partial remission) was considered if the longest diameter of the tumour lesion was decreased by $\geq 30\%$; PD (progressive disease) was considered if the longest tumour diameter was increased by $\geq 20\%$; and SD (stable disease) was considered if the longest tumour diameter was decreased by less than 30% or increased by less than 20%. pCR (pathologic complete remission) was defined as no residual tumour lesion in any breast tissues or lymph nodes. Among them, patients with pCR, CR and PR were regarded as responders, while those with PD or SD were regarded as nonresponders.

Statistical methods

The differences in clinicopathological characteristics between the responders and nonresponders were calculated with the chi-squared test and multivariate logistic regression models, while the Kruskal–Wallis test was used to compare quantitative characteristics. A receiver operating characteristic (ROC) curve was used to assess the cut-off value. Differences were considered to be statistically significant at $P < 0.05$. SPSS version 22.0 software (SPSS Inc, Chicago, USA) was used for all statistical analyses.

Results

Baseline characteristics

The average age of the enrolled patients was 48.5 ± 8.92 years (range, 22 to 72 years), and 435 patients (63.2%) were menopausal. The tumour size was 3.94 ± 2.15 cm at diagnosis and 2.13 ± 1.55 cm after NACT. Two hundred ninety-six patients (42.9%) featured clinically positive lymph nodes. According to IHC and FISH, 669 (97.1%) and 514 (74.6%) patients were ER positive and PR positive, respectively, while 198 (29.0%) patients were Her-2-positive. In this study, 479 (69.6%) patients showed a response to NACT treatment, including 45 with pCR (6.50%), 67 with CR (9.70%) and 367 with PR (53.3%), based on the clinical pathological response. Additional details regarding the patient characteristics are shown in Table 1.

Table 1
Clinicopathological features of the study cohort (n = 689)

Parameters Number(%)	Parameters Number(%)
Age(year)	ER Status
≤40 94(13.8)	Positive 669(97.1)
≥ 40 595(86.2)	Negative 20(2.90)
Menopause	PR Status
Yes 254(36.8)	Positive 534(77.5)
No 435(63.2)	Negative 155(22.5)
Chemotherapy cycles	Her-2
3 18(2.6)	Positive 198(29.0)
4 621(90.1)	Negative 385(55.7)
5–6 50(7.3)	Unknown ² 106(15.3)
Subtypes of Cancer	Ki67(%)
Ductal 673(97.7)	≤14 238(34.5)
Lobular 10(1.40)	≥ 14 451(65.5)
Others 6(0.90)	Subgroup
Tumor Size	ER + PR+ 514(74.6)
≤2 cm 58(8.40)	ER + PR- 155(22.5)
2-4cm 391(56.8)	ER-PR+ 20(2.90)
≥4 cm 240(34.8)	Responders ³
Clinical nodal status	pCR 45(6.50)
Positive 296(42.9)	CR 67(9.70)

Abbreviations: pCR pathologic complete remission, CR complete remission, PR partial remission, SD stable disease, PD progressive disease.

1: The pathology test was given but the results were unknown.

2: IHC test was given and showed Her-2(2+), but not be verified by FISH.

3: pCR, CR and PR were regarded as responders to NACT, while SD or PD was regarded as non-responders to NACT.

Table.2 Univariate analysis of baseline characteristics according to response to NAC

Parameters Number(%)		Parameters Number(%)	
Negative 393(57.1)		PR 367(53.3)	
Histological Grade		SD 189(27.4)	
I 24(3.50)		PD 21(3.50)	
II 451(65.5)			
III 66(9.60)			
Unknown ¹ 148(21.4)			
Characteristic	clinical response		P value
	Res ¹	Non-Res ²	
Age(Y)	47.9 ± 8.81	50.0 ± 9.02	0.019
Tumor size (cm)	4.1 ± 2.11	3.7 ± 1.93	0.003
Histological Grade	16	8	0.752
I	302	150	
II	41	25	
III			
Menopause	162	92	0.012
Yes	317	118	
No			
Clinical nodal status	217	79	0.061
Positive	262	131	
negative			
Molecular subtype	351	163	0.408
ER + PR+	112	43	
ER + PR-			
PR status	367	167	0.427
Positive	112	43	
negative			

Abbreviations: pCR pathologic complete remission, CR complete remission, PR partial remission, SD stable disease, PD progressive disease.

1: The pathology test was given but the results were unknown.

2: IHC test was given and showed Her-2(2+), but not be verified by FISH.

3: pCR, CR and PR were regarded as responders to NACT, while SD or PD was regarded as non-responders to NACT.

Table.2 Univariate analysis of baseline characteristics according to response to NAC

Parameters Number(%)		Parameters Number(%)	
Her2 status positive negative	149 261	49 124	0.062
Ki67 expression (%) ≤14 ≥ 14	144 333	94 118	0.001
1: including pCR, CR and PR;			
2: including PD and SD.			
Abbreviations: pCR pathologic complete remission, CR complete remission, PR partial remission, SD stable disease, PD progressive disease.			
1: The pathology test was given but the results were unknown.			
2: IHC test was given and showed Her-2(2+), but not be verified by FISH.			
3: pCR, CR and PR were regarded as responders to NACT, while SD or PD was regarded as non-responders to NACT.			
Table.2 Univariate analysis of baseline characteristics according to response to NAC			

Univariate and multivariable analysis of clinicopathological characteristics based on the response to NACT

Univariate analysis and multivariate logistic regression models were used to analyse the relationship between treatment response and baseline characteristics (Tables 2 and 3). Histological grade, molecular subtype and PR status did not show a statistically significant association with the response to NACT treatment (all $p > 0.05$). In contrast, a larger tumour size (OR 1.657 CI 1.186–2.313 $p = 0.003$), postmenopausal status (OR 1.458 CI 1.039–2.045 $p = 0.029$) and higher Ki67 index (OR 1.735 CI 1.231–2.444 $p = 0.002$) predicted a better therapy response. In addition, clinical nodal status showed a marginal P value ($p = 0.061$) in the univariate analysis but no statistical significance in the multivariate logistic regression analysis (OR 1.212 CI 0.860–1.707 $p = 0.272$).

Characteristic	clinical response		P value
	Res ¹	Non-Res ²	
Age(Y)	47.9±8.81	50.0±9.02	0.019
Tumor size (cm)	4.1±2.11	3.7±1.93	0.003
Histological Grade			
I	16	8	0.752
II	302	150	
III	41	25	
Menopause			
Yes	162	92	0.012
No	317	118	
Clinical nodal status			
Positive	217	79	0.061
negative	262	131	
Molecular subtype			
ER+PR+	351	163	0.408
ER+PR-	112	43	
PR status			
Positive	367	167	0.427
negative	112	43	
Her2 status			
positive	149	49	0.062
negative	261	124	
Ki67 expression (%)			
<14	144	94	0.001
≥14	333	118	

1: including pCR, CR and PR;

2: including PD and SD.

Table.2 Univariate analysis of baseline characteristics according to response to NAC

Table 3
Multivariate logistic regression models of baseline characteristics according to response to NAC

Characteristic	clinical response		P value
	HR	95%CI	
Reference	0.907		
Tumor size	1.657	1.186–2.313	0.003
Menopause	1.458	1.039–2.045	0.029
Clinical nodal status	1.212	0.860–1.707	0.272
Ki67 expression	1.735	1.231–2.444	0.002

Predictive value of pretreatment and decreased ER and PR expression

The interaction between pretreatment HR expression and clinical response was further evaluated. As shown in Fig. 1, in the postmenopausal subgroup, the patients who exhibited a clinical response showed lower ER expression before NACT (n = 244, p = 0.006) (Fig. 1A and 1B), but this phenomenon was not

observed in the premenopausal subgroup ($n = 425$, $p = 0.157$). Similarly, Figs. 1C and 1D show that in the postmenopausal subgroup patients had higher PR expression prior to NACT, reducing their chances of achieving a response ($n = 182$, $p = 0.05$). Moreover, ROC curve analysis was used to define the optimal cut-off value for primary HR expression in the postmenopausal subgroup (Fig. 2). The area under the curve for pretreatment ER and PR expression were 0.605 ($p = 0.006$) and 0.580 ($p = 0.05$), respectively, and the optimal cut-off values were 87.5% and 65%, respectively (Fig. 2).

Next, we assessed the changes in ER and PR expression in BC patients during NACT treatment. Decreased ER and PR expression was observed after NACT treatment ($p = 0.003$ & $p < 0.001$, Fig. 3A). Moreover, this decreased expression could predict a higher possibility of tumour remission ($p = 0.028$ & $p < 0.001$, Fig. 3B, C). However, the ROC results indicated that decreased ER and PR expression can predict the response to NACT only in the Her-2 negative subgroup; the AUCs were 0.565 ($p = 0.044$, Fig. 4C) and 0.648 ($p < 0.001$, Fig. 4D), respectively, while the cut-off values of ER and PR reduction were 17.5% and 26.5%, respectively.

Discussion

A large number of clinical studies have shown that HR-positive BC patients have a better relative prognosis but are not as sensitive to NACT as HR-negative BC patients [12]. However, NACT is an imperative method for luminal BC patients with relatively late stages. At present, the predictive factors for the efficiency of NACT in HR-positive BC patients have not been thoroughly elucidated, and whether the response to NACT could be predicted by semiquantified ER and PR has been a matter of debate [13].

In our study, patients with larger tumour size (≥ 4 cm) or higher percent expression of Ki67 ($\geq 14\%$) could predict a better outcome after 4-cycle NACT treatment, which was in line with prior studies [8, 10, 11]. Unlike previous research from other institutes, the results of our multivariable logistic regression showed that postmenopausal BC patients are more likely to obtain a better clinical therapy response in NACT compared with premenopausal BCs. To the best of our knowledge, this is the first study to describe the impact of menstrual status on NACT in HR-positive BC patients.

We next assessed the interaction between quantified HR expression and clinical response. Similarly, pretreatment ER and PR expression could be used as predictors of response to NACT only for postmenopausal BC patients. This result is similar to that obtained in Raphael's study, in which he concluded that a lower pretreatment ER expression predicted a higher clinical response rate. However, the effect of menopausal status was not discussed in his study [13]. The possible mechanism is that the oestrogen level may affect the sensitivity of tumour cells to chemotherapy [14]. Furthermore, it is well known that ER+/PR- tumours have a higher level of growth signalling than ER+/PR+ tumours [15, 16]. However, in our study, there was no significant difference when analysing the response to NACT among various ER and PR status subgroups (ER+/PR+ and ER+/PR- subgroups).

In the current study, the changes in ER and PR expression among non-pCR patients who received NACT were also investigated. According to our findings, decreased ER and/or PR levels were found in

approximately 50% of the patients after NACT treatment. However, when considering the therapeutic response of NACT, the predictive roles of decreased ER and PR could only be observed in the Her-2 negative subgroup. This finding may be attributed to differences in tumour cell subtypes, which may activate dissimilar signalling pathways in response to chemotherapy. Another reason for this finding may be that Her-2-positive subtypes have complex growth-promoting mechanisms, which may be affected by molecularly targeted therapy drugs rather than chemotherapy [17].

Our study has the largest number of patients to date among studies exploring the relationship between semiquantitative HR and NACT treatment, but the major limitation of this study is that our data were obtained from only one single breast cancer centre, and the research followed a retrospective design. As is well known, low HR expression is be an adverse factor for endocrine therapy [12], and in this research, we observed a reduction in HR expression due to chemotherapy. Could decreased HR expression be beneficial to whole breast cancer treatment? How does it affect progression-free survival (PFS) or overall survival (OS) in patients? Further studies, especially long-term follow-up studies, should take these questions into consideration.

The results of this study showed that quantified HR expression can serve as a predictor of NACT treatment response in postmenopausal BC patients. Changes in HR expression can also predict the outcomes of patients in the ER+/Her-2 negative subgroup. However, in the premenopausal and ER+/Her-2-positive subgroups, these factors were not observed to have predictive functions. In clinical work, continued chemotherapy should be considered for patients with the aforementioned features.

Conclusion

Semiquantified pretreatment HR expression can predict the response of NACT in postmenopausal BC patients. Decreased ER and PR expression is also associated with a reduction in tumour size in Her-2-negative subtypes treated with NACT. The findings could help in screening effective chemotherapy regimens for these patients.

Abbreviations

BC: Breast Cancer; CI: Confidence Interval; CR: Complete Remission; PR: Partial Remission; PD: Clinical Progressive Disease; SD: Clinical Stable Disease; pCR: Pathological Complete Remission; HR: Hormone Receptor; ER: Oestrogen Receptor; PR: Progesterone Receptor; Her-2: Human Epidermal Receptor2; FISH: Fluorescence In Situ Hybridization; IHC: Immunohistochemistry; NACT: Neoadjuvant Chemotherapy; OR: Odds Ratio; OS: Overall Survival; PFS: Progression-Free Survival; ROC: Receiver Operating Curve.

Declarations

Ethics approval and consent to participate

This article does not contain any studies with human participants performed by any of the authors.

Consent for publication

Not applicable.

Availability of data and material

The datasets are not publicly available due to local regulations but are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests to report.

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Authors' contributions

Sc Liu conceived and designed the overall study. Y Ye and R Chen collected the clinical medical histories of patients and analysed the data. Y Ye and Y Fu wrote the manuscript.

R Chen and Y Fu revised the manuscript. Y Peng, F Luo and Fl Qu helped perform the statistical analyses. Bg Zong, Zr Tang and Yh Wang finished data filtration and helped write the manuscript.

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Figures

1: including pCR, CR and PR (black).
 2. including PD and SD (gray).
 Figure A & B: Expression of ER between pre- and post-menopausal patients.
 Figure C & D: Expression of PR between pre- and post-menopausal patients.
 D*: In this group, 2 patients showed 1% stained of PR in IHC and were excluded.

Figure 1

Expression of HR between cRes and cNon-Res is affected by menstrual status. To show the interaction between HR expression and clinical response affected by menstrual status. Figure 1A and 1B indicated that clinically responsive patients had a lower expression of ER only in the postmenopausal subgroup. Figure 1C and 1D showed that only in the postmenopausal subgroup, the higher expression of PR before NAC treatment had a lower response possibility.

Figure 2

ROC curve for pretreatment ER and PR expression in the postmenopausal subgroup. Showed that the best cut-off value of primary ER and PR expression was 87.5% and 65%, respectively. The area under the curve for pretreatment ER and PR was 0.605 (P=0.006, 95% CI 0.530-0.679) and 0.580 (P=0.05, 95% CI 0.493-0.668), respectively.

A

B¹

C²

1,2: ER expression did not change in 56 patients, while PR expression was unchanged in 25 patients. These patients were not analyzed.

Figure 3

Change of HR expression between biopsy and surgical specimens. Decreased expression of ER and PR between biopsy and surgery specimens was observed, and the P values were 0.028 and less than 0.000, respectively.

Figure 4

ROC curve for the change of ER and PR expression after NAC. Decreased ER and PR can predict the response to NAC treatment in the Her-2 negative subgroup, but the predictive role was lost in Her-2 positive subgroup. The ROC curve showed that decreased ER and PR can predict the response to NAC treatment in the Her-2 negative subgroup, and the cut-off values of ER and PR reduction were 17.5% and 26.5%, respectively.