

Improved Prognosis in HER2-Low Breast Cancer Patients with Reduced Ki67 Index After Neoadjuvant Chemotherapy: A Multi-Center Retrospective Study

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Background: HER2-low breast cancer represents a distinct subgroup with unique clinical characteristics and treatment challenges. Nevertheless, it remains uncertain whether there exists a distinction in δ Ki67 between patients with HER2-low and HER2-zero statuses, and whether the prognosis varies among patients with differing HER2 statuses and δ Ki67.

Methods: We conducted a multi-center retrospective study to investigate the correlation between alterations in Ki67 index following NAC and the prognosis among patients with HER2-low or HER2-zero breast cancer. 3 distinct cohorts comprising patients with HER2-negative breast cancer who underwent NAC were included. Comprehensive clinicopathological data were documented, with particular emphasis on evaluating changes in Ki67 index from baseline to post-NAC. These changes were then correlated with disease-free survival (DFS) through rigorous analysis.

Results: Three cohorts, comprising 403, 315, and 72 patients respectively, were finally included. The study found that δ Ki67 did not show significant associations with other variables and were not identified as independent risk factors for survival. Nevertheless, across the three cohorts, following NAC, HER2-low breast cancer patients with δ Ki67 below the cut-off value demonstrated a better prognosis compared to those with δ Ki67 above the cut-off value. Additionally, their prognosis was also superior to that of HER2-zero breast cancer patients with δ Ki67 below the cut-off value.

Conclusion: Our study demonstrates that HER2-low breast cancer patients with δ Ki67 values below the cut-off point after NAC are associated with improved prognosis. Monitoring δ Ki67 index and HER2 status may help identify patients who are likely to benefit from NAC and guide personalized treatment strategies.

Keywords: HER2-low breast cancer, neoadjuvant chemotherapy, Ki67 index, prognosis, survival

Introduction

Breast cancer remains one of the most prevalent malignancies worldwide, posing a significant health burden and a formidable challenge to healthcare systems.¹ The management of breast cancer has evolved considerably over the years, with neoadjuvant chemotherapy (NAC) emerging as a cornerstone in the treatment paradigm. This approach, which involves administering chemotherapy prior to surgery, offers several advantages, including the potential to downsize tumors, increase the likelihood of breast-conserving surgery, and assess treatment response in vivo.^{2,3}

Despite the widespread adoption of NAC, accurately predicting treatment response and long-term outcomes for individual patients remains a complex task. Traditional clinical and pathological factors, such as tumor size, grade, and hormone receptor status, provide valuable prognostic information but may not fully capture the dynamic changes induced

by chemotherapy within the tumor microenvironment.^{4–8} In recent years, molecular markers have garnered increasing attention for their potential to refine prognostication and guide therapeutic decision-making in breast cancer. Among these, the Ki67 and the human epidermal growth factor receptor 2 (HER2), have emerged as a promising biomarker. The dynamic nature of Ki67 expression in response to NAC presents a unique opportunity to assess treatment response and predict long-term outcomes.^{9–11} Several studies have investigated the prognostic significance of changes in Ki67 levels following NAC, with decreased Ki67 index is significantly associated with better prognosis.^{12–15} Meanwhile, the ongoing discussion surrounding the regrouping of HER2 is currently a focal point of interest and research. Some studies have indicated that HER2-low (defined as an IHC score of 1+ or 2+ without HER2 gene amplification) represents an intrinsic subtype, distinct from HER2-zero (defined as an IHC score of 0).^{16–21} Conversely, additional studies have reached conflicting conclusions.^{22,23}

In this manuscript, we hypothesized that the different changes in Ki67 post NAC in breast cancer patients with HER2-low or HER2-zero predict different prognosis, and we aim to offer novel evidence for stratifying HER2 patients, particularly aiding in personalized treatment decisions and prognostication within the realm of precision oncology.

Methods

Patient Selection and Study Design

This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the ethics committee of the First Affiliated Hospital of Chongqing Medical University (ID: No. 2020–59), as well as approved by the ethics committee of the Chongqing University Three Gorges Hospital (ID: No.2022–129). Informed consent was waived due to the retrospective nature of the study and the use of de-identified patient data.

We retrospectively reviewed 3 patients' cohort: 1) Breast cancer patients at the First Affiliated Hospital of Chongqing Medical University from January 2013 to June 2018. 2) Breast cancer patients at the First Affiliated Hospital of Chongqing Medical University from July 2018 to January 2023; 3) Breast cancer patients at the Chongqing University Three Gorges Hospital from November 2020 to May 2023. The HER2-negative patients who received NAC and without achieving pCR were selected for the subsequent analysis. The patients' clinical characteristics and the pathological index both pre- and post-surgery were recorded. We assessed the relationship of different HER2 statuses and changes in Ki67 with patients' variables, determining whether it constituted an independent risk factor for patient survival outcomes.

Treatment Protocol

Patients who met the criteria were scheduled to begin NAC within a week. The NAC regimen was determined according to the guidelines outlined by the Chinese Society of Clinical Oncology (CSCO) and the National Comprehensive Cancer Network (NCCN). The treatment protocol was mainly TEC (docetaxel 75 mg/m², epirubicin 75 mg/m², and cyclophosphamide 500 mg/m²). The drugs were administered in 21-day cycles. Following completion of 4–8 cycles of NAC, the treatment response was assessed by both clinicians and pathologists. Afterwards, either mastectomy or breast-conserving surgery along with axillary lymphadenectomy was conducted. The post-surgery systemic therapy protocols are outlined as follows. Endocrine Therapy: Patients with estrogen receptor-positive or progesterone receptor-positive tumors are required to undergo hormone therapy. Adjuvant Chemotherapy: The criteria for post-surgical adjuvant chemotherapy include: (1) A high Ki67 index. (2) Triple-negative breast cancer. (3) HER2-positive status. (4) Positive regional lymph nodes. (5) Histological grade III or higher. (6) Genetic testing indicating a high risk of recurrence. (7) A relatively larger tumor size. Post-Mastectomy Radiation Therapy (PMRT): For patients with node-positive disease, all individuals will receive PMRT to the chest wall. In contrast, for node-negative patients with tumors measuring 5 cm or less and clear margins (≥ 1 mm), PMRT is generally not required. However, for high-risk patients, including those with: (1) Central tumors. (2) T3-stage tumor. (3) Tumors larger than 2 cm with fewer than 10 axillary nodes removed. (4) At least one of the following characteristics: (a) grade 3, (b) ER-negative, or (c) lymphovascular invasion, PMRT should be considered.^{24,25}

Pathological Evaluation

The pathological diagnosis was established through interpretation of pathological slides, complemented by the immunohistochemistry (IHC) index derived from both the tumor's core needle biopsy and the surgical resection specimens post NAC. In Cohorts 1 and 2, the IHC results of all patients were tested using related antibodies, which were monoclonal antibodies sourced from Fuzhou Maxim Biotechnologies Co., Ltd., located in Fujian Province, China. The specific product numbers for the antibodies were as follows: ER, Kit-0012; PR, Kit-0013; HER2, Kit-0043; and Ki67, MAB-0672. In Cohort 3, the antibodies were from the Roche. The clone number were as follows: ER, SP1; PR, 1E2; HER2, 4B5; Ki67, 30–9. The change in Ki67 (δKi67) was defined as the post-NAC Ki67 index subtracted from the pre-NAC Ki67 index ($\delta\text{Ki67} = \text{Ki67}_{\text{post-NAC}} - \text{Ki67}_{\text{pre-NAC}}$). ER, PR positivity was characterized by the presence of cells expressing at a percentage greater than 10% on IHC. HER2 status was classified according to the IHC score and the result of FISH. A score of 0 indicated HER2-zero, whereas a score of 1+ or 2+ without ERBB2 gene amplification was classified as HER2-low.²⁶ To ensure the credibility of the results, all pathological slides were independently re-evaluated retrospectively by two pathologists. Moreover, the absence of residual invasive tumor in both the breast and axillary lymph nodes (ypT0ypN0) was regarded as achieving pathological complete response (pCR) following NAC.³

Follow-Up

All patients underwent interviews either through outpatient visits or telephone consultations. For cohort 1, the follow-up period was from discharge until January 31st, 2021. For cohort 2 and cohort 3, the follow-up period was both from discharge until April 30th, 2024. Disease-free survival (DFS) was chosen as the metric for evaluating patient survival duration. DFS was defined as the duration from surgery to disease recurrence, progression, or death from any cause, whichever occurred first.

Statistical Analysis

We used IBM SPSS 23.0 (Chicago, IL, USA), X-tile (Version 3.6.1) and RStudio (R version 4.3.2) software for statistical analysis. The X-tile software was employed to determine the optimal cut-point for changes in the Ki67 index. Descriptive statistics were used to summarize patient characteristics and treatment variables. Kaplan-Meier curves were constructed to estimate DFS probabilities, and differences between groups were compared using Log rank tests. The chi-square test was employed to examine differences between groups for categorical variables, while the Mann–Whitney *U*-test or *t*-test was utilized to analyze differences between groups for continuous variables. Univariate and multivariate logistic regression was used to assess the association between changed Ki67 index and patient characteristics. Univariate and multivariate Cox proportional hazards models were used to assess the independent predictors between changed Ki67 index post-chemotherapy and survival outcomes, adjusting for potential confounders. The survival analysis was conducted using Kaplan-Meier curves, with differences between the groups assessed using the Log rank test. Statistical analysis primarily relied on the “autoReg”, “mice”, “survival”, and “survminer” packages within the R software. A *p*-value less than 0.05 was deemed indicative of statistical significance.

Results

Patient Characteristics

In total, 403, 315, and 72 patients were ultimately selected for cohort 1, cohort 2, and cohort 3, respectively. The median ages were 48.0-year-old, 51.0-year-old, and 49-year-old for cohort 1, 2, and 3, respectively. In cohort 1 and cohort 2, the majority of patients presented with either cT1 or cT2 stage tumors, while over half of the patients had cT3 or cT4 stage tumors. Additionally, most of patients across all three cohorts had either cN0 or cN1 stage tumors. Furthermore, a significant proportion of HER2-negative patients across the three cohorts exhibited HER2-low expression, comprising 83.9%, 63.2%, and 75% of the respective cohorts. The median follow-up times and interquartile ranges (IQRs) for the three cohorts were 51.0 months (IQR: 20.5 to 78.0), 23.0 months (IQR: 15.9 to 36.3), and 18.4 months (IQR: 14.0 to 33.8). The selection process is illustrated in Figure 1, and detailed comparison of patient information across three cohorts is provided in Table 1.

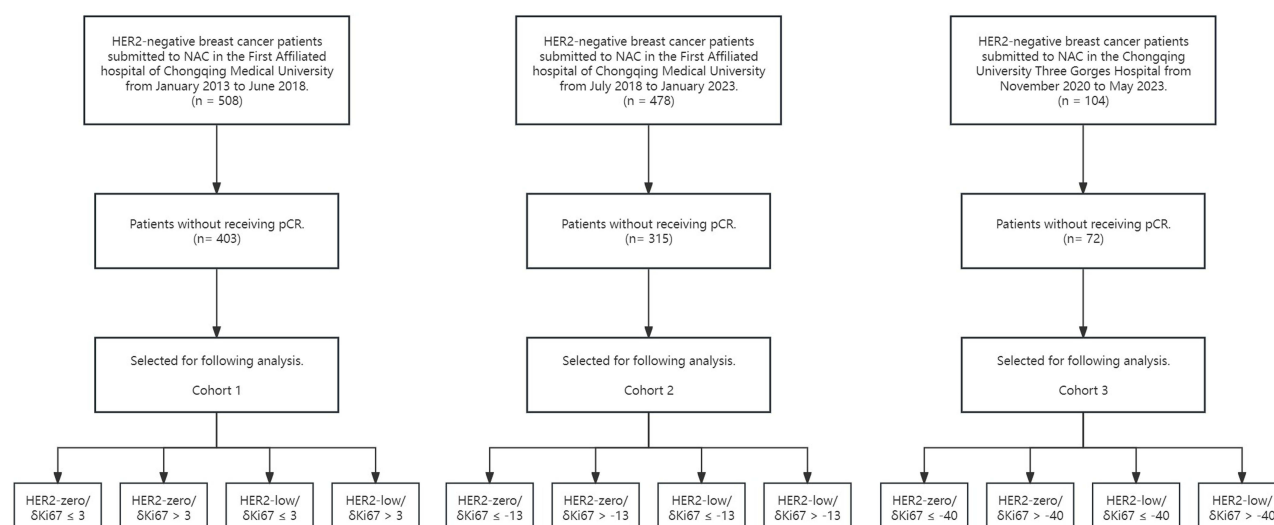


Figure 1 The flowchart of this study.

The Optimal Cut-Point of δ Ki67

In cohort 1, leveraging X-tile and R software alongside comprehensive survival analysis post-stratification, the optimal cut-off value for δ Ki67 was determined to be 3. This indicates that patients experiencing a Ki67 increase of more than 3 post NAC exhibit a poorer prognosis compared to those with a rise of less than 3. The same methodology was applied to calculate the optimal cut-off values of δ Ki67 in Cohorts 2 and 3, resulting in cut-off values of -13 and -40 , respectively (Figure 2A–F).

The Relationship Between δ Ki67 and Clinical Indices, as Well as Its Impact on Patient Prognosis

The outcomes across the three cohorts exhibited notable disparities. Cohort 1 found no significant association between δ Ki67 and the variables studied. In Cohort 2, δ Ki67 was linked to hormone receptors, whereas in Cohort 3, it appeared to be

Table 1 Clinicopathological Variables of the Three Cohorts

		Cohort 1 (N=403)	Cohort 2 (N=315)	Cohort 3 (N=72)
Age	Median (IQR)	48.0 (43.0 to 56.0)	51.0 (44.0 to 57.5)	48.5 (40.5 to 53.0)
Histological type	Invasive ductal carcinoma	389 (96.5%)	296 (94%)	70 (97.2%)
	Others	14 (3.5%)	19 (6%)	2 (2.8%)
T	T1+T2	307 (76.2%)	223 (70.8%)	34 (47.2%)
	T3+T4	96 (23.8%)	92 (29.2%)	38 (52.8%)
N	N0+N1	330 (81.9%)	200 (63.5%)	60 (83.3%)
	N2+N3	73 (18.1%)	115 (36.5%)	12 (16.7%)
ER	negative	130 (32.3%)	101 (32.1%)	16 (22.2%)
	positive	273 (67.7%)	214 (67.9%)	56 (77.8%)
PR	negative	204 (50.6%)	154 (48.9%)	26 (36.1%)
	positive	199 (49.4%)	161 (51.1%)	46 (63.9%)
HER2	HER2-zero	65 (16.1%)	116 (36.8%)	18 (25%)
	HER2-low	338 (83.9%)	199 (63.2%)	54 (75%)
Ki67	Median (IQR)	20.0 (10.0 to 30.0)	30.0 (20.0 to 42.5)	30.0 (20.0 to 60.0)
Change in ER	Median (IQR)	0.0 (−10.0 to 0.0)	0.0 (−5.0 to 0.0)	0.0 (−10.0 to 0.0)
Change in PR	Median (IQR)	0.0 (−10.0 to 0.0)	0.0 (−13.5 to 0.0)	−1.5 (−40.0 to 0.0)
Change in Ki67	Median (IQR)	−3.0 (−15.0 to 0.0)	−10.0 (−24.0 to 0.0)	−10.0 (−27.5 to 0.0)
DFS	Median (IQR)	51.0 (20.5 to 78.0)	23.0 (15.9 to 36.3)	18.4 (14.0 to 33.8)

Abbreviations: IQR, interquartile range; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; DFS, disease-free survival.

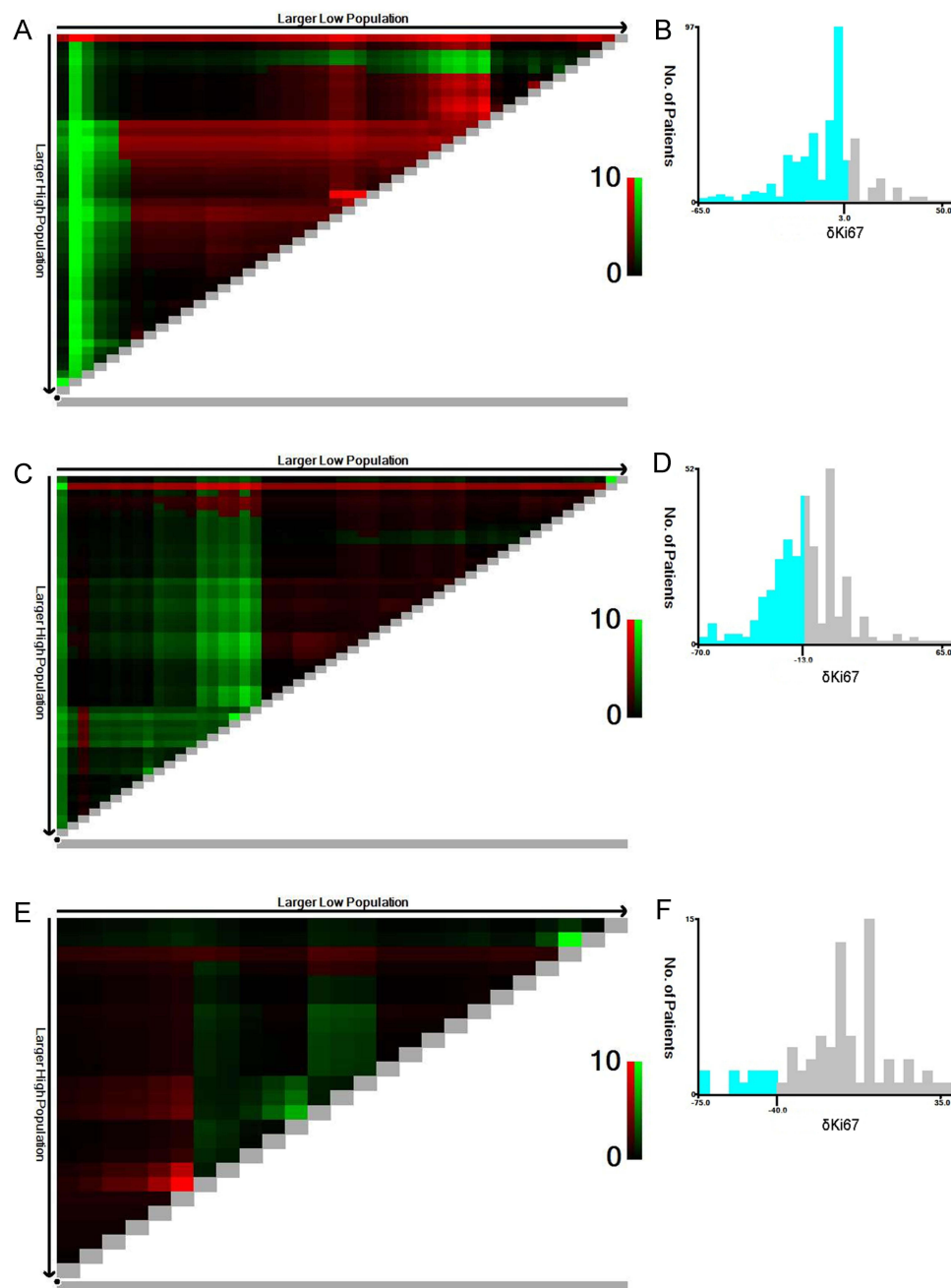


Figure 2 The optimal cut-point for δKi67 in Cohort 1 (**A** and **B**), Cohort 2 (**C** and **D**), and Cohort 3 (**E** and **F**) was calculated by X-tile.

correlated with N-stage. Cumulatively, our analysis did not identify a significant association between the observed changes and the variables under investigation (Table 2). Furthermore, univariate and multivariate analyses were performed across all three cohorts, revealing that δKi67 did not emerge as an independent prognostic risk factor (Table 3).

Survival Analysis

In cohort 1, based on the δKi67 index and the HER2 status, the patients were divided into 4 groups: 1) HER2-zero/ $\delta\text{Ki67} \leq 3$; 2) HER2-zero/ $\delta\text{Ki67} > 3$; 3) HER2-low/ $\delta\text{Ki67} \leq 3$; 4) HER2-low/ $\delta\text{Ki67} > 3$. Survival analysis revealed a significantly higher survival rate in group 3 compared to both group 1 ($p = 0.018$) and group 4 ($p = 0.046$). This finding is visually represented by the Kaplan-Meier curves shown in Figure 3A. In cohort 2, patients were categorized into four groups based on the following

Table 2 The Relationship Between δ Ki67 and Individual Clinicopathological Variables Within Each of the Three Cohorts

		Cohort 1			Cohort 2			Cohort 3		
		δ Ki67 ≤ 3 (N=304)	δ Ki67 > 3 (N=99)	p	δ Ki67 ≤ -13 (N=141)	δ Ki67 > -13 (N=174)	p	δ Ki67 ≤ -40 (N=11)	δ Ki67 > -40 (N=61)	p
Age	Median (IQR) or Mean $\pm$ SD	47.0 (43.0 to 56.5)	49.0 (43.0 to 54.0)	0.867	51.0 (43.0 to 57.0)	51.0 (45.0 to 58.0)	0.468	45.5 $\pm$ 9.2	47.3 $\pm$ 9.2	0.551
Histological type	Invasive ductal carcinoma	291 (95.7%)	98 (99%)	0.220	135 (95.7%)	161 (92.5%)	0.340	11 (100%)	59 (96.7%)	1.000
	Others	13 (4.3%)	1 (1%)		6 (4.3%)	13 (7.5%)		0 (0%)	2 (3.3%)	
T	T1+T2	226 (74.3%)	81 (81.8%)	0.167	101 (71.6%)	122 (70.1%)	0.865	6 (54.5%)	28 (45.9%)	0.841
	T3+T4	78 (25.7%)	18 (18.2%)		40 (28.4%)	52 (29.9%)		5 (45.5%)	33 (54.1%)	
N	N0+N1	250 (82.2%)	80 (80.8%)	0.865	91 (64.5%)	109 (62.6%)	0.818	6 (54.5%)	54 (88.5%)	0.019
	N2+N3	54 (17.8%)	19 (19.2%)		50 (35.5%)	65 (37.4%)		5 (45.5%)	7 (11.5%)	
ER	negative	92 (30.3%)	38 (38.4%)	0.168	29 (20.6%)	72 (41.4%)	<0.001	3 (27.3%)	13 (21.3%)	0.965
	positive	212 (69.7%)	61 (61.6%)		112 (79.4%)	102 (58.6%)		8 (72.7%)	48 (78.7%)	
PR	negative	148 (48.7%)	56 (56.6%)	0.213	60 (42.6%)	94 (54%)	0.056	3 (27.3%)	23 (37.7%)	0.747
	positive	156 (51.3%)	43 (43.4%)		81 (57.4%)	80 (46%)		8 (72.7%)	38 (62.3%)	
HER2	HER2-zero	48 (15.8%)	17 (17.2%)	0.867	44 (31.2%)	72 (41.4%)	0.081	3 (27.3%)	15 (24.6%)	1.000
	HER2-low	256 (84.2%)	82 (82.8%)		97 (68.8%)	102 (58.6%)		8 (72.7%)	46 (75.4%)	

Abbreviations: IQR, interquartile range; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; DFS, disease-free survival.

Table 3 Univariate and Multivariate Analyses for DFS

Cohort 1			HR (95% CI, P-value), Univariate analysis	HR (95% CI, P-value), Multivariate analysis
Age	Mean ± SD	48.9 ± 9.6	1.00 (0.98–1.02, p=0.949)	1.00 (0.98–1.02, p=0.925)
Histological type	Invasive ductal carcinoma	389 (96.5%)		
	Others	14 (3.5%)	1.50 (0.61–3.70, p=0.374)	1.60 (0.64–4.04, p=0.317)
T	T1+T2	307 (76.2%)		
	T3+T4	96 (23.8%)	1.22 (0.77–1.92, p=0.398)	1.21 (0.76–1.94, p=0.418)
N	N0+N1	330 (81.9%)		
	N2+N3	73 (18.1%)	1.74 (1.11–2.74, p=0.017)	1.76 (1.11–2.79, p=0.015)
ER	negative	130 (32.3%)		
	positive	273 (67.7%)	0.60 (0.40–0.90, p=0.013)	0.68 (0.40–1.16, p=0.159)
PR	negative	204 (50.6%)		
	positive	199 (49.4%)	0.66 (0.44–0.99, p=0.044)	0.88 (0.52–1.51, p=0.643)
HER2	HER2-zero	65 (16.1%)		
	HER2-low	338 (83.9%)	0.60 (0.37–0.97, p=0.037)	0.68 (0.42–1.10, p=0.117)
Ki67	Mean ± SD	24.6 ± 17.8	1.00 (0.99–1.01, p=0.521)	1.00 (0.99–1.01, p=0.995)
δKi67	Mean ± SD	0.2 ± 0.4	1.54 (1.00–2.37, p=0.049)	1.49 (0.96–2.33, p=0.078)
Cohort 2			HR (95% CI, P-value), Univariate analysis	HR (95% CI, P-value), Multivariate analysis
Age	Mean ± SD	50.4 ± 10.1	1.03 (1.00–1.06, p=0.034)	1.03 (1.00–1.06, p=0.047)
Histological type	Invasive ductal carcinoma	296 (94.0%)		
	Others	19 (6.0%)	0.29 (0.04–2.09, p=0.219)	0.24 (0.03–1.73, p=0.156)
T	T1+T2	223 (70.8%)		
	T3+T4	92 (29.2%)	1.17 (0.67–2.03, p=0.586)	1.10 (0.62–1.95, p=0.754)
N	N0+N1	200 (63.5%)		
	N2+N3	115 (36.5%)	2.10 (1.24–3.54, p=0.006)	1.72 (1.00–2.96, p=0.052)
ER	negative	101 (32.1%)		
	positive	214 (67.9%)	0.49 (0.29–0.83, p=0.007)	1.18 (0.57–2.44, p=0.654)
PR	negative	154 (48.9%)		
	positive	161 (51.1%)	0.38 (0.21–0.66, p<0.001)	0.44 (0.21–0.91, p=0.027)
HER2	HER2-zero	116 (36.8%)		
	HER2-low	199 (63.2%)	0.69 (0.41–1.16, p=0.160)	1.02 (0.58–1.80, p=0.944)
Ki67	Mean ± SD	34.0 ± 20.8	1.02 (1.00–1.03, p=0.011)	1.01 (1.00–1.03, p=0.050)
δKi67	Mean ± SD	0.6 ± 0.5	1.32 (0.77–2.26, p=0.318)	1.56 (0.84–2.88, p=0.156)
Cohort 3			HR (95% CI, P-value), Univariate analysis	HR (95% CI, P-value), Multivariate analysis
Age	Mean ± SD	47.1 ± 9.1	1.02 (0.94–1.11, p=0.635)	1.11 (1.01–1.22, p=0.029)
Histological type	Invasive ductal carcinoma	70 (97.2%)		
	Others	2 (2.8%)		0.00 (0.00–Inf, p=1.000)
T	T1+T2	34 (47.2%)		
	T3+T4	38 (52.8%)	5.47 (0.66–45.44, p=0.116)	6.51 (0.51–83.46, p=0.150)
N	N0+N1	60 (83.3%)		
	N2+N3	12 (16.7%)	8.28 (1.84–37.22, p=0.006)	20.09 (0.62–646.09, p=0.090)
ER	negative	16 (22.2%)		
	positive	56 (77.8%)	0.05 (0.01–0.45, p=0.007)	0.00 (0.00–Inf, p=0.999)
PR	negative	26 (36.1%)		
	positive	46 (63.9%)	0.11 (0.01–0.90, p=0.040)	NA
HER2	HER2-zero	18 (25.0%)		
	HER2-low	54 (75.0%)	0.43 (0.10–1.93, p=0.272)	2.61 (0.13–50.89, p=0.526)
Ki67	Mean ± SD	38.8 ± 26.0	1.03 (1.00–1.07, p=0.045)	1.02 (0.94–1.12, p=0.577)
δKi67	Mean ± SD	0.8 ± 0.4	0.47 (0.09–2.45, p=0.373)	2.65 (0.05–139.22, p=0.630)

Abbreviations: SD, standard deviation; HR, hazard ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; DFS, disease-free survival.

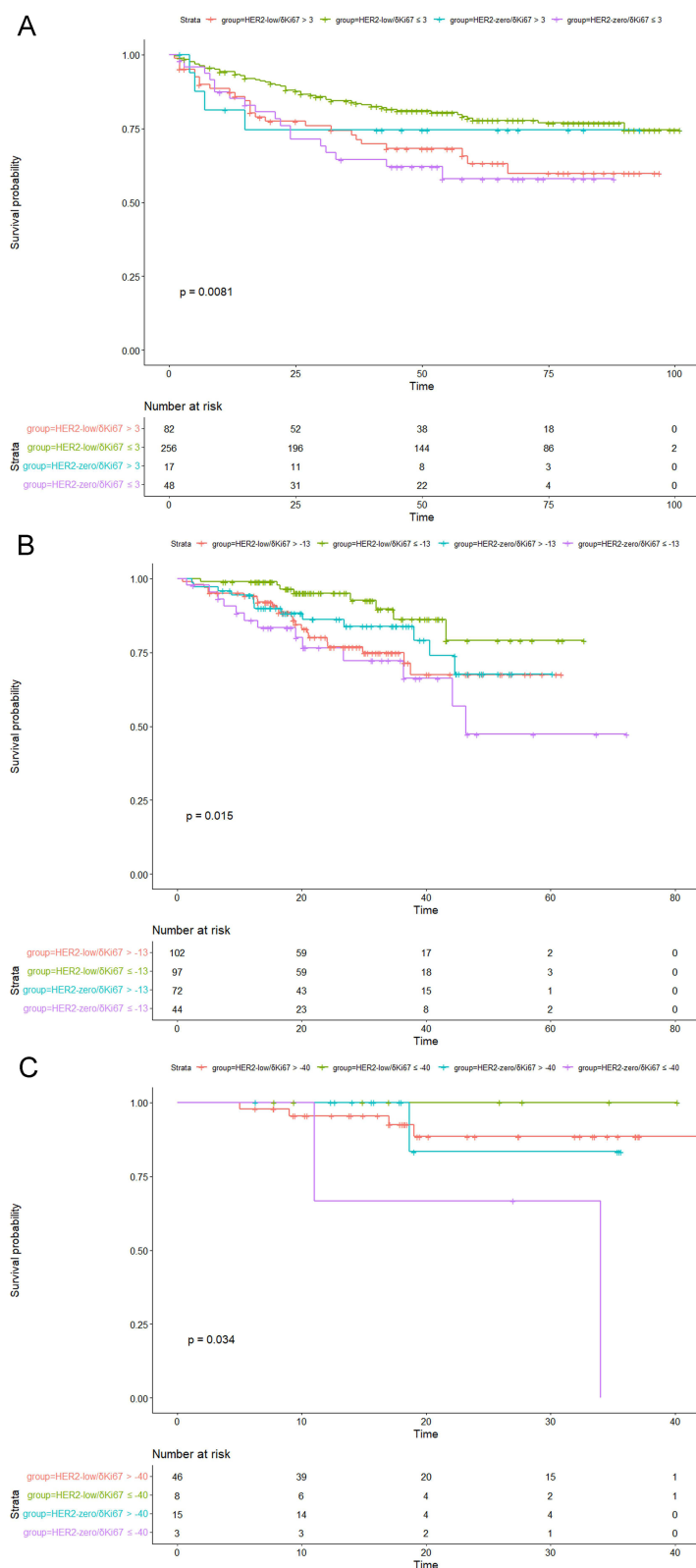


Figure 3 Comparison of DFS among patients with different HER2 status and δ Ki67 in Cohort 1 (**A**), Cohort 2 (**B**), and Cohort 3 (**C**).

criteria: 1) HER2-zero/ δ Ki67 ≤ -13 ; 2) HER2-zero/ δ Ki67 > -13 ; 3) HER2-low/ δ Ki67 ≤ -13 ; 4) HER2-low/ δ Ki67 > -13 . Consistent with cohort 1, similar results were found in cohort 2. The survival rate in group 3 was significantly higher than in both group 1 ($p = 0.02$) and group 4 ($p = 0.011$). This is illustrated by the Kaplan-Meier curves presented in Figure 3B. Additionally, in

cohort 3, patients were stratified into four groups based on the following criteria: 1) HER2-zero/ $\delta\text{Ki67} \leq -40$; 2) HER2-zero/ $\delta\text{Ki67} > -40$; 3) HER2-low/ $\delta\text{Ki67} \leq -40$; 4) HER2-low/ $\delta\text{Ki67} > -40$. It was observed that the survival rate in group 3 was significantly higher than in group 1 ($p = 0.046$). Although no significant difference was noted when comparing group 3 to both group 1 and group 4, patients in group 3 exhibited higher survival rates than those in these two groups. The Kaplan-Meier curves are depicted in Figure 3C.

Discussion

For years, researchers are dedicated to studying the differences between HER2-low and HER2-zero breast cancer and trying to explore the underlying reasons for this difference.^{19–22,27–34} Researchers have overlooked the fact that the changes in tumor markers before and after NAC may also reflect the characteristics of different subtypes of tumors. In this study, we aimed to explore the role of the changes in Ki-67 index pre and post NAC.

We first analyzed the characteristics of patients in the three cohorts. Subsequently, we determined the optimal cut-off value of δKi67 for each cohort and stratified patients within each cohort into four groups based on HER2 status and the cut-off value of δKi67 . Our findings suggested that HER2-low breast cancer patients with δKi67 below the cut-off value post NAC generally exhibited the most favorable prognosis. Specifically, we observed a significantly higher survival rate in these patients compared to those with δKi67 above the cut-off value. Additionally, their prognosis was also superior to that of HER2-zero breast cancer patients with δKi67 below the cut-off value. In Cohort 3, patients with HER2-low/ $\delta\text{Ki67} \leq -40$ also demonstrated a better prognosis compared to those with HER2-low/ $\delta\text{Ki67} > -40$, although this difference did not reach statistical significance. This may be attributed to the relatively small sample size within Cohort 3, potentially influencing the final outcome.

This finding is consistent with previous studies suggesting that a decline in Ki67 index post-chemotherapy may reflect a favorable response to treatment and a less aggressive tumor phenotype.^{10–12} Previously, Gunter et al observed that post-NAC, individuals with a higher Ki67 index faced an elevated risk of recurrence and mortality compared to those with a lower index.¹⁴ They suggested that assessing the change in Ki67 levels before and after NAC could offer valuable insights for clinical prognostication. Paula et al have highlighted a significant association between Ki67 reduction and improved DFS and OS. Their multivariate analysis revealed that the absence of Ki67 reduction significantly elevated the risk ratio for both recurrence and mortality.¹² Tan et al similarly affirmed a notable correlation between changes in Ki67 levels and prognostic outcomes, as evidenced by both univariate and multivariate analyses.¹⁵ Thereafter, we hypothesized that the alteration in Ki67 levels before and after NAC may contribute to the significant variance in prognosis observed between patients with HER2-low and HER2-zero breast cancer.

In our study, the decrease in Ki67 index post NAC may be particularly beneficial in HER2-low expression breast cancer, where the tumor cells may be significantly different from the HER2-zero tumor cells. One potential explanation for this disparity could be related to the underlying biology of HER2-low and HER2-zero breast cancers. Moreover, the decrease in Ki67 index post NAC suggested a reduction in tumor cell proliferation, which was generally associated with a better response to treatment and improved prognosis. In HER2-low tumors, this decrease in Ki67 index might reflect a more effective suppression of proliferation pathways, leading to better disease control and outcomes compared to HER2-zero tumors where the absence of HER2 expression may not confer the same sensitivity to treatment-induced changes in proliferation.

Another factor to consider is the potential presence of additional molecular alterations or tumor microenvironment characteristics that could influence treatment response and prognosis independently of HER2 status and Ki67 index. For example, differences in hormone receptor status, tumor-infiltrating lymphocytes, or genomic profiles may contribute to the observed differences in prognosis between these subgroups of patients.

Furthermore, the combination of decreased Ki67 index and HER2-low status may represent a subgroup of patients with a more indolent disease course and better prognosis following NAC. It has been reported that within the HER2-low expression subgroup, the expression level of Ki67 was notably lower compared to both HER2-zero and HER2-positive patients with breast cancer.³⁴ Hence, perhaps the interaction between Ki67 index and HER2 status highlighted the heterogeneous nature of breast cancer, emphasizing the necessity for personalized treatment strategies. By incorporating both pre- and post-NAC molecular markers into clinical decision-making, clinicians could better identify patients who are likely to derive the most benefit from NAC and tailor treatment strategies accordingly.

Our study holds significant implications for the tailored management and timely intervention of breast cancer patients. Specifically, HER2-negative individuals, notably those with triple-negative breast cancer, stand to gain substantial benefits from these findings. Nevertheless, it is important to acknowledge the limitations and challenges. First, the retrospective nature of our study imposes inherent limitations, including the potential for selection bias, incomplete data capture, and confounding variables that were not accounted for in the analysis. Second, variations among the cohorts resulted in substantial differences in the calculated optimal cut-off values of δKi67 . Consequently, our conclusions can only assert that patient prognosis improves following a certain degree of Ki67 decline. The absence of precise key data limited the guiding significance of this study for future clinical applications. Third, certain markers, such as P53, were not within the scope of this study, and we did not collect the corresponding data. Nevertheless, we plan to further investigate these and other variables based on the findings of this study in our future research.

Besides, it was also important to point out that there were significant differences in certain characteristics among the three cohorts of patients from the two clinical centers, particularly in T stage, N stage, and HER2 status, which were significantly different. These differences might be attributed to several factors. Firstly, advancements in breast cancer screening technology have enabled earlier and more accurate detection of the disease. Additionally, increased public awareness and education regarding breast cancer have encouraged more women to participate in screening programs. This heightened awareness has contributed to a rise in the detection rate of HER2-negative cases. In addition, the differences observed between various cohorts from different medical centers might be attributed to several factors: 1) Different hospitals may employ varying patient selection criteria, leading to discrepancies in the characteristics of hospitalized patients. 2) The geographical location and demographic characteristics of patients can influence the incidence and types of breast cancer. 3) Variations in follow-up duration and sample size between hospitals may affect the comparison of clinical characteristics and outcomes. 4) The experience and professional background of clinicians in different hospitals regarding the diagnosis and treatment of breast cancer may differ, which could impact patient management and treatment outcomes. 5) Patients' socioeconomic status—such as income, education level, and health insurance—may influence their healthcare-seeking behavior and treatment choices, resulting in differences in the characteristics of the various cohorts. Differences in these variables can also cause fluctuations in results.

Despite these differences, we consistently observed similar results across all three cohorts, suggesting that there was indeed a cut-off value for the δKi67 index that correlated with improved survival outcomes for HER2-low breast cancer patients below this threshold following NAC. Further research is warranted to validate our findings, especially calculate the precise cut-off value of δKi67 index, as well as to elucidate the underlying mechanisms driving the relationship between δKi67 index, HER2 status, and prognosis in breast cancer. Additionally, prospective studies are needed to evaluate the potential utility of these molecular markers as predictive biomarkers for treatment response and long-term outcomes in clinical practice.

Conclusion

Overall, our study demonstrated that HER2-low breast cancer patients with δKi67 below the cut-off value after NAC were associated with improved prognosis. The biological mechanism required further researches. Moreover, the complex interplay between HER2 status, Ki67 index, and other molecular and tumor-related factors underscores the need for further investigation to elucidate the underlying mechanisms. And incorporating comprehensive molecular profiling and tumor microenvironment assessments may help refine risk stratification and guide personalized treatment strategies for patients with HER2-low and HER2-zero breast cancer subtypes.

Data Sharing Statement

The data are available and can be requested from the corresponding author.

Ethics Approval and Informed Consent

This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the ethics committee of the First Affiliated Hospital of Chongqing Medical University (ID: No. 2020–59), as well as

approved by the ethics committee of the Chongqing University Three Gorges Hospital (ID: No.2022-129). Informed consent was waived due to the retrospective nature of the study and the use of de-identified patient data.

Consent for Publication

We confirmed that the details of any images and recordings can be published.

Author Contributions

All authors made a significant contribution to the work reported, whether in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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