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Biomarker alteration following chemotherapy-based systemic therapy in de novo metastatic breast cancer

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ABSTRACT

Introduction: It is unclear whether the expression of biomarkers such as estrogen receptor (ER), progesterone receptor(PR), human epidermal growth factor receptor-2(HER2), and Ki-67 proliferation index changes following chemotherapy-based systemic therapy(CST) in patients with de novo metastatic breast cancer(dnMBC). The study aimed to investigate the expression of the biomarkers before and after CST and its impact on the prognosis of dnMBC patients.

Methods: Using hospital-based database, we conducted a retrospective cohort study on dnMBC patients who received CST between February 2010 and December 2017. Based on clinicopathological data, changes in pathological findings(ER,PR,HER-2,Ki-67) following CST were examined. The effect of biomarker conversion on prognosis was evaluated. The primary outcome was overall survival(OS). Kaplan-Meier method and log-rank test was used for survival analyses.

Results: The study included 192 female patients. The change rates of ER,PR,HER-2 and Ki-67 before and after CST were 9.9 %,17.2 %,6.2 % and 25.5 % respectively. Among them, the change in negative-to-positive expression of Ki-67 was the most common type of discordance observed. There was no statistical difference in OS between patients with changes in the four biomarkers and patients with no changes in the biomarkers(all $p > 0.05$). Interestingly, positive conversion of ER and PR, as well as persistent positive HER2 and Ki-67, were significantly associated with poor prognosis($p < 0.001$,$p < 0.001$; $p = 0.029$,$p < 0.001$). Family history, initial metastatic site, and tumor grade were independent variables related to survival($p = 0.002$,$p < 0.001$,$p < 0.001$).

Conclusions: Changes in ER, PR, HER2, and Ki-67 status were observed in patients following CST. Positive conversion of ER and PR, and persistent positive expression of HER2 and Ki-67 may indicate a poor prognosis. Further research is needed to determine whether biomarker expression investigations are needed following CST to optimize treatment options and improve survival.

1. Introduction

Breast cancer remains a major health problem for women worldwide, with the highest incidence and the second highest mortality rate in 2023 [1]. Neoadjuvant chemotherapy (NAC) has become a standard treatment for breast cancer, which is conducive to reducing tumour stage, eliminating micrometastatic disease, and assessing the response of the tumor to systemic therapy [2]. For de novo metastatic breast cancer (dnMBC), chemotherapy-based systemic therapy (CST) remains the mainstay for dnMBC patients and the therapeutic option for these patients in the neoadjuvant setting. But it is a cornerstone of treatment rather than an adjunct therapy in the actual sense. As with other breast cancer, treatment options and plans for this disease are based on the presence of related biomarkers, such as estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (HER2), and Ki-67 proliferation index. Core needle biopsy (CNB) is an essential measure to confirm the diagnosis and determine the presence or absence of immunohistochemical (IHC) markers such as hormone receptors (HR) and HER2, which are important prognostic indicators and key factors in the treatment decision-making [3]. Therefore,

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pretreatment biopsies that include CNB or excision of suspicious lesions are very valuable in determining treatment plans and providing prognostic information. This is not only important but essential for dnMBC.

In previous studies, although some investigators have reported no change in biomarker expression following NAC [4 5], there is growing evidence that has indicated the discordance in the expression of ER, PR, HER2, and Ki-67 status before and after NAC [6–8], and that this conversion may be a potential prognostic factor[9 10]. Recently, retrospective analysis showed that receptor expression discordance was not only statistically significant, but also associated with poor survival [8]. Somewhat regrettably, prospective studies have not evaluated the effects of receptor expression discordance throughout therapy on patient survival [6]. Indeed, many studies have found differences in the expression of biomarkers in pre-NAC biopsies versus post-NAC surgical materials [6–13]. However, biomarker changes following NAC and the relevance of these changes to prognosis remain controversial.

As for dnMBC, its particularity is reflected in the fact that there are both primary lesions and metastases, and the two may have different biomarker characteristics [14]. Moreover, CST is the most effective and important treatment, and local therapies including surgery may be used as adjuvant therapy. Nevertheless, in dnMBC patients, the prognosis is relatively poor, and it is critical to find potential biomarkers to distinguish patients with different long-term outcomes. Studies have found that chemotherapy affects tumor biology directly or indirectly and causes receptor discordance in breast cancer [8 15]. However, it is rarely reported in patients with dnMBC. What's more, the impact of these conversions on subsequent treatment options and how they affect patient outcomes remains unclear. In this retrospective study, we evaluated the expression of ER, PR, HER2, and Ki67 before and after CST in a cohort of patients in Cangzhou, China. We hypothesized that discordance in biomarker expression might be associated with prognosis in dnMBC patients.

2. Methods

2.1. Study design and patients

This was a retrospective cohort study conducted at Hebei Cangzhou Hospital of Integrated Traditional Chinese and Western Medicine in China. Data of patients with dnMBC treated between February 2010 and December 2017 were reviewed. The study was conducted in accordance with the Declaration of Helsinki and was approved by the regional Institutional Review Board (approval number: 2017-AF29-058). Written informed consent was obtained from all patients included in the analysis. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guideline for cohort studies [16]. Patients were included in the analysis if they met the following criteria:1) histopathology and immunohistochemistry (IHC) were performed by core needle biopsy or surgical specimen to confirm the diagnosis; 2) After receiving at least one cycle of CST, histopathological and IHC were performed again; 3) Clinicopathological and follow-up data were complete. Exclusion criteria included: 1) incomplete data on clinicopathology, IHC score, imaging examinations, treatment, and follow-up; 2) distant metastases were pathologically confirmed non-breast origin.

2.2. Clinicopathologic data

We collected clinicopathologic data from the institutional database of consecutive patients with dnMBC who underwent CST at our hospital from February 2010 to December 2017. Detailed data on ER、PR、HER2 和Ki67 status were abstracted from pathology records, which are derived from IHC tests of Core needle biopsies or surgical excision specimens. As recommended by the ASCO/CAP guidelines [17], positive ER and PR status was set at 1 % of invasive tumor cells with positive nuclear staining. Tumors were defined as HER2 overexpression in cases of membrane staining of 3+ or amplified fluorescence in situ hybridization (FISH), while HER2 negativity was defined in cases of 0 (no membrane staining) or 1+ or 2+ (negative by in ISH assay) scores. Ki-67 expression was divided into positive and negative expression groups with a cutoff of 20 %. Tumor subtypes were defined based on the expression of HR and HER2 as follows: Luminal (HR+/HER2−), Luminal-HER2 (HR+/HER2+), HER2-rich (HR−/HER2+), and triple-negative (ER- and PR- and HER2-, TNBC). Here, we defined the threshold for HR (ER and PR) changes to be 1 %.

2.3. Treatment

All enrolled patients received more than one cycle of CST, including anthracycline-based regimens, taxane-based regimens, anthracycline- and taxane-based regimens, and taxane-and capecitabine-based regimens, in combination with endocrine therapy, targeted therapy, surgery (excision of primary lesion, mastectomy, etc.), or radiation therapy whenever indicated. The following baseline clinicopathological parameters were evaluated by ER, PR, HER2 and Ki-67 status: age at diagnosis, family history, menopausal status, initial metastatic sites, tissue specimen sites, pathological materials, tumor histology, tumor grade, CST cycles, chemotherapy regimens chemotherapy, endocrine therapy, targeted therapy, as well as radiation therapy. Overall survival (OS), as primary endpoint, was defined as the time from date of diagnosis to the last follow-up or death. Follow-up data for survival analysis were retrospectively collected from patients' medical records or through interviews, telephone or internet. Patients who were alive after the cut-off date were censored.

2.4. Statistical analysis

To compare patient clinicopathologic characteristics, continuous variables were presented as a median with range or a mean with SD and categorical variables were presented as number and percentage. The Wilcoxon rank sum test was used to compare continuous variables, and the Chi-square test or Fisher exact test were used to compare categorical variables, as appropriate. The Kaplan-Meier method was used to calculate survival outcomes, and subgroups were compared by the log-rank test. The hazard ratio and 95 % confidence intervals (CI) were calculated for each variable using the Cox univariate model. Cox proportional hazards models were performed to evaluate the correlation between the alteration of receptor status and survival risk and to identify statistically significant prognostic factors. Concordance analysis of biomarkers status before and after CST were assessed by Cohen's Unweighted Kappa. Analyses were conducted using Stata software (version 18.0, STATA Corp., College Station, Texas, USA), and 2-sided p values < 0.05 were considered statistically significant.

3. Results

The study included 192 female patients. The mean age was 49.1 ± 10.6 years (range: 21–82). 47.9 % of the patients were over 50 years of age and 44.8 % were postmenopausal. During a median follow-up of 75.0 months, 138 of 192 patients (71.9 %) died, with a median survival of 39 months. In this study, 55.2 % of patients had Luminal subtype tumors, 16.1 % of patients had Luminal-HER2 tumors, 16.7 % had HER2-rich tumors, and 12.0 % had triple negative breast cancer. Following the treatment, 53.1 % (102/192) of patients receiving CST had at least one altered biomarker status. Changes in ER, PR, HER2 and Ki-67 status were observed in 19 (9.9 %), 33 (27.2 %), 12 (6.2 %) and 49 (25.5 %) cases, respectively. And their corresponding concordance rate was 90.1 % (k = 0.773), 82.8 % (k = 0.653), 93.8 % (k = 0.859) and 74.5 % (k = 0.391). Clinical and pathological characteristics of the patients are shown in Table 1. Changes in biomarker status following CST are shown in Table 2.

The change rate of ER status was 9.9 %. ER status was unchanged in

Table 1
Clinical and pathological characteristics of the patients.

Characteristics	Biological subtypes					
	Patients	HR+/HER2−	HR+/HER2+	HR−/HER2+	TNBC	P-value
	N = 192 (%)	106 (55.2)	31 (16.1)	32 (16.7)	23 (12.0)	
Age(SD)	49.052(10.667)	49.255(10.537)	45.871(10.230)	50.094(11.269)	50.957(10.793)	0.281
Menopausal status						
Premenopausal	106 (55.2)	59 (55.7)	23 (74.2)	14 (43.8)	10 (43.5)	0.057
Postmenopausal	86 (44.8)	47 (44.3)	8 (25.8)	18 (56.2)	13 (56.5)	
Family history						
No	139 (72.4)	74 (69.8)	22 (71.0)	27 (84.4)	16 (69.6)	0.427
Yes	53 (27.6)	32 (30.2)	9 (29.0)	5 (15.6)	7 (30.4)	
Initial metastatic sites						
Bone alone	96 (50.0)	65 (61.3)	15 (48.4)	9 (28.1)	7 (30.4)	0.001
Single organ	51 (26.6)	22 (20.8)	5 (16.1)	15 (46.9)	9 (39.1)	
Multiple organs	41 (21.4)	19 (17.9)	9 (29.0)	6 (18.8)	7 (30.4)	
Brain	4 (2.1)	0 (0.0)	2 (6.5)	2 (6.2)	0 (0.0)	
Tissue specimen sites						
Primary lesions	170 (88.5)	90 (84.9)	29 (93.5)	31 (96.9)	20 (87.0)	0.222
Extramammary lesions	22 (11.5)	16 (15.1)	2 (6.5)	1 (3.1)	3 (13.0)	
Pathological materials						
Core biopsy	33 (17.2)	22 (20.8)	5 (16.1)	5 (15.6)	1 (4.3)	0.297
Surgical excision	159 (82.8)	84 (79.2)	26 (83.9)	27 (84.4)	22 (95.7)	
Histology						
Ductal	179 (93.2)	102 (96.2)	26 (83.9)	29 (90.6)	22 (95.7)	0.095
Mixed	13 (6.8)	4 (3.8)	5 (16.1)	3 (9.4)	1 (4.3)	
Tumor grade						
Well differentiated	22 (11.5)	18 (17.0)	0 (0.0)	3 (9.4)	1 (4.3)	0.127
Moderately differentiated	66 (34.4)	36 (34.0)	10 (32.3)	10 (31.2)	10 (43.5)	
Poorly differentiated	104 (54.2)	52 (49.1)	21 (67.7)	19 (59.4)	12 (52.2)	
CST cycles						
4–8	22 (11.5)	13 (12.3)	2 (6.5)	3 (9.4)	4 (17.4)	
9–12	122 (63.5)	71 (67.0)	18 (58.1)	20 (62.5)	13 (56.5)	
>12	48 (25.0)	22 (20.8)	11 (35.5)	9 (28.1)	6 (26.1)	0.629
Chemotherapy regimens						
AT-based	132 (68.8)	106 (100.0)	6 (19.4)	13 (40.6)	7 (30.4)	<0.001
A-based	19 (9.9)	0 (0.0)	0 (0.0)	19 (59.4)	0 (0.0)	
T-based	25 (13.0)	0 (0.0)	25 (80.6)	0 (0.0)	0 (0.0)	
TC-based	16 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	16 (69.6)	
Endocrinotherapy						
No	70 (36.5)	5 (4.7)	10 (32.3)	32 (100.0)	23 (100.0)	<0.001
Yes	122 (63.5)	101 (95.3)	21 (67.7)	0 (0.0)	0 (0.0)	
Targeted therapy						
No	161 (83.9)	103 (97.2)	16 (51.6)	20 (62.5)	22 (95.7)	<0.001
Yes	31 (16.1)	3 (2.8)	15 (48.4)	12 (37.5)	1 (4.3)	
Initial ER status						
Negative	58 (30.2)	3 (2.8)	0 (0.0)	32 (100.0)	23 (100.0)	<0.001
Positive	134 (69.8)	103 (97.2)	31 (100.0)	0 (0.0)	0 (0.0)	
Initial PR status						
Negative	80 (41.7)	14 (13.2)	11 (35.5)	32 (100.0)	23 (100.0)	<0.001
Positive	112 (58.3)	92 (86.8)	20 (64.5)	0 (0.0)	0 (0.0)	
Initial HER2 status						
Negative	129 (67.2)	106 (100.0)	0 (0.0)	0 (0.0)	23 (100.0)	<0.001
Positive	63 (32.8)	0 (0.0)	31 (100.0)	32 (100.0)	0 (0.0)	
Initial Ki-67 status						
Negative	59 (30.7)	43 (40.6)	5 (16.1)	5 (15.6)	6 (26.1)	0.009
Positive	133 (69.3)	63 (59.4)	26 (83.9)	27 (84.4)	17 (73.9)	

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; Ki-67, proliferation index; TNBC, triple negative breast cancer; CST, chemotherapy-based systemic therapy.

90.1 % of patients. Thirteen of 134 ER positive patients (9.7 %) became ER negative, while six of 58 ER negative patients (10.3 %) became ER positive. ER was consistently positive in 90.3 % of patients and consistently negative in 89.6 % of patients (Table 2). The 5-year survival rate was 44.9 % in patients whose ER status was positive and did not change, 43.1 % in patients whose ER status was positive and turned negative, and 16.2 % in patients whose ER status was negative and did not change (Table 2). Survival rates of the ER negative group who remained negative after CST were statistically significantly lower than those who remained positive (Fig. 1A; Table 2). Patients with ER alterations did not differ significantly in OS compared to those without (Fig. 1A).

According to the PR status, the change rate of PR status after CST was 17.2 % (33/192). 82.8 % of patients had no change in PR status (Table 2). Of 112 PR-positive patients, 22 (19.6 %) became PR-negative, and of 80 PR-negative patients, 11 (13.7 %) became positive after CST. PR was consistently positive in 80.3 % of patients and consistently negative in 86.2 % of patients (Table 2). The 5-year survival rate was 46.5 % in those who were positive and did not change, 21.0 % in those who were negative and did not change, 48.7 % in those who changed from positive to negative, and 18.2 % in those who changed from negative to positive. Survival rates of the groups with PR change from negative to negative and negative to positive were statistically significantly lower than those with positive to negative (Fig. 1B; Table 2). No statistically significant difference was found in OS in patients with PR changes compared with those without (Fig. 1B).

There was no change in the HER2 status in 93.8 % of the patients

Table 2
Changes in biomarker status and survival following chemotherapy-based systemic therapy.

Biomarker status	Patients (%)	Disconcordance (%)	Concordance (%)	K value	5-year survival (%)
ER			90.1	0.773	
Remained positive	121 (63.0 %)				44.9
Remained negative	52 (27.1 %)				16.2
Positive to negative	13 (6.8 %)				43.1
Negative to positive	6 (3.1 %)	9.9			n/a
PR		17.2	82.8	0.653	
Remained positive	90 (46.9 %)				46.5
Remained negative	69 (35.9 %)				21.0
Positive to negative	22 (11.5 %)				48.7
Negative to positive	11 (5.7 %)				18.2
HER2		6.2	93.8	0.859	
Remained positive	58 (30.2 %)				23.7
Remained negative	122 (63.5 %)				39.2
Positive to negative	5 (2.6 %)				80
Negative to positive	7 (3.6 %)				42.8
Ki-67		25.5	74.5	0.391	
Remained positive	110 (57.3 %)				24.7
Remained negative	33 (17.2 %)				61.9
Positive to negative	23 (12.0 %)				46.4
Negative to positive	26 (13.5 %)				41.9
Total					
Unchanged	90 (46.9 %)				31.7
Changed	102 (53.1 %)				40.8

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; Ki-67, proliferation index; K of concordance, Cohen's unweighted Kappa.

following CST. The change rate of HER2 status was only 6.2 % (12/192) (Table 2). Of 63 HER2-positive patients, 5 (2.6 %) became HER2-negative, and of 129 HER2-negative patients, 7 (3.6 %) became positive after CST (Table 2). Five-year survival was 23.7 % in HER2 positive and unchanged, 80.0 % in HER-2 positive and changed to negative, 42.8 % in HER2 negative and changed to positive, and 39.2 % in HER-2 negative and unchanged. There was no statistical difference in OS between the HER2-changed and unchanged groups (Fig. 1C). However, the survival rate of the positive-to-negative subgroup was significantly higher than that of the other three subgroups (Fig. 1C). There was a statistical difference in OS between patients who were consistently positive for HER2 and those who became positive (Fig. 1C).

The change rate of Ki-67 status after CST was 25.5 % (49/192). Ki-67 status was unchanged in 74.5 % of patients (Table 2). Twenty-three of 133 Ki67 positive patients (17.3 %) became Ki-67 negative, while 26 of 59 Ki-67 negative patients (44.1 %) became Ki-67 positive. Ki-67 was consistently positive in 82.7 % of patients and consistently negative in 55.9 % of patients (Table 2). The 5-year survival rate was 24.7 % in those who were positive and did not change, 61.9 % in those who were negative and did not change, 46.4 % in those who changed from positive to negative, and 41.9 % in those who changed from negative to positive. The Ki-67 persistently negative group had the highest survival rate, while the persistently positive group had significantly lower survival rate than the three other groups. The difference in OS between them was statistically significant (Fig. 1D). No statistically significant difference was found in OS between patients with changes in Ki-67 and those without changes in Ki-67 (Fig. 1D).

Univariate analysis identified that the conversion of ER, PR, HER2 and Ki-67 status, and family history, initial metastatic sites, pathological materials, tumor grade, and chemotherapy regimens were significantly associated with OS, and biological subtypes were also correlated with OS. In multivariate Cox regression analyses, the conversion of ER, PR, HER2 and Ki-67 status, and family history, initial metastatic sites, and tumor differentiation were significantly associated with OS (Table 3).

4. Discussion

The major finding in the current study is that the expressions of receptor markers ER, PR, HER2 and proliferation index Ki-67 change following CST treatment in dnMBC patients. Further, although we did not find a statistical association between these biomarker discordance and survival outcomes, their positive conversion may indicate a poor prognosis. In addition, univariate and multifactorial analyses suggest that changes in these biomarker status, family history, initial metastatic site, and tumor grade may be prognostic factors.

Our study demonstrates the instability of biomarker expression in dnMBC patients treated with CST. In this study, 53.1 % of patients receiving CST had alterations in the expression of at least one biomarker, and the expression of ER, PR, HER2 and Ki-67 showed varying proportions, respectively. These changes support the hypothesis that the discordance in biomarker expression do exist and may be elicited by CST. In HR, the change rate of ER is significantly lower than that of PR, and the low expression tendency of both is consistent. The study by Gupta et al. showed that the change rate of ER was 8.7 % and that of PR was 17.4 %, and the change rate of PR was higher than that of ER [18], which was consistent with our results. It is well known that ER is a more dominant determinant for HR positivity. Thus, it is not rational that ER change is not significant and PR change is significant. The exact reasons for this are unknown. For HER2, the 6.2 % conversion rate is low and much lower than the conversion rate reported in the literature[2 19]. In fact, previous studies on the changes of HR and HER-2 status after NAC have shown conflicting results. Studies conducted by Mohan SC et al. and Lee S et al. showed changes in tumor receptor expression after NAC [7 12], while Kasami M et al. concluded in their study that there was no receptor inconsistency in patients following NAC [5]. In this study, we also found differences in increased and decreased expression of the altered receptor or in the conversion between negative and positive. The conversion of negative to positive HR and the loss of HER2-positive were significantly different from previous findings[8 18]. As for Ki-67 status, existing studies have shown that Ki-67 status changes generally after NAC [20 21], which is consistent with our findings. Similar to other studies [22], we found that Ki-67 expression was significantly decreased in 12.0 % of patients, and significantly increased in 13.5 % of patients following CST. Overall, the discordance rate of biomarker expression in this study was consistent with the literature.

In this study, we found that patients who had a positive HR conversion and continued to be negative had worse OS than those who had a negative HR conversion and unchanged HR expression. ER positive patients who remained unchanged after treatment had the highest survival rate, those who changed from positive to negative had the second highest survival rate, and those who changed from negative to positive had a survival time of less than 3 years. Similar to changes in ER status, the survival rate of PR-positive to negative patients was higher than that of PR-negative and unchanged groups and PR-negative to positive groups. However, previous studies have shown that negative HR conversion is associated with reduced OS and is therefore a poor prognostic indicator [19 21]. Clearly, our findings are inconsistent with previous

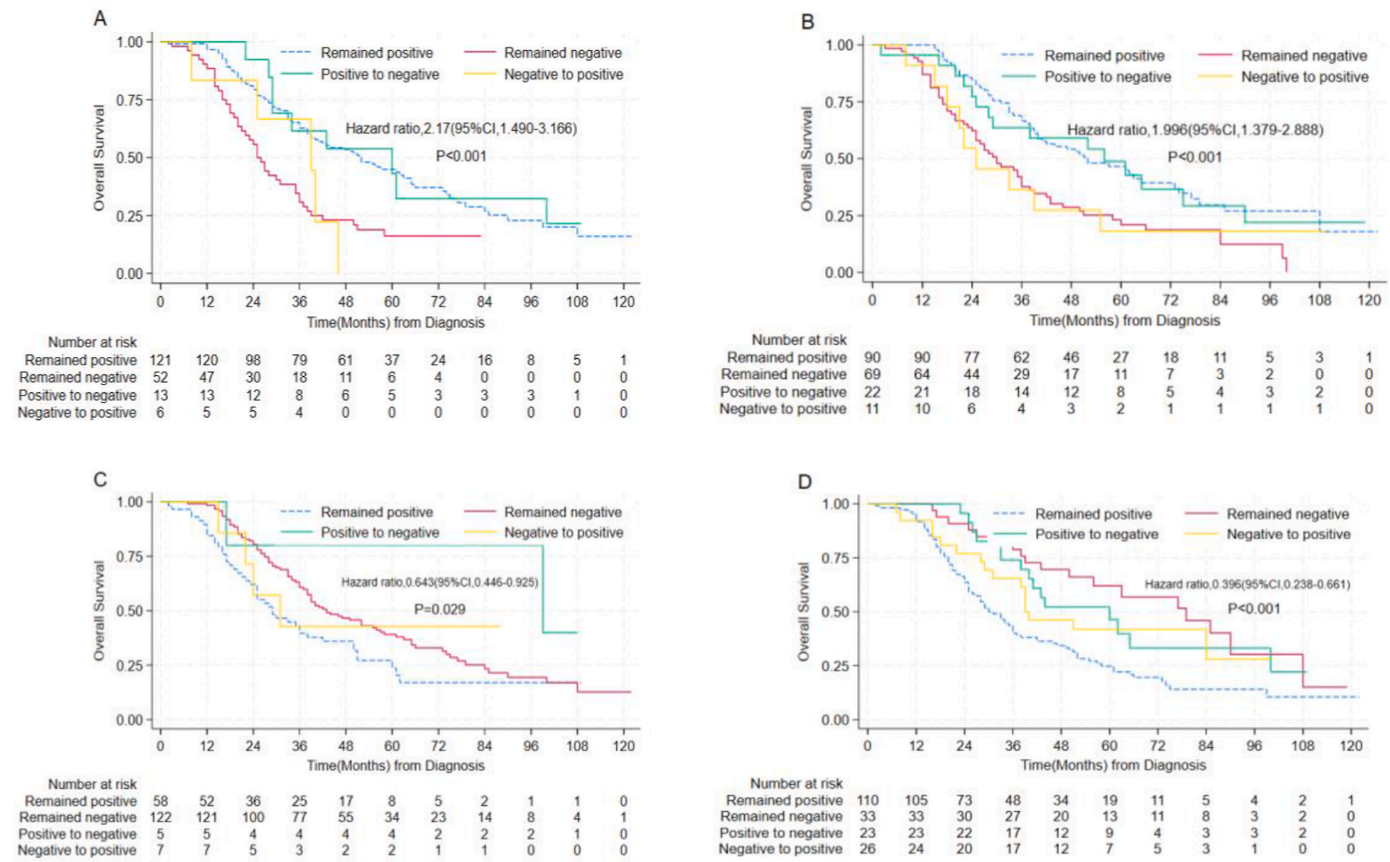

Fig. 1. Kaplan-Meier curve of overall survival in patients with ER, PR, HER2 and Ki-67 discordance. (A) ER; (B) PR; (C) HER2; (D) Ki-67.

studies in that a change from negative to positive HR status is associated with poorer survival, while a change from positive to negative HR status is associated with a better prognosis. One plausible explanation is that in our study, 91.0 % of ER-positive patients and 94.6 % of PR-positive patients received endocrine therapy, which is bound to have a favorable impact on survival outcomes.

We also found that in dnMBC patients, HER2-positive loss was associated with longer survival, although the incidence was low and changes in HER2 status did not significantly affect prognosis. On the contrary, Ahn et al. reported that positive-to-negative change in HER2 expression was more common than negative-to-positive change after NAC [22]. Also, Tural et al. showed that HER2-positive loss was an in dependent risk factor for worse DFS [23]. In contrast, 2.6 % of cases in our cohort changed from positive to negative and less than 3.6 % of cases changed from negative to positive, but the survival rate was as high as 80.0 %. We believe that the mechanism of this difference should be due to the use of targeted therapies. To date, consequences on adjuvant treatment reported in literature were mainly related to the switch of HR or HER2 status from negative to positive, allowing adding further therapy [21]. This is basically consistent with our results. Obviously, especially for dnMBC patients, positive expression after CST should be more clinically significant.

The effect of Ki-67 status conversion on prognosis is still controversial. Several studies have shown that high expression of Ki-67 after NAC is one of the important predictors of clinical prognosis [21,23–26]. Our study showed that there was no statistical difference between the discordance of Ki-67 status and survival following CST, however, the change of Ki-67 status from negative to positive may indicate a poor prognosis. One possible mechanism could explain the increased expression of Ki-67 and its resulting poor prognosis. Chemotherapy kills active proliferating tumor cells and indirectly promotes the active metabolism of residual tumor cells in G0 stage or re-enters the division cycle [19]. In this case, Ki-67 can be re-expressed in tumor cells. In addition, the association between Ki-67 high expression and chemotherapy resistance may also explain the increase in Ki-67 expression. These results suggest that re-evaluation of Ki-67 status may be necessary following CST in dnMBC patients.

Currently, the frequency of biomarker changes in daily clinical practice after CST, as well as their actual impact on treatment, remains unknown. Several studies have produced mixed results on this topic [7,27–29], and although some authors have recommended retesting biomarkers after NAC [15], the actual benefits are still debated, with an international working group on pathology recommending against it [30]. The main limitation is that not all pathology laboratories systematically replicate the biological properties after NAC, and few pathology laboratories routinely test for Ki67 due to the lack of standardization and reproducibility of assessment. Despite these challenges, this study suggests that for dnMBC patients, biomarker expression investigation following CSA may allow patients to adjust or change their treatment regimen, while providing an opportunity to optimize treatment options and reevaluate prognosis.

A major strength of this study is that we investigated and evaluated changes in the biomarkers HR, HER2, Ki-67 following CST and how these changes relate to survival outcomes, which has not been reported before. However, we acknowledge multiple limitations in the current study. First, this is a retrospective study, which is inevitably biased due to the completeness of data collection and the accuracy of follow-up information. Further, the sample size is relatively small, and to some extent there may be challenges of limited statistical power and insufficient ability to identify significant differences. Second, although all patients receive CST, patients may receive different chemotherapy regimens or in combination with other adjuvant therapies such as

Table 3
Univariate and multivariate Cox regression analyses of overall survival.

Variables		Univariate analysis		Multivariate analysis	
	Patients (n)	Hazard ratio (95 % CI)	P	Hazard ratio (95 % CI)	P
Age			0.613		
≤50 years	100	1		1	
>50 years	92	0.916 (0.654–1.284)	0.614	1.162(0.568–2.377)	0.679
ER status			**<0.001**		
Remained positive	121	1		1	
Remained negative	52	2.172(1.490–3.166)	**<0.001**	0.587(0.154–2.234)	0.436
Positive to negative	13	0.888(0.444–1.775)	0.739	0.407(0.177–0.936)	**0.034**
Negative to positive	6	1.964(0.790–4.886)	0.146	0.557(0.112–2.759)	0.474
PR status			**0.001**		
Remained positive	90	1		1	
Remained negative	69	1.996(1.379–2.888)	**<0.001**	3.162(1.579–6.332)	**0.001**
Positive to negative	22	1.024(0.579–1.812)	0.934	1.681(0.887–3.186)	0.111
Negative to positive	11	1.901(0.938–3.851)	0.075	1.156(0.433–3.087)	0.772
HER2 status			**0.027**		
Remained positive	58	1		1	
Remained negative	122	0.643(0.446–0.925)	**0.018**	0.819(0.233–2.870)	0.755
Positive to negative	5	0.232(0.055–0.964)	**0.044**	0.179(0.037–0.868)	**0.033**
Negative to positive	7	0.613(0.220–1.711)	0.351	1.069(0.215–5.307)	0.935
Ki-67 status			**<0.001**		
Remained positive	110	1		1	
Remained negative	33	0.396(0238–0.661)	**<0.001**	0.373(0.207–0.672)	**0.001**
Positive to negative	23	0.506(0.292–0.877)	**0.015**	0.556(0.303–1.020)	0.058
Negative to positive	26	0.594(0.349–1.014)	0.056	0.654(0.364–1.175)	0.156
Menopausal status			0.191		
Premenopausal	106	1		1	
Postmenopausal	86	0.796 (0.566–1.121)	0.193	0.672(0.327–1.382)	0.281
Family history			**0.028**		
No	139	1		1	
Yes	53	0.653(0.439–0.970)	**0.035**	0.496(0.316–0.780)	**0.002**
Initial metastatic sites			**<0.001**		
Bone alone	96	1		1	
Single organ	51	1.207(0.798–1.826)	0.371	1.081(0.656–1.781)	0.759
Multiple organs	41	2.795(1.857–4.253)	**<0.001**	2.898(1.799–4.666)	**<0.001**
Brain	4	9.376(3.280–26.798)	**<0.001**	7.840(1.895–32.442)	**0.004**
Pathological materials			**0.004**		
Core biopsy	33	0.518(0.340–0.791)	**0.002**	0.641(0.377–1.090)	0.101
Surgical excision	159				
Tumor grade			**0.041**		
Well differentiated	22	1		1	
Moderately differentiated	66	0.496(0.292–0.842)	**0.009**	0.293(0.152–0.566)	**<0.001**
Poorly differentiated	104	0.642(0.394–1.044)	0.074	0.423(0.227–0.786)	**0.007**
CST cycles			0.449		
4–8	22	1		1	
9–12	122	0.828(0.485–1.411)	0.488	0.691(0.386–1.234)	0.212
>12	48	1.053(0.581–1.909)	0.863	0.939(0.489–1.805)	0.852
Chemotherapy regimens			**0.006**		
AT-based	132	1		1	
A-based	19	1.535(0.872–2.703)	0.137	0.460(0.173–1.223)	0.120
T-based	25	1.339(0.816–2.198)	0.248	1.344(0.370–4.873)	0.653
TC-based	16	2.831(1.622–4.939)	**<0.001**	1.428(0.515–3.962)	0.493
Histology			0.733		
Ductal	179	1		1	
Mixed	13	1.135(0.554–2.321)	0.729	0.608(0.274–1.347)	0.220
Biological subtypes			**<0.001**		
HR+/HER2−	106	1		1	
HR+/HER2+	32	1.335(0.826–2.157)	0.237	0.528(0.074–3.727)	0.522
HR−/HER2+	31	1.946(1.220–3.104)	**0.005**	1(omitted)	
TNBC	23	2.728(1.661–4.482)	**<0.001**	1.601(0.307–8.343)	0.576

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; Ki-67, proliferation index; CST, chemotherapy-based systemic therapy; A-based,anthracycline; T-based,taxane; AT-based, anthracycline and taxane; TC-based,taxane and capecitabine.

endocrine therapy or targeted therapy. Therefore, this may introduce bias into the research. In addition, we did not use therapeutic response as study endpoint, which should be a shortcoming. We acknowledge that therapeutic response, including pathological response, is an important prognostic determinant of CST in dnMBC patients. We will consider this in future researches. Although our results are hypothesis-generating due to the retrospective nature of this study and the relatively small number of patients, it helps to fine-tune and improve treatment plans and optimize treatment strategies.

5. Conclusions

In summary, the discordance rates of ER, PR, HER2 and Ki-67 before and after CST were 9.9 %, 17.2 %, 6.2 % and 25.5 %, respectively. Negative-to-positive Ki-67 expression changes were the most common type of discordance observed. In this study, we did not observe a statistical correlation between changes in these biomarkers and survival outcomes. However, we found that positive conversion of ER and PR, as well as persistent positive HER2 and Ki-67 may be associated with poor prognosis in dnMBC patients. In addition to these altered biomarkers, family history, initial metastatic site, and tumor grade may be independent prognostic factors. In dnMBC patients, biomarker expression investigations following CST may be appropriate, especially in patients who are initially HR negative. Further studies are needed to clarify these issues and determine whether biomarker status needs to be reevaluated following CST to optimize treatment options and improve survival.

CRediT authorship contribution statement

Lingjun Kong: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Chongxi Ren:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Informed consent statement

Written informed consent has been obtained from the patients to publish this paper.

Consent for publication

All authors give consent for the publication of this manuscript.

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki and approved by Hebei Cangzhou Hospital of Integrated Traditional Chinese Medicine and Western Medicine Research Ethics Board (2017-AF29-058).

Data availability

The datasets used and analyzed during the current study are stored in corresponding author of this paper and are available upon request.

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Declaration of competing interest

The authors declare no potential conflicts of interest.

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Abbreviations

ER	estrogen receptor
PR	progesterone receptor
HR	hormone receptor
HER2	human epidermal growth factor receptor 2
Ki-67	proliferation index
TNBC	triple-negative breast cancer
CNB	core needle biopsy
IHC	immunohistochemistry
FISH	fluorescence in situ hybridization
OS	overall survival
CI	confidence interval
CST	chemotherapy-based systemic therapy
A-based	anthracycline
T-based	taxane
AT-based	anthracycline and taxane
TC-based	taxane and capecitabine.

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