Original Article

Prognostic Impact of Real-World Immunohistochemical Changes in Breast Cancer Treated with Neoadjuvant Chemotherapy

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Abstract

This real-world study of 369 breast cancer patients revealed immunohistochemical profile changes in 41.7% following neoadjuvant chemotherapy, with negative prognostic impact. Patients with IHC changes showed worse disease-free survival (HR = 1.95, *P* = .001) and overall survival (HR = 1.82, *P* = .001), particularly those converting to triple-negative phenotype (HR = 3.42, *P* < .0001). Post-treatment biomarker reassessment is essential for personalized adjuvant therapy planning.

Purpose: To evaluate the rate and types of immunohistochemical (IHC) changes after neoadjuvant chemotherapy (NAC) and their influence on disease-free survival (DFS) and overall survival (OS) in breast cancer patients, with a focus on conversions such as HR+/HER-2+ to HR-/HER-2- and their implications for treatment adjustments. **Methods:** This retrospective cohort study included 369 female patients aged 18 years or older with nonmetastatic breast cancer treated with NAC between January 2011 and January 2023. Patients who did not achieve complete pathological response were evaluated for changes in IHC profiles, including hormone receptor (HR) status, HER-2 expression, and Ki-67 index. Prognostic outcomes were assessed using Kaplan-Meier survival analysis and multivariate Cox regression models.

Abbreviations: ASCO, American Society of Clinical Oncology; BC, breast cancer; CAP, College of American Pathologists; CTCs, circulating tumor cells; ctDNA, circulating tumor DNA; DFS, disease-free survival; ER, estrogen receptor; ESMO, European Society for Medical Oncology; FFPE, formalin-fixed paraffin-embedded; FISH, fluorescence in situ hybridization; HER-2, human epidermal growth factor receptor 2; HR, hormone receptor; IHC, immunohistochemical; ISPE, International Society for Pharmacoepidemiology; ISPOR, International Society for Pharmacoeconomics and Outcomes Research; MOC, Manual de Oncologia Clínica; NAC, neoadjuvant chemotherapy; NCCN, National Comprehensive Cancer Network; OS, overall survival; pCR, pathological complete response; PR, progesterone receptor; RWD, real-world data; T-DM1, trastuzumab emtansine; TNBC, triple-negative breast cancer.

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Results: IHC changes were observed in 41.7% of patients. Among those initially classified as HR-/HER-2-, 50.9% gained HR expression, and 14.1% acquired HER-2 expression. In HR+/HER-2+ cases, 70.8% experienced a loss of HER-2 expression. Patients with HER-2+ tumors exhibited more frequent IHC changes compared to HER-2- cases ($P < .0001$). After a median follow-up of 47.7 months, local recurrences occurred in 10.3% of patients, distant metastases in 29.5%, and 25.5% had died. Patients with IHC changes demonstrated significantly worse DFS and OS ($P = .002$), with the poorest outcomes associated with conversion to HR-/HER-2- ($P < .001$). **Conclusion:** Post-NAC IHC changes are common and associated with poor prognosis, especially in patients losing HR and HER-2 expression. Monitoring IHC shifts is critical for guiding personalized treatment and improving prognostic evaluation.

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Keywords: HER-2, Hormone receptors, Immunohistochemical profile

Introduction

Breast cancer (BC) is the most prevalent malignancy in women worldwide, including Brazil,[1-3] with significant variability in biological characteristics and clinical outcomes.[4] This heterogeneity reflects the presence of distinct tumor subtypes, each with unique prognostic and therapeutic implications.[5,6] The emergence of transcriptomic studies in the early 2000s introduced an intrinsic molecular classification of BC, dividing it into 5 key subtypes—Luminal A, Luminal B, HER2-enriched, Basal-like, and Normal-like – based on specific gene expression patterns and their associated prognoses.[5,6] Treatment strategies are guided by traditional histopathological and immunohistochemical (IHC) markers, including estrogen receptor (ER), progesterone receptor (PR), HER-2 status. The proliferation marker Ki-67 is also frequently assessed in some settings and may aid in risk stratification. Although intrinsic molecular classification is based on gene expression profiling, in daily clinical practice the immunohistochemical profile (ER, PR, HER2, Ki-67) plays an important role, alongside TNM staging, in guiding initial treatment decisions.[5-7]

The impact of neoadjuvant chemotherapy (NAC) on tumor characteristics, particularly hormone receptor status and HER2 expression, remains incompletely understood, especially in resource-constrained healthcare settings. While some small cohort studies reported no significant changes or alterations in these markers,[8] others documented changes as high as 46% in ER status following treatment.[9] Similarly, findings on HER-2 status vary widely, ranging from no observed alterations[10] to up to 43% changes post-therapy.[9] These inconsistencies have resulted in a lack of consensus in clinical practice globally, particularly in settings with resource constraints where treatment decisions may have significant economic implications.

Despite the growing interest in IHC changes following NAC, significant knowledge gaps remain, especially regarding the comprehensive analysis of subtype conversions and their prognostic implications in real-world settings. Most studies have focused on changes in individual markers rather than analyzing the complex patterns of subtype transitions and their impact on clinical outcomes. Furthermore, the clinical significance of these alterations in healthcare systems with limited access to newer targeted therapies remains poorly understood. There is a critical need for comprehensive real-world data that can inform clinical practice in diverse healthcare environments, particularly in middle-income countries where resource constraints may influence treatment decisions.

The KEYNOTE-522 trial demonstrated that the addition of pembrolizumab to NAC significantly improved pathological complete response (pCR) and survival outcomes in TNBC patients, highlighting the importance of addressing residual disease.[11] Additionally, the KATHERINE trial showed that switching from trastuzumab to T-DM1 in HER-2-positive patients with residual disease after NAC resulted in improved Overall Survival (OS).[12] NAC not only reduces tumor burden and facilitates surgical interventions but also induces biological changes within the tumor microenvironment, which may be reflected in alterations to IHC profiles. These changes, such as shifts in hormonal receptor (HR) status or HER-2 expression, are increasingly recognized as potential prognostic indicators.[10,13] Evidence suggests that such alterations may directly impact patient outcomes, including Disease-free Survival (DFS) and OS.[14,15] For instance, loss of HR positivity or HER-2 expression has been associated with poorer outcomes,[16] while favorable changes, such as the acquisition of HR positivity, may correlate with improved survival.[13,15]

This study aims to evaluate the frequency and patterns of immunohistochemical changes after NAC in breast cancer patients from a real-world perspective, with particular emphasis on conversions to and from the HR-/HER2- phenotype. Additionally, we seek to determine the prognostic significance of these alterations on DFS and OS, providing insights that may guide personalized treatment strategies in settings where access to newer targeted therapies remains limited. By incorporating these findings into clinical practice, we aim to enhance prognostic evaluation and treatment planning for breast cancer patients receiving NAC, particularly in resource-constrained healthcare environments.

Methods

Study Design

This retrospective cohort study utilized real-world data (RWD) from the Hospital do Servidor Público Estadual (HSPE), a tertiary referral hospital in Brazil. The analysis was based on secondary data from the institutional database and was conducted following approval from the ethics committee. The study followed ISPE/ISPOR guidelines for exploratory real-world research.[17] Data

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from women diagnosed and treated at the hospital between January 2011 and December 2023 were included.

Patients Selection

This study included women aged ≥18 years with nonmetastatic breast cancer who underwent NAC followed by surgery (2011-2023). Eligibility required complete medical records covering diagnosis, treatment, and follow-up. Exclusion criteria included metastatic disease at diagnosis, enrollment in other clinical trials, loss to follow-up, and cases outside the study period. This ensured a homogeneous cohort for assessing NAC's impact on immunohistochemical changes and prognostic outcomes.

Treatment Protocol

NAC regimens were tailored to the pretreatment IHC profile. Patients with HR+/HER2- tumors received sequential anthracycline-cyclophosphamide followed by taxane (AC-T) regimens consisting of doxorubicin (60 mg/m²) and cyclophosphamide (600 mg/m²) every 3 weeks for 4 cycles, followed by paclitaxel (80 mg/m²) weekly for 12 weeks or docetaxel (75 mg/m²) every 3 weeks for 4 cycles.

Patients with HER2+ tumors, regardless of HR status, received the same chemotherapy regimen with the addition of trastuzumab (8 mg/kg loading dose, followed by 6 mg/kg every 3 weeks). Due to resource constraints in our healthcare system during the study period, pertuzumab was not available for neoadjuvant use. Patients with HR-/HER2- tumors additionally received carboplatin (AUC 5-6) every 3 weeks or weekly (AUC 1.5) in select cases.

During the study period (2011-2023), the AC regimen was administered every 3 weeks rather than in a dose-dense schedule. This decision reflected institutional protocol preferences and logistical constraints in the public healthcare system, where supportive measures such as routine use of granulocyte-colony stimulating factor (G-CSF) were not always readily available. As such, the conventional schedule was considered more feasible and widely implemented in our setting.

At our institution, treatment decisions after NAC are made based on a combination of the pre-NAC and post-NAC IHC profiles. This practice has been consistently adopted since 2011 as part of a multidisciplinary tumor board strategy. The following standardized approach was implemented:

1. For patients with HR+ disease, either pre- or post-NAC, standard endocrine therapy was prescribed according to menopausal status and risk (tamoxifen for premenopausal, aromatase inhibitors for postmenopausal) for a minimum of 5 years.[18,19]
2. Patients with HER2+ tumors, either pre- or post-NAC, completed a full year of trastuzumab therapy. Notably, patients who experienced loss of HER2 positivity after NAC still received complete anti-HER2 therapy based on their initial status, while those who gained HER2 positivity post-NAC were initiated on trastuzumab in the adjuvant setting.[20]
3. Patients with HR-/HER2- tumors post-NAC, particularly those who converted from HR+ to HR-/HER2-, received adjuvant capecitabine (1000-1250 mg/m² twice daily, days 1-14 every 21 days for 6-8 cycles) when feasible, based on emerging evidence from the CREATE-X trial.[36]
4. Extended adjuvant therapy (beyond 5 years) was considered for high-risk HR+ patients based on clinicopathological factors and treatment tolerance.[18,19]

Treatment decisions were documented in the medical records, allowing for retrospective assessment of whether therapy adjustments were made based on pre-NAC or post-NAC IHC profiles. This approach enabled us to evaluate not only the biology of IHC changes but also their influence on clinical management within a resource-constrained healthcare setting.

Pathological Complete Response Definition

Pathological complete response (pCR) was defined as the absence of residual invasive disease in the breast and lymph nodes (ypT0ypN0).[21]

Pathological and Immunohistochemical Analysis

In this retrospective study, we analyzed formalin-fixed paraffin-embedded (FFPE) tissue specimens obtained at 2 distinct timepoints: pretreatment core needle biopsies and post-NAC surgical specimens. All specimens underwent standardized processing with fixation in 10% neutral buffered formalin for 6 to 72 hours to preserve antigen integrity. Histological sections (4 μm thickness) were prepared for IHC analysis on positively charged slides. In accordance with the current WHO classification,[22] tumors previously referred to as invasive ductal carcinoma (IDC) are now categorized as invasive carcinoma of no special type (NST). In our institution, this terminology is routinely adopted in pathology reports.

IHC protocols strictly adhered to ASCO/CAP guidelines throughout the study period. Despite variations in antibody manufacturers over time due to institutional procurement practices, rigorous quality control measures were implemented to minimize inter-batch variability.[20] For each IHC run, positive and negative controls were included and evaluated before interpreting patient samples. The following primary antibodies were used: anti-ER (clone SP1), anti-PR (clone 1E2), anti-HER2 (clone 4B5), and anti-Ki-67 (clone MIB-1). Antigen retrieval was performed using either heat-induced epitope retrieval (HIER) in citrate buffer (pH 6.0) or EDTA buffer (pH 9.0), according to the manufacturer's recommendations for each antibody.

A change in IHC status was defined using the following criteria:

1. Hormone receptor (HR) status change: A shift from ≥1% positive nuclear staining to < 1% (loss), or from < 1% to ≥ 1% (gain) for either ER or PR, in accordance with current ASCO/CAP guidelines.[20]
2. HER2 status change: A shift from positive (3+ by IHC or 2+ with FISH amplification) to negative (0, 1+, or 2+ without FISH amplification), or vice versa. Specifically, for all cases with HER2 2+ immunohistochemistry, fluorescence in situ hybridization (FISH) was systematically performed in accordance with ASCO/CAP guidelines to determine gene amplification status. Only those confirmed as amplified by FISH were considered HER2-positive in our analyses. This approach ensured accurate classification of HER2 status and minimized misclassification bias.[21]

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3. Ki-67 index change: A transition across the established threshold of 14%, as per St. Gallen consensus guidelines (from < 14% to ≥ 14% or vice versa).[21, 23]

At our institution, reassessment of the immunohistochemical profile after NAC is part of routine clinical practice and has been systematically performed since 2011 for all patients who did not achieve pCR. This standard approach was not introduced specifically for this study but was already integrated into institutional protocols, allowing for consistent evaluation of IHC changes over time and enabling retrospective analysis of real-world treatment decisions based on both pre- and post-NAC profiles.

Statical Analyses

The primary aim of the study was to assess the presence or absence of changes in the immunohistochemical pattern in patients who received NAC. Patients were thus divided into groups with changes and without changes, correlating clinical data and outcomes with post-NAC changes. Chi-square tests were used for categorical variables, while Student's t-tests assessed differences between groups for normally distributed data.

To visualize shifts in molecular profiles pre- and post-NAC, a Sankey diagram was employed, providing a clear representation of subtype transitions. This helped identify major tumor profile changes following treatment.

Survival analysis included OS, defined as the time from curative surgery to death, and DFS, measured from curative surgery to the first recurrence (local, regional, or systemic) or death. Kaplan-Meier curves with log-rank tests assessed survival differences, while ANOVA compared mean values of quantitative factors related to OS and DFS. A *P*-value < .05 was considered statistically significant.

Ethics

This study was conducted in compliance with the ethical principles of the Declaration of Helsinki (https://www.wma.net/policies-post/wma-declaration-of-helsinki/) and was approved by the Research Ethics Committee of HSPE (CAAE 80127724.1.0000.5463) through Plataforma Brazil (https://plataformabrasil.saude.gov.br/login.jsf). Due to its retrospective nature, the requirement for informed consent was waived, ensuring the confidentiality and anonymity of patient data through record anonymization.

Results

From January 2011 to January 2023, 540 patients underwent NAC at HSPE. Of these, 171 were excluded (114 due to achieving pCR, 25 due to incomplete data, 22 due to loss to follow-up, and 10 due to participation in clinical trials), leaving 369 patients included in the study. The selection process is detailed in Figure 1.

Sociodemographic Characteristics

The clinical and pathological characteristics of the 369 patients are summarized in Table 1. The mean age was 55.7 years, with the majority being postmenopausal (67.2%) and presenting with Invasive carcinoma of no special type (NST) (94.3%). HR+ was detected in 61.5% of cases, while HER-2+ were observed in 27.1%. The distribution of HER-2 scores was as follows: 0 (6.5%), 1+ (38.5%), 2+ (41.2%), and 3+ (13.8%). Elevated Ki67 levels (> 14%) were identified in 69.5% of tumors.

The tumor subtypes were distributed as follows: HR-/HER2- (28.7%), HR-/HER2+ (9.8%), HR+/HER2- (44.2%), and HR+/HER2+ (17.3%). The majority of tumors were classified as T2 (46.1%) or T3 (30.6%), with lymph node involvement present in 70.2% of patients. Clinical staging revealed that 55.0% of patients were stage III, 42.0% stage II, and 3.0% stage I.

Comparison of Clinical and Pathological Characteristics of Patients With Changes in IHC Profiles Post-NAC

Of the 369 patients included in the study, 154 (41.7%) experienced changes in their IHC profiles after NAC, while 215 (58.3%) maintained their original profiles. Table 2 presents a comparison of patients who experienced IHC profile changes versus those who maintained their original IHC status after NAC. The mean age of patients with IHC changes was 55.3 years, compared to 55.6 years in those without changes, with no statistically significant difference ($P = .986$). Regarding menopausal status, 36.9% of patients with IHC changes were premenopausal, compared to 28.3% of those without changes. Conversely, 63.1% of postmenopausal patients exhibited IHC changes, compared to 71.7% without changes. This difference was not statistically significant ($P = .112$).

Analysis of histological subtypes revealed that 94.8% of patients in both groups (with and without IHC changes) had invasive ductal carcinoma (IDC), while invasive lobular carcinoma (ILC) was observed in 3.3% of each group. Special subtypes (metaplastic, mucinous, papillary) accounted for 1.9% in both groups, with no statistically significant difference ($P = .998$).

IHC analysis showed that 43.5% of patients with changes were HR+, compared to 74.4% of those without changes, while 56.4% of patients with changes were HR-, compared to 25.6% without changes, demonstrating a statistically significant difference ($P < .0001$).

In terms of HER-2 status, 40.9% of patients with changes converted from HER-2+ to HER-2-, compared to 82.8% of patients who maintained HER-2+. Among those who converted to HER-2+, 59.1% were initially HER-2-, compared to 17.2% who remained HER-2-, with a significant difference ($P < .0001$).

HER-2 scores also varied significantly ($P = .002$). In the group with changes, 3.2% had a score of 0, 35.1% had 1+, 40.9% had 2+, and 20.8% had 3+. In the group without changes, 8.8% had a score of 0, 40.9% had 1+, 41.4% had 2+, and 8.8% had 3+.

Ki67 analysis revealed that 27.9% of patients with IHC changes had Ki67 levels ≤ 14%, compared to 31.6% of those without changes. Conversely, 72.1% of patients with changes had Ki67 > 14%, compared to 68.4% of patients without changes. This difference was not statistically significant ($P = .515$).

Significant differences were observed in the distribution of IHC subtypes ($P < .001$). Among patients with changes, 44.8% were HR-/HER-2-, 11.7% HR-/HER-2+, 14.3% HR+/HER-2-, and 29.2% HR+/HER2+. In contrast, among those without changes, 17.2% were HR-/HER-2-, 8.4% HR-/HER-2+, 65.2% HR+/HER-2-, and 9.3% HR+/HER-2+.

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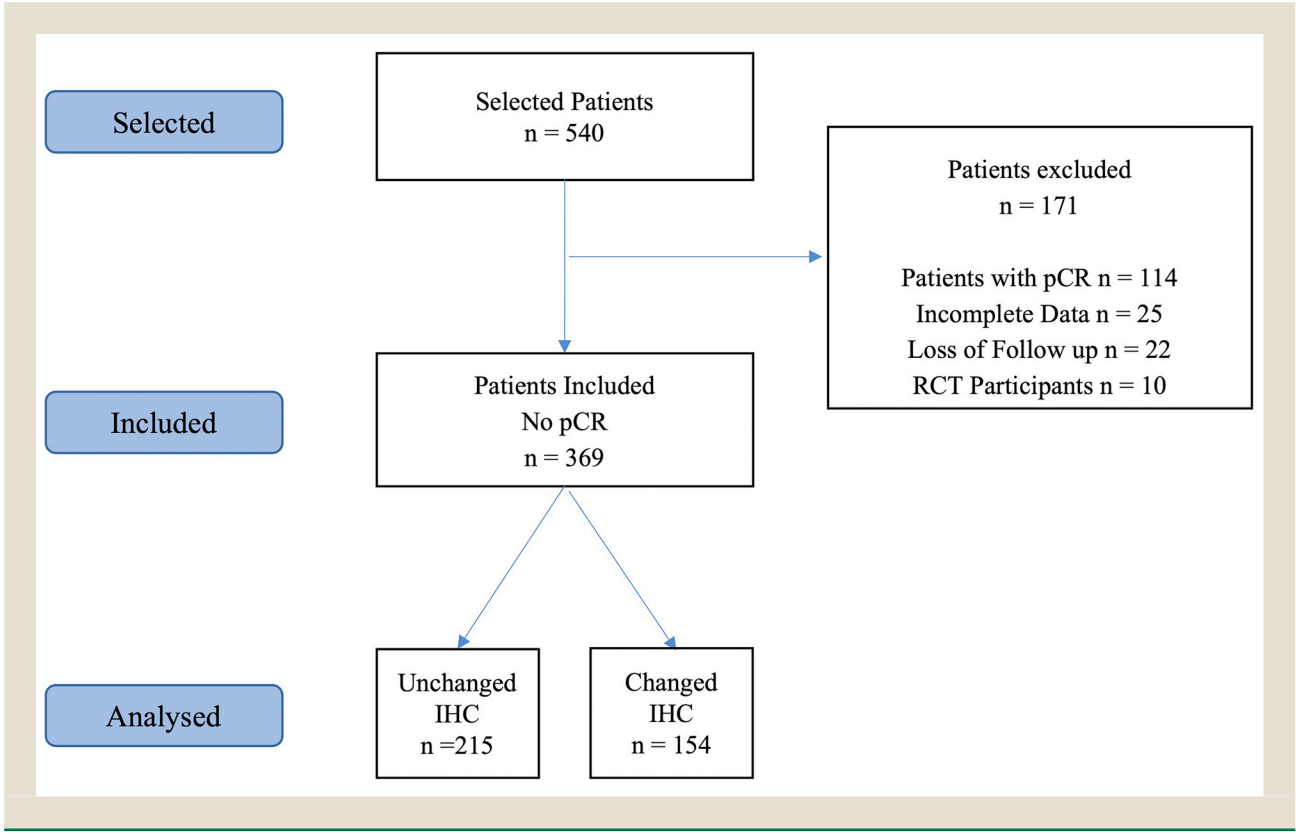
Figure 1 Flowchart with included patients. Abbreviations: IHC = immunohistochemical, pCR = pathological complete response; RCT = randomize clinical trial.



Tumor size (cT) showed no significant differences between the groups ($P = .315$). Among patients with IHC changes, 0.6% were T0, 4.5% T1, 50.0% T2, 26.6% T3, and 18.2% T4, compared to 0.0%, 7.0%, 53.3%, 33.5%, and 16.2%, respectively, in patients without changes.

Lymph node involvement (cN) also showed no significant differences ($P = .617$). Among patients with IHC changes, 33.1% were N0, 32.5% N1, 32.5% N2, and 1.9% N3, compared to 27.4%, 34.9%, 34.9%, and 3.3% in patients without changes.

Similarly, clinical staging (TNM) did not differ significantly between groups ($P = .304$). Patients with IHC changes were distributed as follows: 1.9% stage IA, 22.7% stage IIA, 24.7% stage IIB, 31.2% stage IIIA, 17.5% stage IIIB, and 1.9% stage IIIC. This is compared to 3.7%, 15.8%, 22.3%, 39.5%, 15.3%, and 3.3%, respectively, among patients without IHC changes. The detailed data are presented in Table 2.

Additionally, exploratory interaction analyses were conducted to assess whether age or clinical stage modified the association between IHC changes and outcomes. No statistically significant interactions were observed for age ($P = .28$ for OS, $P = .33$ for DFS) or disease stage ($P = .41$ for OS, $P = .38$ for DFS). These findings suggest that the negative prognostic impact of IHC changes remained consistent across different age groups and stages. Detailed results are presented in Supplemental Table 1.

Comparison of Pre- and Post-NAC Immunohistochemical Changes

Significant shifts in immunohistochemical (IHC) profiles were observed following neoadjuvant chemotherapy (NAC), indicating dynamic changes in tumor biology in response to systemic treatment ($P < .0001$). Among patients initially classified as HR-/HER2-, a considerable proportion transitioned to HR+/HER2- (14.6%) and HR-/HER2+ (4.1%). Of those originally HR-/HER2+, 4.9% retained this phenotype, while 1.9% converted to HR+/HER2+. In the HR+/HER2- group, 38.2% maintained the same profile post-treatment, and no cases shifted to HER2-positive subtypes. For patients initially categorized as HR+/HER2+, 12.0% became HR+/HER2-, while 5.1% retained the original profile.

Changes in individual biomarker expression were also statistically significant. ER expression showed conversion from negative to positive in 24.1% of cases, while 14.0% of ER-positive tumors became negative ($P < .001$). For PR, 40.7% of PR-negative tumors converted to positive, and 37.4% of PR-positive tumors lost expression ($P < .001$). HER2 status changed in 18.1% of HER2-negative cases, which became positive post-NAC, whereas 25.4% of HER2-positive tumors were reclassified as negative ($P < .001$).

These findings, summarized in Table 3 and illustrated in Figure 2, highlight the importance of reassessing IHC profiles after NAC to guide subsequent therapeutic decisions and improve prognostic accuracy.

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Table 1 Clinical and Pathological Characteristics of Patients Before NAC

Characteristics	n = 369	%	*P*-Value[a]
Age (mean years [SD])	55.7 [2.5]	-	-
Status menopausal			
Premenopausal	121	32.8	.997
Post-menopausal	248	67.2	
Histological type			
Invasive carcinoma of no special type (NST)	348	94.3	.375
Invasive lobular carcinoma (ILC)	12	3.2	
Special subtypes (metaplastic, mucinous, papillary)	9	2.5	
Immunohistochemistry			
HR+	227	61.5	.995
HR-	142	38.5	
HER-2+	100	27.1	.999
HER-2-	269	72.9	
HER-2 score			
0	24	6.5	.999
1+	142	38.5	
2+	152	41.2	
3+	51	13.8	
Ki-67			
Up to 14%	111	30.6	.836
Above 14%	258	69.5	
Subtypes			
HR-/HER-2-	106	28.7	.999
HR-/HER-2+	36	9.8	
HR+/HER-2-	163	44.2	
HR+/HER-2+	64	17.3	
Tumor size (cT)			
T0	1	0.3	.999
T1	22	6.0	
T2	170	46.1	
T3	113	30.6	
T4			
Lymph node involvement (cN)			
N0	110	29.8	.999
N1	125	33.9	
N2	124	33.6	
N3	10	2.7	
Clinical stage (TNM)			
IA	11	3.0	.999
IIA	69	18.7	
IIB	86	23.3	
IIIA	133	36.0	
IIIB	60	16.3	
IIIC	10	2.7	

Abbreviations: HR = hormone receptor; n = sample size; N = lymph nodes; SD = standard deviation.
[a] *P*-value chi-square.

Correlation Between Immunohistochemical Profile Changes and Survival

The Kaplan-Meier analysis for overall survival demonstrated a statistically significant difference between patients with changed versus unchanged IHC status (log-rank $P = .001$). The calculated hazard ratio (HR) is approximately 1.82 (95% CI: 1.27-2.60), indicating that patients with changed IHC status have an 82% higher risk of death compared to those with unchanged status. Patients with unchanged IHC status exhibited better OS, with a mean survival of 117.8 months, compared to patients with IHC

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Table 2 Clinical and Pathological Characteristics of Patients With and Without Immunohistochemical Profile Changes After Neoadjuvant Chemotherapy

Characteristics	Immunohistochemical Change		*P*-Value
	Yes n = 154 n (%)	No n = 215 n (%)	
Age (mean years)	55.3	55.6	.986
Status Menopausal			
Premenopausal	60 (36.9)	61 (28.3)	.112
Post-menopausal	104 (63.1)	154 (71.7)	
Histological type			
Invasive carcinoma of no special type (NST)	146 (94.8)	204 (94.8)	.998
Invasive lobular carcinoma (ILC)	5 (3.3)	7 (3.3)	
Special subtypes (metaplastic, mucinous, papillary)	3 (1.9)	4 (1.9)	
Immunohistochemistry			
HR+	67 (43.5)	160 (74.5)	< .001
HR-	87 (56.5)	55 (25.5)	
HER-2+	63 (40.9)	178 (82.8)	
HER-2-	91 (59.1)	37 (17.2)	
HER-2 score			
0	5 (3.2)	19 (.,8)	.002
1+	54 (35.1)	88 (40.9)	
2+	63 (40.9)	89 (41.4)	
3+	32 (20.8)	19 (8.8)	
Ki-67			
Up to 14%	43 (27.9)	68 (31.6)	.515
Above 14%	111 (72.1)	147 (68.4)	
Subtypes			
HR-/HER-2-	69 (44.8)	37 (17,2)	< .001
HR-/HER-2+	18 (11.7)	18 (8.4)	
HR+/HER-2-	22 (14.3)	140 (65.2)	
HR+/HER-2+	45 (29.2)	20 (9.3)	
Tumor size (cT)			
T0	1 (0.6)	0 (0.0)	.315
T1	7 (4.5)	15 (7.0)	
T2	77 (50.0)	93 (53.3)	
T3	41 (26.6)	72 (33.5)	
T4	28 (18.2)	35 (16.2)	
Lymph node involvement (cN)			
N0	51 (33.1)	59 (27.4)	.617
N1	50 (32.5)	75 (34.9)	
N2	50 (32.5)	74 (34.9)	
N3	3 (1.9)	7 (3.3)	
Clinical stage (TNM)			
IA	3 (1.9)	8 (3.7)	.304
IIA	35 (22.7)	34 (15.8)	
IIB	38 (24.7)	48 (22.3)	
IIIA	48 (31.2)	85 (39.5)	
IIIB	27 (17.5)	33 (15.3)	
IIIC	3 (1.9)	7 (3.3)	

Abbreviations: HR = hormone receptor; n = sample size; N = lymph nodes.
* *P*-value chi-square.

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Table 3 Comparison of IHC Profiles and Biomarkers Pre- and Post-NAC

IHC Profile Pre-NAC	IHC Profile Post-NAC				*P*-Value			
	HR-/HER-2-n (%)	HR-/HER-2+n (%)	HR+/HER-2-n (%)	HR+/HER-2+n (%)				
HR-/HER-2-	37 (10.0%)	15 (4.1%)	54 (14.6%)	0 (0.0%)	< .0001			
HR-/HER-2+	11 (3.0%)	18 (4.9%)	0 (0.0%)	7 (1.9%)				
HR+/HER-2-	22 (6.1%)	0 (0.0%)	141 (38.2%)	0 (0.0%)				
HR+/HER-2+	0 (0.0%)	0 (0.0%)	46 (12.0%)	19 (5,1%)				
IHC Biomarker		**EP**		**PR**		**HER-2**		***P*-Value**
		-	+	-	+	-	+	
ER	-	123 (75.9%)	29 (14.0%)					< .001
	+	39 (24.1%)	178 (86.0%)					
PR	-			108 (59.3%)	70 (37.4%)			< .001
	+			74 (40.7%)	117 (62.6%)			
HER-2	-					254 (81.9%)	15 (25.4%)	< .001
	+					56 (18.1%)	44 (74.6%)	

Abbreviations: ER = estrogen receptor; HR = hormonal receptor; PR = progesterone receptor.

changes who demonstrated a mean survival of 81.5 months. The median survival in the changed IHC group was 84 months, with an interquartile range (IQR) of 70 months. At 60 months (5-year follow-up), approximately 76% of patients with unchanged IHC status remained alive, compared with 64% of patients with IHC changes, highlighting the worse prognosis associated with immunohistochemical alterations.

The DFS analysis also revealed a statistically significant difference between groups (log-rank $P = .001$). The calculated HR is approximately 1.95 (95% CI: 1.33-2.85), indicating that patients with changed IHC status have a 95% higher risk of disease recurrence compared to those with unchanged status. Patients with unchanged IHC status presented better DFS, with a mean of 167.2 months, while patients with IHC changes demonstrated a mean DFS of 80.6 months. The median disease-free survival in the changed IHC group was 84 months, with an interquartile range (IQR) of 74 months. At 60 months of follow-up, approximately 76% of patients with unchanged IHC status remained DFS, compared with only 62% of patients with IHC changes, confirming the negative impact of IHC alterations on disease recurrence. These findings are illustrated in Figures 3 and 4.

IHC profiles following neoadjuvant chemotherapy NAC were associated with heterogeneous effects on OS and disease-free survival DFS across breast cancer subtypes. Among patients with HR-/HER2- tumors, alterations in IHC status were not associated with significant differences in OS (mean 70.8 vs. 64.9 months; $P = .909$) or DFS (mean 69.4 vs. 63.8 months; $P = .937$) prior to NAC, nor post-NAC (OS mean 80.9 vs. 64.9 months, $P = .337$; DFS mean 90.3 vs. 63.8 months, $P = .362$; Supplemental Figure 1). Conversely, in patients with HR-/HER2+ tumors, IHC changes were associated with significantly inferior outcomes after NAC, with a mean OS of 37.4 months versus 99.8 months ($P = .003$) and a mean DFS of 36.6 months versus 99.0 months ($P = .006$; Supplemental Figure 2), although pre-NAC differences were not statistically significant (OS mean 90.9 vs. 99.8 months, $P = .050$; DFS mean 90.4 vs. 99.0 months, $P = .099$).

In the HR+/HER2- subgroup, changes in IHC profile correlated with significantly reduced DFS both pre-NAC (mean 80.3 vs. 172.7 months; $P = .004$) and post-NAC (mean 81.5 vs. 116.5 months; $P = .006$; Supplemental Figure 3), although OS was not significantly different (pre-NAC OS mean 83.2 vs. 116.4 months, $P = .104$; post-NAC OS mean 81.5 vs. 72.7 months, $P = .075$). Among patients with HR+/HER2+ tumors, no statistically significant differences in OS or DFS were observed according to IHC stability (all $P > .2$; Supplemental Figure 4). These findings highlight the prognostic relevance of molecular subtype migration after NAC, particularly in HR-/HER2+ and HR+/HER2- tumors, where alterations in IHC profiles were associated with adverse long-term outcomes. Detailed Kaplan-Meier survival curves stratified by subtype and IHC profile stability are available in the Supplementary Material.

Multivariate Analysis of Prognostic Factors

To determine whether IHC changes represent an independent prognostic factor, we performed multivariate Cox regression analysis incorporating established clinicopathological variables.

Notably, the conversion to HR-/HER2- phenotype exhibited the strongest negative prognostic impact (HR 3.42, 95% CI 1.87-6.25, $P < .0001$ for OS), followed by loss of HR expression (HR 2.78, 95% CI 1.54-5.02, $P = .0007$) and loss of HER2 expression (HR 2.13, 95% CI 1.22-3.71, $P = .008$). These findings confirm that specific patterns of biomarker conversion independently influence survival outcomes beyond traditional prognostic factors (Figure 5).

Discussion

This study provides a detailed analysis of IHC profile changes after NAC and their possible prognostic implications in breast cancer patients. In a meta-analysis that included 2847 patients from

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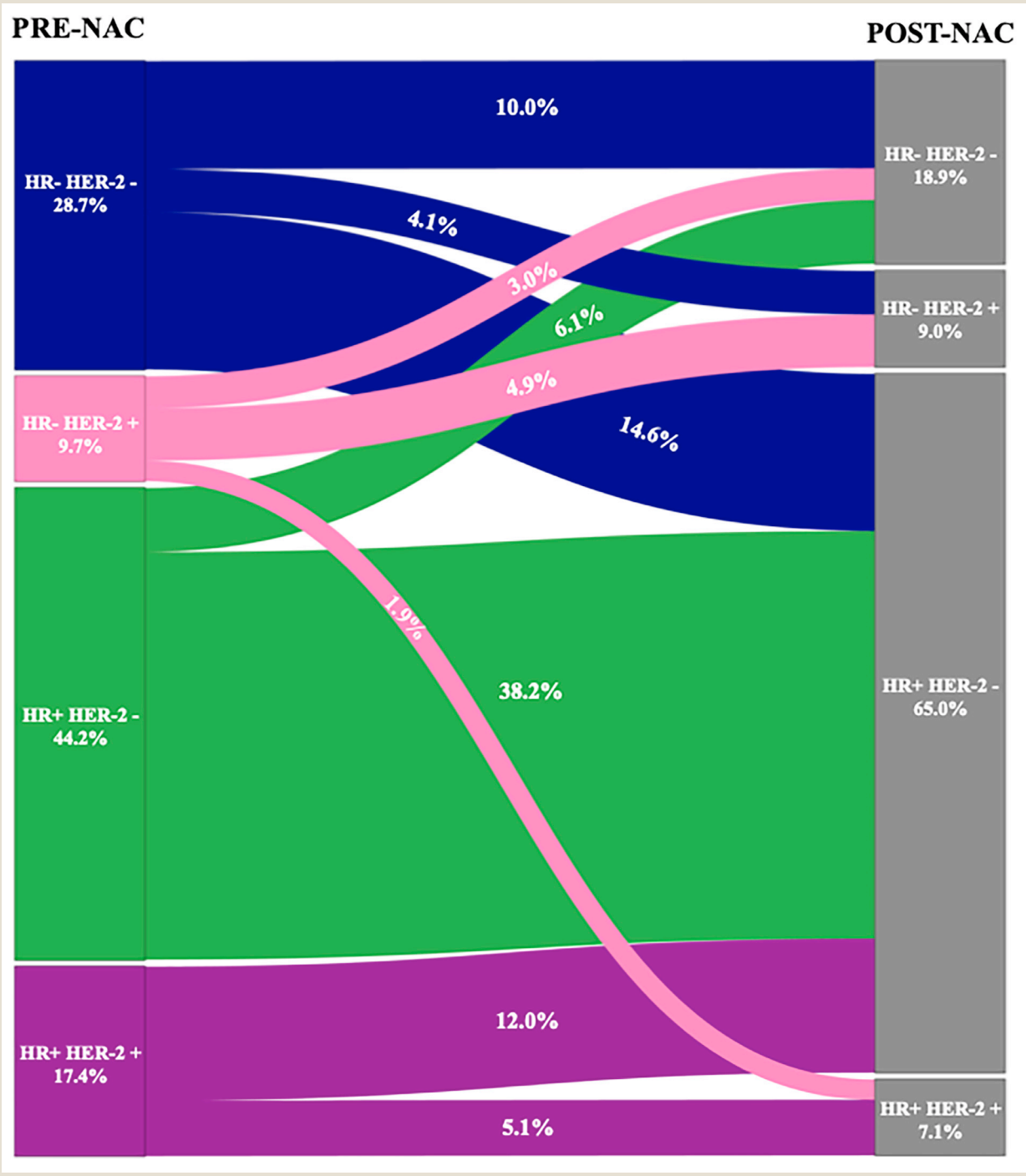
Figure 2 Sankey diagram representing IHC profile changes pre- and post-NAC. Abbreviations: NAC = neoadajuvant chemotherapy.



8 studies, Li et al. also noted an association between biomarker profile change and worse clinical outcomes.[24] The findings underscore the dynamic and complex nature of breast tumor biology under systemic treatment, with important implications for prognosis and therapeutic management. Our results reveal that 41.7% of patients exhibited IHC changes after NAC, suggesting a possible impact of tumor heterogeneity and treatment-related modulation of biomarker expression. These IHC shifts may reflect true biological changes, but also underscore the relevance of sampling variability and clonal selection within heterogeneous tumors These changes

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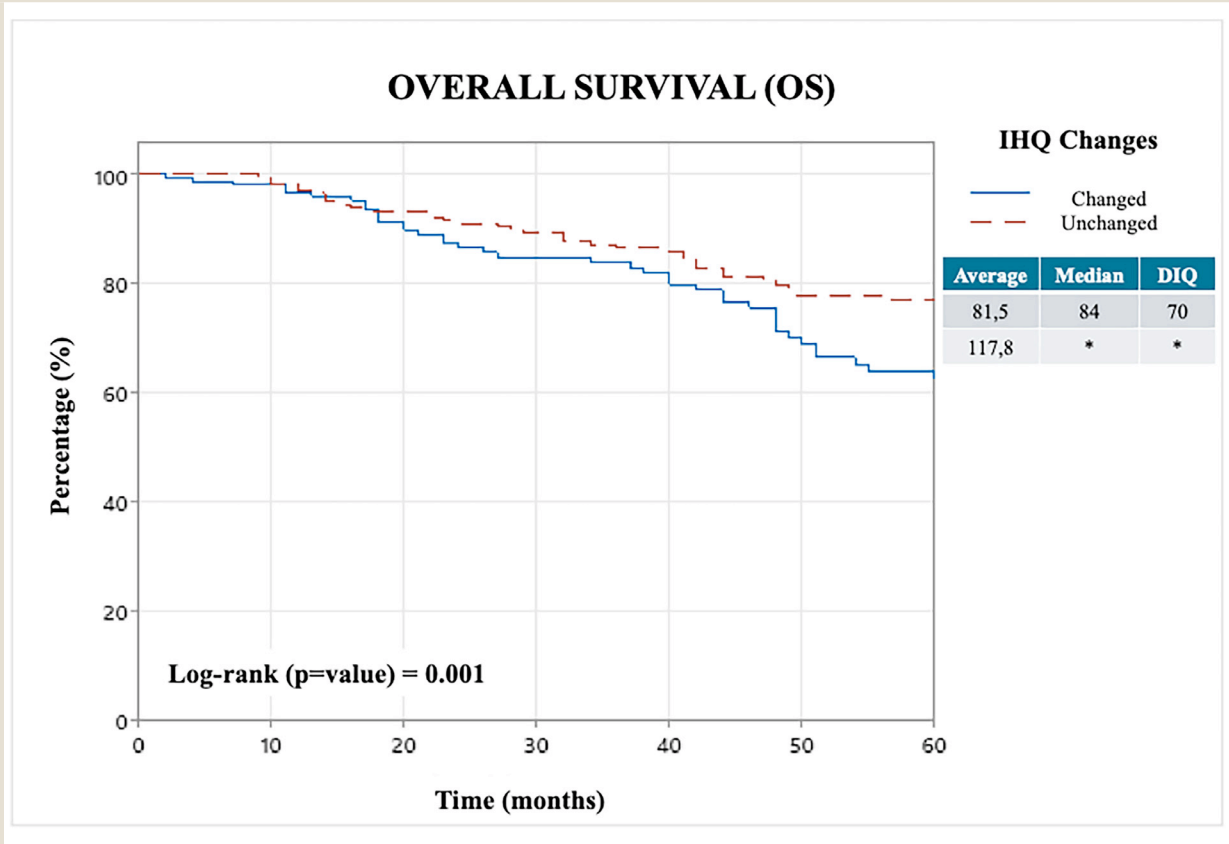
Figure 3 Impact of IHC profile changes on overall survival.



were most pronounced in HR and HER2 statuses, supporting the role of NAC in both tumor downstaging and molecular reprogramming. Specifically, 56.4% of patients with IHC changes became HR-negative post-NAC, compared to only 25.6% in the unchanged group, while 40.9% experienced HER2 status conversions. These observations align with studies reporting HR losses between 28.8% and 63% and significant HER2 alterations post-therapy.[25-27]

A notable finding was the transition of 43.5% of initially HR-positive tumors to HR-negative status in patients with IHC changes. Chen et al. similarly reported HR-positive to HR-negative conversions in 15.2% of their cohort.[10] These shifts can be attributed to several biological mechanisms, including intratumoral heterogeneity and the selective targeting of chemosensitive tumor cells, leaving behind resistant clones with altered biomarker profiles.[25] The concept of tumor heterogeneity highlights the coexistence of multiple cellular subpopulations within a single tumor, each with distinct phenotypic and molecular characteristics. As NAC targets the more proliferative and chemosensitive clones, less responsive subpopulations—often associated with aggressive features—may dominate the residual disease, leading to the observed IHC changes.[26,28] The study also demonstrated significant shifts across IHC subtypes, reinforcing the necessity of reassessing residual disease post-NAC to guide subsequent therapy decisions. For example, 31.4% of HR+/HER2- tumors lost HR expression and transitioned to HR-/HER2- while 26.9% of HER2-positive tumors gained HR expression, transitioning to HR+/HER2+. Within the HR-/HER2- group, 45.5% acquired HER2 positivity, and 22.5% gained HR expression. Lim et al.[29] similarly observed substantial subtype migrations, including HR+/HER2- tumors converting to HR-/HER2- (10.3%) and HR-/HER2- tumors gaining HR positivity (34.6%). Patients with post-NAC IHC changes had significantly worse outcomes, as illustrated by Kaplan-Meier survival curves. Among the 94 patients who died, 33.8% had IHC changes compared to 19.5% in the unchanged group ($P = .002$). This finding is consistent with previous studies highlighting the association of biomarker discordance, such as HR loss or HER2 conversion, with poorer survival outcomes.[30] However, favorable changes, such as HR-to-HR+ conversions, were linked to improved OS and DFS, emphasizing the dual prognostic nature of these shifts.[28,31,32] Managing patients with IHC changes is challenging due to inconsistent guidelines. For example, newly acquired HER2 positivity often prompts the initiation of anti-HER2 therapy, whereas the loss of HER2 expression presents a more complex scenario. Although some guidelines recommend continuing anti-HER2 agents to address residual heterogeneity, robust evidence supporting this strategy is limited.[31,33-35] The KATHERINE trial has demonstrated the OS

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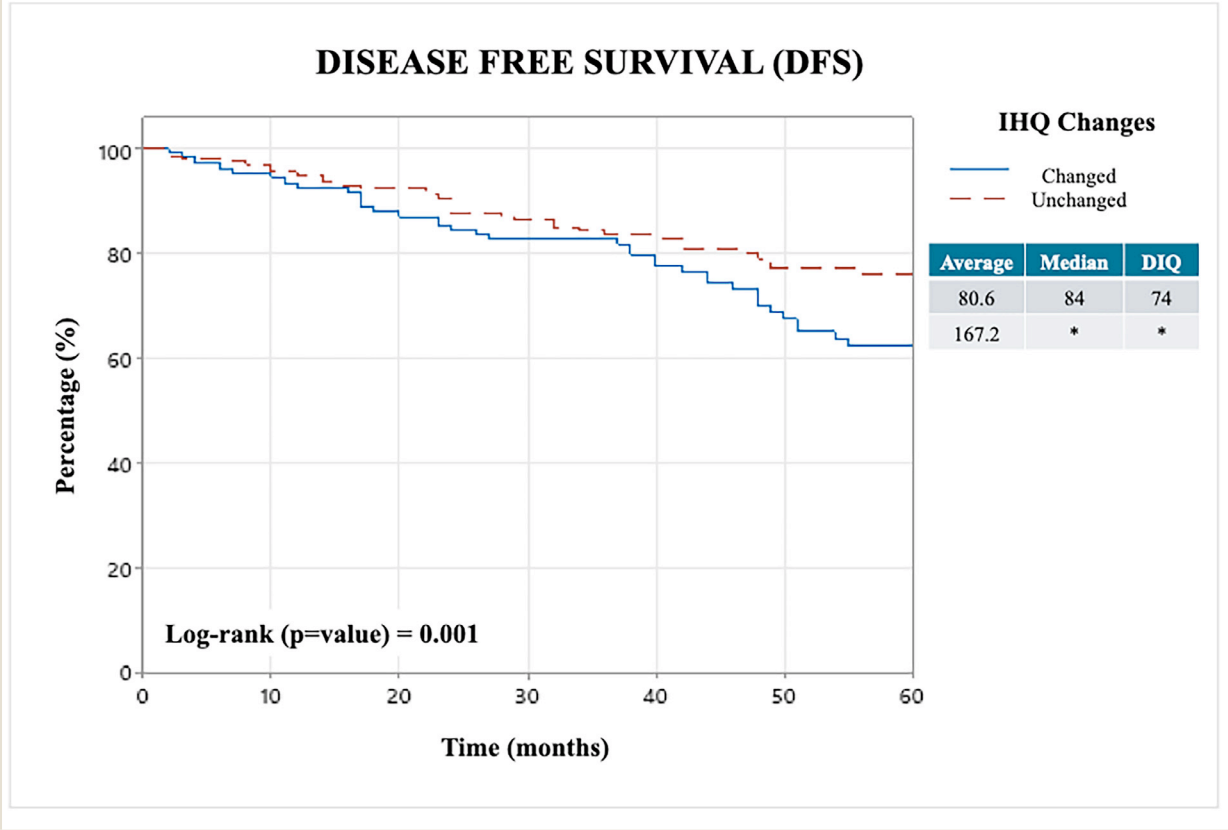
Figure 4 Impact of IHC profile changes on disease-free survival.



benefit of adjuvant T-DM1 in HER2-positive patients with residual disease after NAC, but its applicability in cases of HER2 loss remains uncertain.[12] Further studies are needed to determine whether HER2-targeted therapies should be continued in patients with post-NAC HER2 conversion.

However, recent findings from a sub-analysis of the KATHERINE trial have demonstrated that adjuvant T-DM1 remains effective even in patients whose residual disease was HER2-negative after neoadjuvant therapy. This evidence supports the continuation of HER2-targeted therapy based on the initial HER2 status, regardless of loss of HER2 expression post-NAC, and reinforces the rationale for maintaining treatment in the presence of potential intratumoral heterogeneity.[35]

Similarly, while patients transitioning to HR-/HER2- typically receive capecitabine in the adjuvant setting, the optimal management of HR-negative conversions is unclear. Our study underscores the need for tailored therapeutic strategies. Patients with HER2-negative tumors converting to HER2-positive were treated with Trastuzumab as the treatment could be beneficial in addressing residual microscopic disease and managing intratumoral diversity,[12] while those shifting to HR-/HER2- received capecitabine[36] and continued endocrine therapy when appropriate. The higher conversion rate observed in our study (43.5%) may reflect differences in patient selection, NAC regimens, or IHC evaluation criteria.

Emerging technologies, such as liquid biopsies, offer an opportunity to capture IHC-related changes dynamically and noninvasively by analyzing circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and other biomarkers shed into the bloodstream. Liquid biopsy is particularly useful for detecting intratumoral heterogeneity and identifying minimal residual disease, which may not be fully detected through conventional tissue biopsy.[37-39] For example, a study by Ignatiadis et al. demonstrated the utility of ctDNA in predicting relapse after NAC and tracking molecular changes longitudinally.[40] By complementing traditional IHC assessments, liquid biopsy could allow oncologists to monitor evolving biomarker profiles and tailor therapies more precisely during the adjuvant phase. This real-time monitoring has the potential to detect early signs of resistance, enabling clinicians to adapt therapeutic regimens before clinical recurrence, thereby improving patient outcomes.

Despite these individualized approaches, major clinical guidelines, including NCCN, ESMO, and MOC, lack clear recommendations for managing post-NAC IHC changes.[41-43] This gap highlights the critical need for prospective clinical trials to establish evidence-based guidelines.

This lack of clear guidance underscores the critical need for robust clinical studies to address these uncertainties and provide evidence-based strategies to optimize adjuvant treatment in patients experi-

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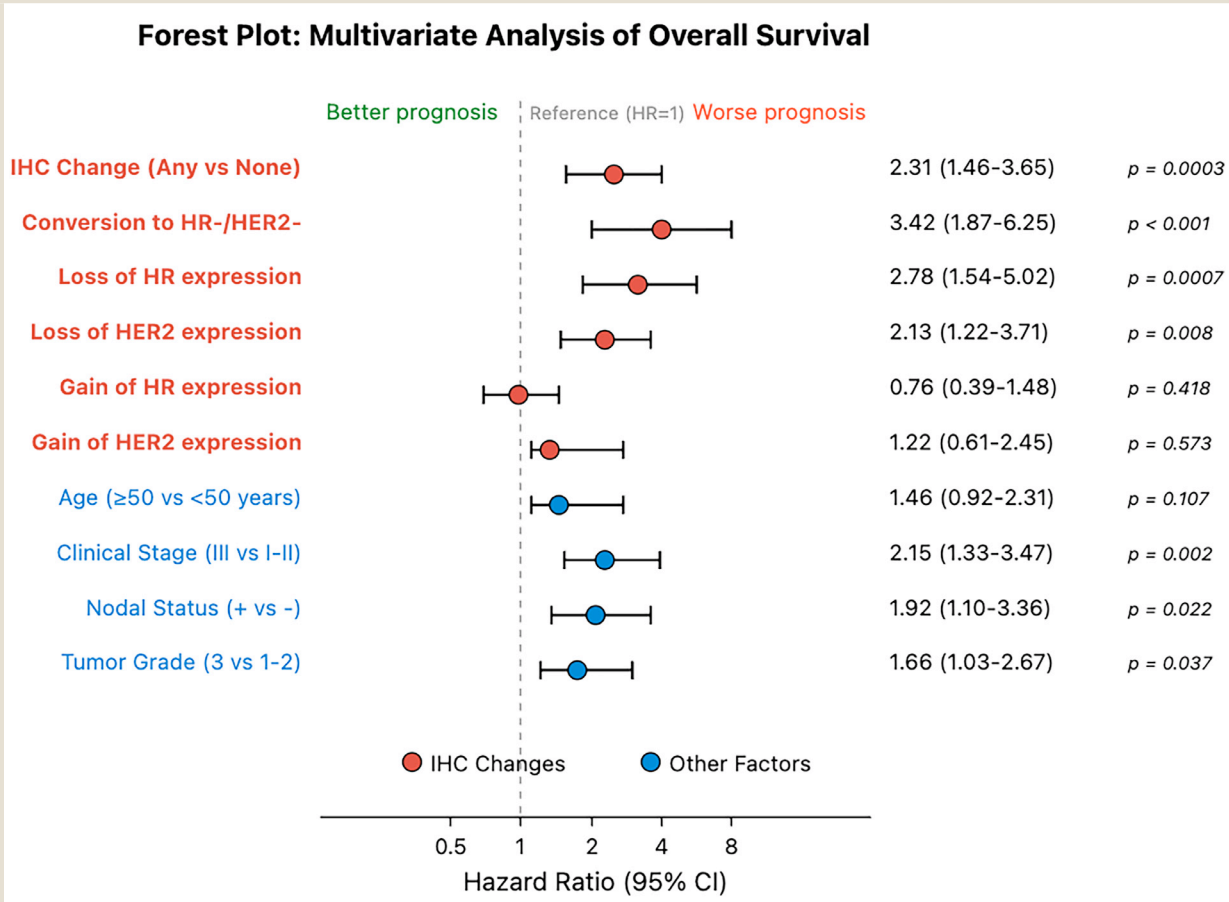
Figure 5 Forest plot multivariate analysis of prognostic factors.



encing IHC profile changes following NAC. These studies would help bridge the gap between observed clinical practices and guideline recommendations, ensuring that patient management was both standardized and individualized.

Although RWD provide valuable insights into clinical outcomes outside controlled experimental settings, several limitations should be acknowledged. Unlike clinical trials, RWD are subject to heterogeneity in treatment protocols, variations in diagnostic methods, and inconsistent follow-up durations, all of which may introduce bias and affect the generalizability of findings. The retrospective nature of this study may have introduced selection bias, particularly due to the exclusion of patients with incomplete data or loss to follow-up. Additionally, its design limits the ability to establish causal relationships between IHC changes and survival outcomes. Prospective validation is crucial to determine whether these biomarker shifts are independent prognostic factors or merely surrogate markers of tumor response. Additionally, the single-center nature of the study limits generalizability to broader populations. Variability in IHC assessment, including differences in sampling techniques and antibody brands, may have influenced biomarker consistency. Moreover, the relatively short follow-up period may not fully capture late recurrences or long-term outcomes. Future research should prioritize prospective, multicenter studies to validate these findings and investigate the mechanisms driving IHC changes. Clinical trials assessing tailored adjuvant therapies based on post-NAC biomarker shifts are essential to bridge the gap between clinical practices and guideline recommendations.

Our findings reinforce the prognostic value of post-NAC IHC shifts and underscore the urgent need for standardized therapeutic strategies in this setting. These alterations were associated with poorer survival outcomes, highlighting their potential as predictive markers of treatment response. Future prospective trials are essential to determine whether adapting treatment based on IHC changes can improve patient outcomes, bridging the gap between clinical observations and evidence-based guidelines. Addressing these gaps through robust clinical studies may refine personalized treatment approaches and enhance long-term patient survival.

Conclusion

This study demonstrates that neoadjuvant chemotherapy induces significant IHC profile changes in 41.7% of patients with invasive breast cancer, which are associated with poorer survival outcomes and increased mortality (33.8% vs. 19.5% in patients without changes). Shifts in hormone receptor and HER2 status reflect tumor

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resistance and aggressive disease, emphasizing the need for systematic post-NAC biomarker monitoring. Tailoring therapeutic strategies based on post-treatment IHC alterations could enhance patient outcomes. Future prospective tials should evaluate the prognostic significance of these changes and determine whether treatment modifications can improve long-term survival, providing stronger evidence to refine clinical guidelines and optimize personalized care.

Clinical Practice Points

- Immunohistochemical (IHC) profile changes occur in 41.7% of breast cancer patients after neoadjuvant chemotherapy, highlighting the dynamic nature of tumor biology under treatment pressure.
- Post-NAC IHC reassessment should be standard practice, as changes in hormone receptor and HER2 status have significant prognostic implications and may guide adjuvant therapy decisions.
- Patients who convert to HR-/HER2- phenotype have the poorest prognosis (HR 3.42, $P < .0001$), suggesting these patients may benefit from more aggressive adjuvant treatment strategies, such as capecitabine.
- Loss of HR expression (HR 2.78, $P = .0007$) and loss of HER2 expression (HR 2.13, $P = .008$) are also associated with worse outcomes, warranting careful consideration in treatment planning.
- Despite losing HER2 expression after NAC, continuing anti-HER2 therapy based on initial status may be beneficial in addressing potential residual microscopic disease, although this requires further validation.
- These findings are particularly relevant in resource-constrained healthcare settings, where optimizing treatment allocation based on post-NAC biomarker status could improve patient outcomes while managing limited resources.

Disclosure

AM received honoraria from Roche, AstraZeneca, Novartis, Exact Siences, and Eli Lilly. The other authors have no conflicts of interest to declare.

CRediT authorship contribution statement

Marcelo Antonini: Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **André Mattar:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Marcelo Madeira:** Writing – review & editing, Methodology. **Letícia Xavier Félix:** Writing – review & editing, Formal analysis. **Julio Antonio Pereira de Araújo:** Writing – review & editing, Formal analysis. **Francisco Pimentel Cavalcante:** Writing – review & editing, Formal analysis. **Felipe Zerwes:** Writing – review & editing, Methodology. **Fabricio Palermo Brenelli:** Writing – review & editing, Methodology. **Antonio Luis Frasson:** Writing – review & editing. **Eduardo Camargo Millen:** Writing – review & editing, Formal analysis. **Marina Diógenes Teixeira:** Writing – review & editing, Investigation. **Larissa Chrispim de Oliveira:** Writing – review & editing, Investigation. **Marcellus do Nascimento Moreira Ramos:** Writing – review & editing, Investigation. **Gil Facina:** Writing – review & editing, Investigation. **Rogério Fenile:** Writing – review & editing, Investigation. **Henrique Lima Couto:** Writing – review & editing, Data curation. **Sabrina Monteiro Rondelo:** Writing – review & editing. **Leonardo Ribeiro Soares:** Writing – review & editing, Data curation. **Ruffo de Freitas Junior:** Writing – review & editing, Data curation. **Renata Arakelian:** Writing – review & editing, Writing – original draft. **Vitoria Rassi Mahamed Rocha:** Writing – review & editing, Writing – original draft. **Renata Montarroyos Leite:** Writing – review & editing. **Luiz Henrique Gebrim:** Writing – review & editing, Writing – original draft, Supervision.

Ethical Approval and Consent to Participate

This study was conducted in compliance with the ethical principles of the Declaration of Helsinki (https://www.wma.net/policies-post/wma-declaration-of-helsinki/) and was approved by the Research Ethics Committee of Hospital do Servidor Publico Estadual (CAAE 80127724.1.0000.5463) through Plataforma Brazil (https://plataformabrasil.saude.gov.br/login.jsf) . Due to its retrospective nature, the requirement for informed consent was waived, ensuring the confidentiality and anonymity of patient data through record anonymization.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request. Requests should be directed to Prof. Marcelo Antonini at drantonini@uol.com.br.

Data Availability

All data generated or analyzed during this study are included in this published article. Additional datasets are available from the corresponding author upon request

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Supplementary Material

Table S1 Interaction Analysis Between IHC Change and Clinical Variables

Variable	Interaction With IHC Change (OS)	Interaction With IHC Change (DFS)
Age (continuous)	HR = 1.03 (95% CI 0.97-1.09), $P = .28$	HR = 1.02 (95% CI 0.96-1.08), $P = .33$
Clinical Stage (I/II vs. III)	HR = 1.12 (95% CI 0.85-1.48), $P = .41$	HR = 1.10 (95% CI 0.83-1.45), $P = .38$

Figure S1 Overall and disease-free survival according to IHC profile stability in HR-/HER2- tumors.
Legend: Overall survival (A) and disease-free survival (B) according to IHC profile stability in HR-/HER2- tumors before and after neoadjuvant chemotherapy (NAC). Kaplan-Meier curves comparing patients with stable versus changed immunohistochemical profiles. No statistically significant differences were observed in either OS or DFS.

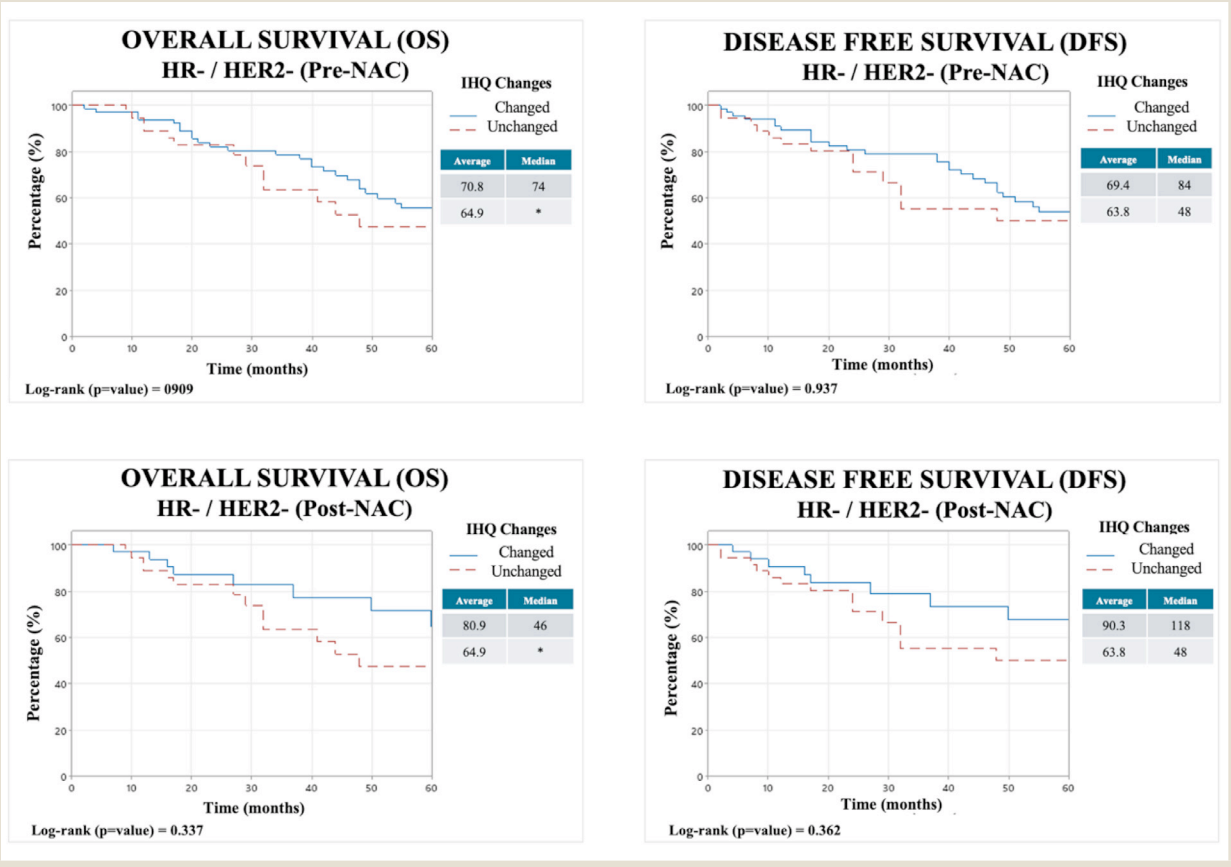

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Figure S2 Overall and disease-free survival according to IHC profile stability in HR+/HER2+ tumors.
Legend: Overall survival (A) and disease-free survival (B) according to IHC profile stability in HR+/HER2+ tumors before and after neoadjuvant chemotherapy (NAC). No statistically significant differences in OS or DFS were observed between patients with stable and changed profiles.

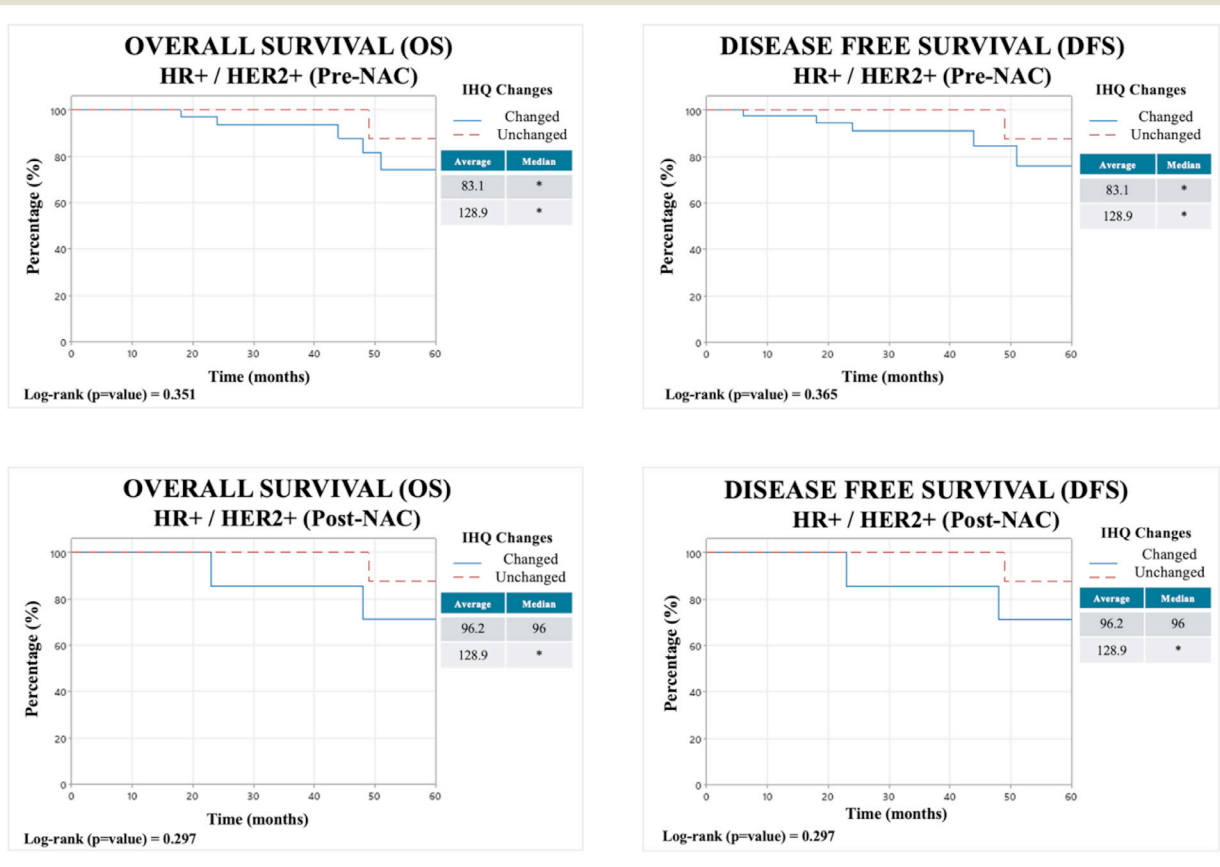

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Figure S3 Overall and disease-free survival according to IHC profile stability in HR-/HER2+ tumors.
Legend: Overall survival (A) and disease-free survival (B) according to IHC profile stability in HR-/HER2+ tumors before and after neoadjuvant chemotherapy (NAC). Patients with changed profiles post-NAC exhibited significantly worse OS ($P = .003$) and DFS ($P = .006$) compared to those with stable profiles.

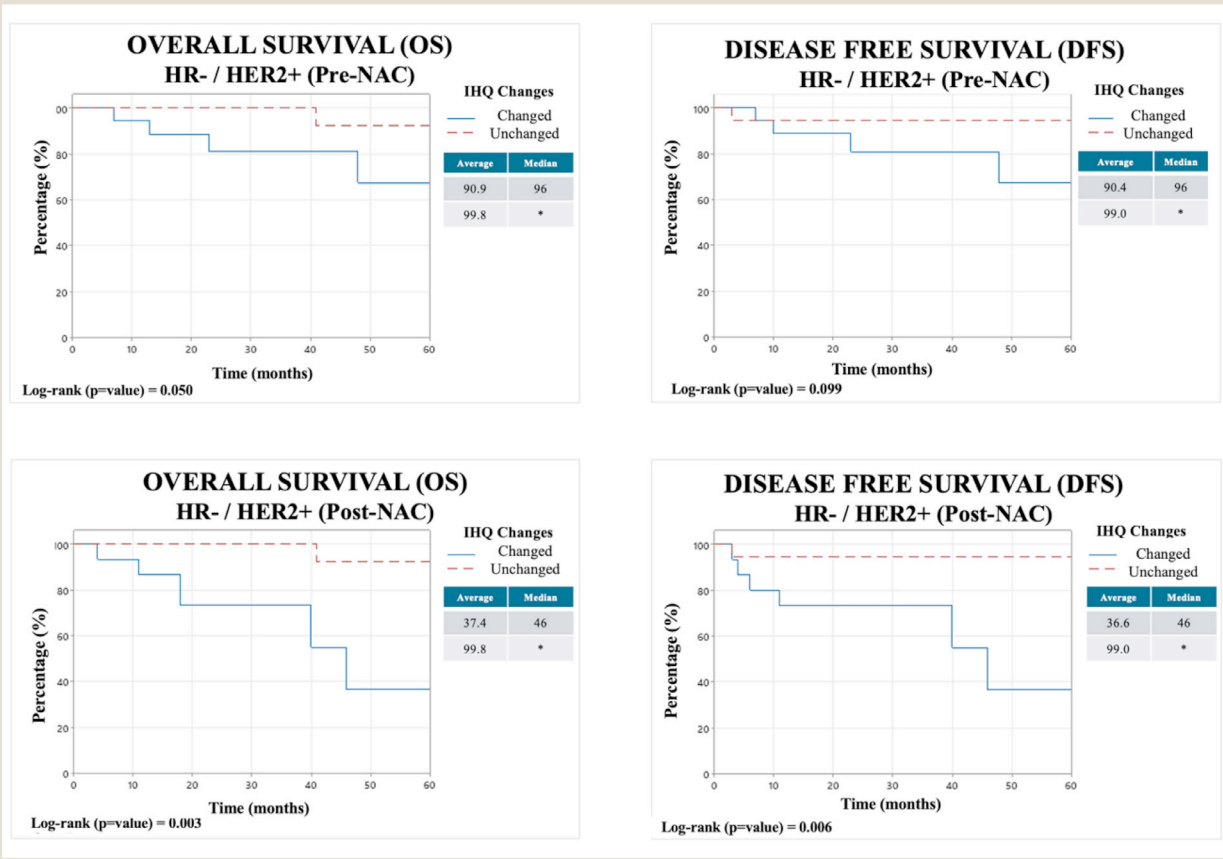

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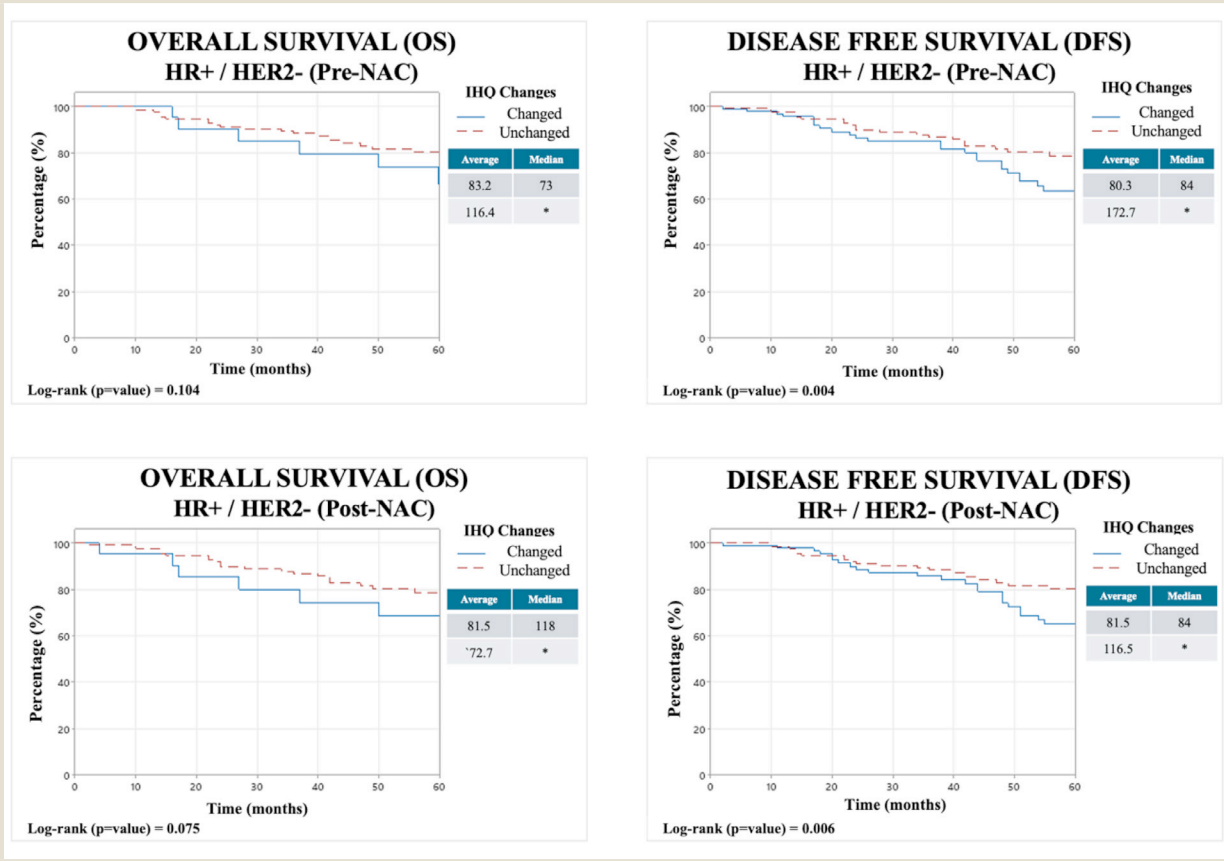
Figure S4 Overall and disease-free survival according to IHC profile stability in HR+/HER2- tumors.
Legend: Overall survival (A) and disease-free survival (B) according to IHC profile stability in HR+/HER2- tumors before and after neoadjuvant chemotherapy (NAC). Changes in IHC profile were associated with significantly reduced DFS pre-NAC ($P = .004$) and post-NAC ($P = .006$), with no significant difference observed for OS.



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