




Exploring Real-World HER2-Low Data in Early-Stage Triple-Negative Breast Cancer: Insights and Implications

Jesse Lopes da Silva ¹, Giselle de Souza Carvalho¹, Lucas Zanetti de Albuquerque¹, Fabiana Resende Rodrigues², Priscila Valverde Fernandes ², Daniel Kischinhevsky¹, Andreia Cristina de Melo ¹

¹Division of Clinical Research and Technological Development, Brazilian National Cancer Institute, Rio de Janeiro, Brazil; ²Division of Pathology, Brazilian National Cancer Institute, Rio de Janeiro, Brazil

Correspondence: Jesse Lopes da Silva, Brazilian National Cancer Institute (INCA), Clinical Research Division, 37 André Cavalcanti Street, 5th Floor, Annex Building, Rio de Janeiro, 20231-050, Brazil, Tel/Fax +55 21 32076585, Email jesse.silva@inca.gov.br

Purpose: This study aimed to compare the clinical behavior, clinicopathological and sociodemographic characteristics of patients with early-stage triple-negative breast cancer (TNBC) who belong to the HER2-low and HER2-zero subgroups.

Patients and Methods: This study involved a thorough search in the internal database of a single Brazilian institution to identify women with TNBC who underwent neoadjuvant chemotherapy (NACT) followed by curative surgery within the period from January 2010 to December 2014. HER2 analysis through immunohistochemistry (IHC) and, if required, amplification by in situ hybridization, was conducted using core biopsy samples. The study assesses outcomes of residual cancer burden (RCB), event-free survival (EFS), and overall survival (OS).

Results: A total of 170 cases were analyzed, with a mean age of 51.4 years (standard deviation, SD 11.2). The HER2 status was categorized as IHC 0, 1+, or 2+ in 80 (47.1%), 73 (42.9%), and 17 (10%) patients, respectively. No significant differences were observed in the prevalence of clinical pathological characteristics among the subgroups. The absence of significant results for clinicopathological and demographic features hindered the multivariate analysis of HER2 subgroups. Similarly, no significant differences were found in the RCB, EFS, and OS outcomes between HER2 subgroups.

Conclusion: The findings of this study suggest that, in early-stage TNBC, the clinical behavior and survival outcomes of the HER2-low subgroup may not differ significantly from those of the HER2-zero subgroup.

Keywords: HER2 status, HER2-low, biomarker, triple-negative breast cancer

Introduction

Boundaries of breast cancer subtypes definition based on Human Epidermal Growth Factor Receptor 2 (HER2) status have never been so intensely debated so far. The immunohistochemistry classification of breast cancer, which has traditionally been used for systemic treatment sequencing and surgical treatment strategies, still relies on the status of hormone receptors and HER2.^{1,2} HER2 status has conventionally been classified dichotomously, based on the expression of immunohistochemistry (IHC) and amplification by in situ hybridization (ISH), as either positive or negative.³ Triple-negative breast cancer (TNBC) is a subtype of breast cancer that accounts for approximately 15% of cases and is characterized by a lack of expression of the estrogen receptor (ER), progesterone receptor (PR) and HER2. This subtype is associated with aggressive clinical behavior and a high risk of early visceral recurrence.⁴

Recent emerging findings from the DESTINY-Breast04 trial have led the oncology community to crack the rigid dichotomous HER2 classification, boosting a great shift toward the introduction into clinical practice of the HER2-low concept, a subgroup opportunely defined by IHC 0 or 1+, or even IHC 2+ with a negative ISH assay.⁵ Notably, the study demonstrated significant clinical benefits of trastuzumab deruxtecan (T-DXd), an HER2-directed antibody-drug conjugate (ADC), in patients with heavily treated HER2-low metastatic breast cancer, including those with hormone receptor-

negative disease (traditionally known as triple-negative breast cancer).⁵ The efficacy of T-DxD can be attributed to its high cytotoxic payload and potent bystander-killing effect, leading to the release of cytotoxic payload from dead antigen-positive cells and subsequent elimination of surrounding antigen-negative tumor cells, resulting in a strong paracrine effect.⁶ These findings have paved the way for new treatment options for this previously overlooked group of patients.⁷

Currently, scarce data in the literature that reliably describe the demographic and clinical characteristics of patients with different levels of HER2 expression to better personalize the treatment of these patients is available.^{8,9} Tumor heterogeneity is a major contributor to the divergent clinical outcomes observed in TNBC, leading to varied responses to neoadjuvant chemotherapy and survival outcomes.¹⁰ Retrospective studies in early breast cancer have shown conflicting results regarding the clinical behavior of HER2-low compared to HER2-zero, as determined through genomic expression profiling tests.^{11–13} Therefore, the evaluation of the HER2-low subgroup in TNBC is of paramount importance to determine whether it represents a distinct subtype. This would enable the design of new clinical trials with targeted treatment options and the development of more accurate assays for HER2 status diagnosis.

The present study aimed to assess the demographic and clinicopathological features, along with chemosensitivity and survival outcomes, of women with locally advanced TNBC who received neoadjuvant chemotherapy (NACT) followed by curative surgery, according to the distinct levels of HER2 expression.

Materials and Methods

Study Design and Ethical Considerations

This investigation is a retrospective cohort study that adhered strictly to the guidelines of the STrengthening the Reporting of OBServational studies in Epidemiology (STROBE). It was approved by the local Institutional Review Board and conducted according to the principles of Good Clinical Practice guidelines.

Patient Selection

To be eligible for inclusion in this study, patients were required to meet specific criteria, which were as follows: a) being female and over 18 years of age; b) confirmation of the histopathological diagnosis of TNBC by the Division of Pathology of the Brazilian National Cancer Institute (DIPAT-INCA) based on the criteria outlined in the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines;^{2,14} tumors with an ER and PR score of less than 1%, as well as a HER2 score of 0/1+ or 2+ with negative ISH; c) having stage II–III breast cancer as defined by the 8th American Joint Committee on Cancer (AJCC);¹⁵ and d) receiving NACT with anthracycline-taxane-based regimens followed by surgery with curative intent at the Brazilian National Cancer Institute (INCA). Patients who had previously received antineoplastic agents or had second primary or unresectable breast tumors after neoadjuvant treatment were excluded from the study.

Immunohistochemistry

The entire tissue sections of the core biopsy samples were subjected to analysis, with immunostaining for ER (clone EP1, Dako, prediluted), PR (clone PgR636, Dako, prediluted), and HER2 (clone SP3, Cell Marque, diluted 1:500).¹⁶ Negative immunostaining scores were validated per the guidelines established by the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP). In cases where the HER2 immunostaining score was indeterminate (score 2+), ISH was performed with results that were defined as the ratio of HER2 gene amplification to the chromosome 17 enumeration probe (CEP17). Therefore, negative ISH was identified when the HER2/CEP17 ratio was < 2.0 , and the HER2 copy number signals/cell was < 4 . An HER2 immunostaining score of 0 corresponded to a HER2 IHC-negative score, whereas a HER2 immunostaining score of 1+ or 2+ with negative ISH corresponded to a HER2-low score. Ki67 was evaluated using nuclear staining with a mouse monoclonal antibody (SP6 clone, Cell Marque) at a dilution of 1:500.

Other Pathological and Clinical Variables

Data derived from electronic medical records and clinical charts were used in the analyses. The following variables were obtained: age at diagnosis, ethnicity categorized as either Caucasian or non-Caucasian as per the Brazilian Institute of

Geography and Statistics (IBGE),¹⁷ body mass index (BMI), clinical stage (II–III), response to neoadjuvant chemotherapy (NACT) as indicated by residual cancer burden (RCB), histological type, Elston histological grade (1: low grade; 2: moderate grade; 3: high grade), lymphovascular invasion (LVI), perineural invasion (PI), type of NACT (5-fluorouracil + doxorubicin + cyclophosphamide followed by paclitaxel or docetaxel [FAC-T] or doxorubicin + cyclophosphamide followed by paclitaxel or docetaxel [AC-T]) and type of surgery (radical or conservative, type of axillary approach). By established protocols, RCB scores were stratified into a standard 4-level categorical variable denoted as RCB “classes” 0, 1, 2, and 3, with an incremental increase in score corresponding to a greater residual disease burden.¹⁸

Statistical Analysis

Event-free survival (EFS) was defined as the time interval from the date of diagnosis to the earliest occurrence of disease progression, all-cause mortality, or discontinuation of treatment for initiation of complementary therapy due to inadequate response to standard neoadjuvant chemotherapy (NACT). Overall survival (OS) was calculated from the date of diagnosis until death from any cause, or until the last day of data collection. Categorical data were presented as absolute counts (n) and proportions (%), while continuous data were expressed as means with standard deviation (SD) or medians with interquartile range (IQR), following the Shapiro–Wilk test for normality. Logistic regression analysis was employed to evaluate the association between HER2 level and each variable evaluated. The Kaplan-Meier method was utilized to estimate EFS and OS for the HER2 subgroups, and compared using the Log rank test. Crude hazard ratios (HRs) were calculated for each factor using Cox proportional hazards model. The multivariate analysis would be performed by including all variables associated with response and survival outcomes at a p-value < 0.20 and selecting the most appropriate model using the Akaike Information Criterion. A p-value < 0.05 was considered statistically significant, and missing data were excluded from the analysis. Statistical analyzes were conducted using the R environment version 4.2.1.

Results

A total of 235 patients were selected from the institutional database. Following the exclusion of patients with essential missing data, primarily due to scarce or unavailable tumor samples, 170 cases of women with locally advanced TNBC met the inclusion criteria (Figure 1). The main characteristics of the patients are presented in Table 1. The HER2 status was determined by immunohistochemistry (IHC) and was identified as IHC 0 in 80 patients (47.1%), IHC 1+ in 73 patients (42.9%), and IHC 2+ in 17 patients (10%) (Figure 2). The mean age (standard deviation, SD) was 51.4 (11.2) years, with minimal variation observed between the groups.

Upon evaluating the clinicopathological variables, no noteworthy differences in the frequency distribution were discerned among subgroups (please refer to Table 1). Remarkably, individuals classified as obese, that is, those with a BMI surpassing 30, were more commonly represented in the HER2 IHC 0 group (37.5%). Furthermore, this trend was also observed in the HER2 IHC 0 group in relation to Caucasian ethnicity (50%), grade 3 tumors (73.8%), and tumors with metaplastic histology (8.8%). Concerning ethnicity, the prevalence of non-Caucasian women was slightly higher in patients with HER2 IHC 2+ (58.8%), in which there was also a higher frequency of LVI (23.5%), PNI (11.8%), and mean Ki67 expression (51.2%, SD 36.3). Regrettably, the absence of significant associations between any of the clinicopathological and demographic characteristics evaluated with the different levels of HER2 has rendered multivariate analysis unfeasible.

As presented in Table 2, there was no statistically significant difference in the treatment modality administered to patients across the three HER2 subgroups. Concerning systemic neoadjuvant therapy, the majority of patients in this study (67.6%, 115 cases) received the AC-T regimen, whereas 24.7% (42 cases) received FAC-T. The majority of patients underwent radical surgery (96.5%, 164 cases) and axillary dissection (85.9%, 146 cases).

In terms of the response to NACT, there was no significant difference observed among the HER2 subgroups. However, Residual Cancer Burden (RCB) of 0/1 showed a trend toward higher frequency in HER2 IHC 2+ (47.1%) in comparison to HER2 IHC 1+ (31.5%) and HER2 IHC 0 (25%), as demonstrated in Table 1. The median follow-up period for this study was 62.5 months (95% Confidence Interval (CI) 60.2–67.9). Univariate analysis for Event-Free Survival (EFS) revealed no significant difference among the subgroups, with the 3-year EFS estimate for HER2 IHC 0 being 64.2% (95% CI 54.3–75.8%), for HER2 IHC 1+ being 60.7% (95% CI 50.3–73.2%), and for HER2 IHC 2+ being

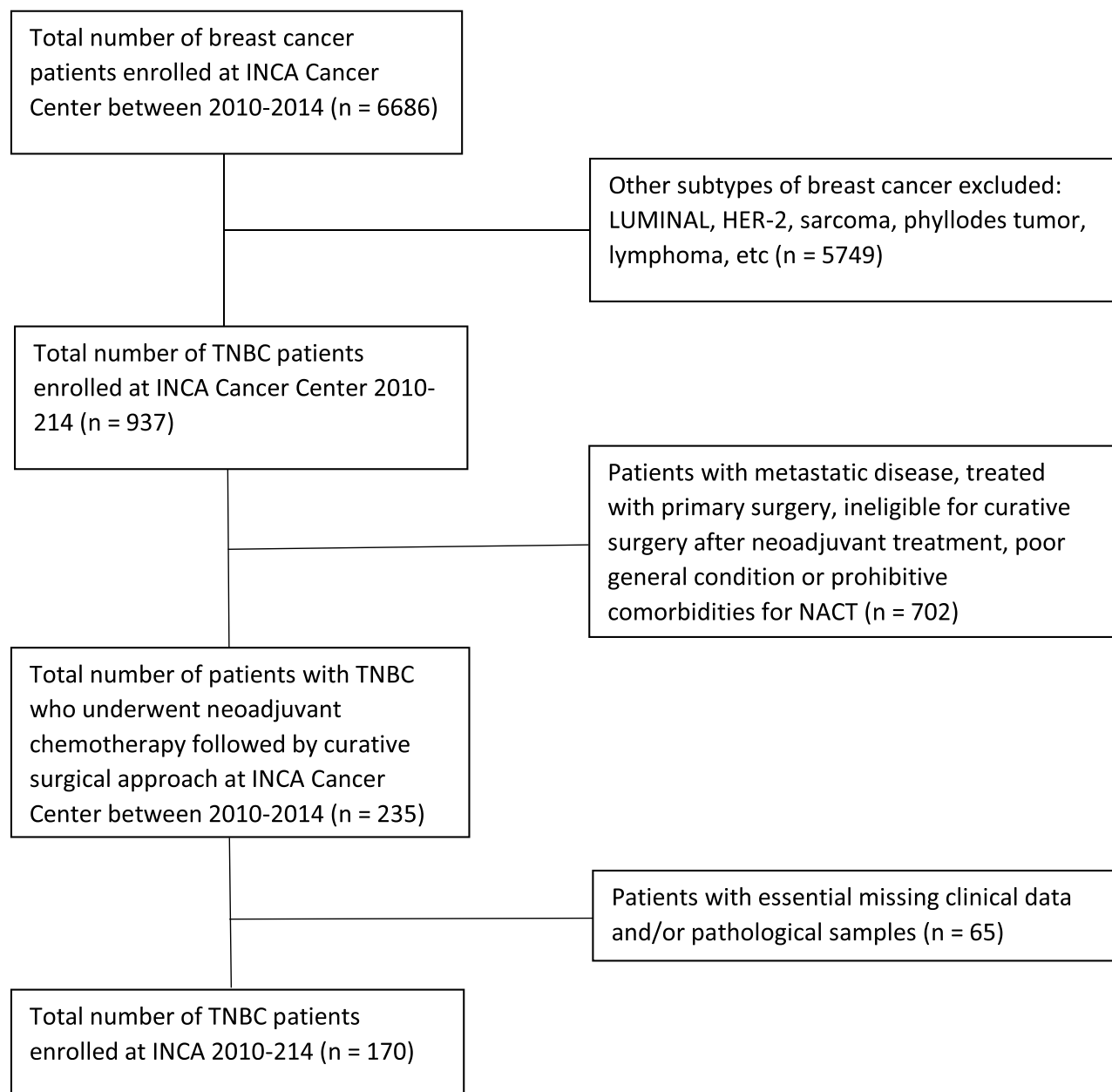


Figure 1 Study design.

58.23% (95% CI 38.7–87.5%), as shown in [Figure 3](#). Similarly, the univariate analysis for 3-year Overall Survival (OS) did not indicate any meaningful differences among the HER2 subgroups, with the 3-year OS estimate for HER2 IHC 0 being 66.5% (95% CI 56.5–78.2%), for HER2 IHC 1+ being 73.2% (95% CI 63.6–84.3%), and for HER2 IHC 2+ being 69.0% (95% CI 49.7–95.8%), as depicted in [Figure 4](#).

Discussion

The categorization of the HER2 biomarker into a binary status has been subject to significant scrutiny in light of recent clinical trials, which have demonstrated promising response rates and improved survival outcomes in patients with HER2-low tumors treated with anti-HER2 ADCs.¹⁹ This emerging evidence has led proponents to advocate for a more nuanced understanding of HER2 expression as a continuous spectrum biomarker.^{20,21} However, there remains an unmet need to comprehensively characterize the extensive tumor heterogeneity observed in the context of TNBC.²² The primary

Table 1 Demographic and Clinical-Pathological Characteristics by HER2 Status

Characteristic	HER2 Status			Total (%)	Crude p-value
	IHC 0 (%)	IHC 1+ (%)	IHC 2+ (%)		
Number of patients (%)	80 (47.1)	73 (42.9)	17 (10.0)	170	-
Mean age, years (SD)	51.7 (11.8)	51.4 (10.5)	50.0 (11.8)	51.4 (11.2)	0.849
Mean BMI kg/m ² (SD)	29.2 (5.7)	27.7 (5.8)	28.6 (7.2)	28.5 (5.9)	0.324
Stratified BMI kg/ m ² (%)					
BMI ≤ 30	48 (60.0)	54 (74.0)	11 (64.7)	113 (66.5)	0.163
BMI > 30	30 (37.5)	17 (23.3)	5 (29.4)	52 (30.6)	-
Missing	2 (2.5)	2 (2.7)	1 (5.9)	5 (2.9)	-
Race/Ethnicity					
White	40 (50.0)	33 (45.2)	7 (41.2)	80 (47.1)	0.778
Non-white	40 (50.0)	38 (52.1)	10 (58.8)	88 (51.8)	-
Missing	0 (0.0)	2 (2.7)	0 (0.0)	2 (1.2)	-
Clinical stage					
II	14 (17.5)	11 (15.1)	3 (17.6)	28 (16.5)	0.913
III	66 (82.5)	62 (84.9)	14 (82.4)	142 (83.5)	-
Tumor grade					
I	0 (0.0)	3 (4.1)	0 (0.0)	3 (1.8)	0.223
2	21 (26.2)	22 (30.1)	7 (41.2)	50 (29.4)	-
3	59 (73.8)	48 (65.8)	10 (58.8)	117 (68.8)	-
Histologic subtype					
IDC	73 (91.2)	70 (95.9)	17 (100.0)	160 (94.1)	0.264
Metaplastic carcinoma	7 (8.8)	3 (4.1)	0 (0.0)	10 (5.9)	-
LVI					
Absent	54 (67.5)	45 (61.6)	10 (58.8)	109 (64.1)	0.916
Present	17 (21.2)	16 (21.9)	4 (23.5)	37 (21.8)	-
Missing	9 (11.2)	12 (16.4)	3 (17.6)	24 (14.1)	-
PNI					
Absent	49 (61.2)	48 (65.8)	9 (52.9)	106 (62.4)	0.723
Present	9 (11.2)	6 (8.2)	2 (11.8)	17 (10.0)	-
Missing	22 (27.5)	19 (26.0)	6 (35.3)	47 (27.6)	-
Mean Ki67 expression (SD)	46.1 (31.3)	45.8 (28.4)	51.2 (36.3)	46.5 (30.5)	0.805
RCB					
0/1	20 (25.0)	23 (31.5)	8 (47.1)	51 (30.0)	0.184
2/3	60 (75.0)	50 (68.5)	9 (52.9)	119 (70.0)	-

Notes: The RCB score followed the standard four-level categorical variable (RCB “classes” 0, 1, 2, and 3).

Abbreviations: IHC, Immunohistochemistry; SD, Standard deviation; BMI, Body mass index; IDC, Invasive ductal carcinoma; LVI, Lymphovascular invasion; PNI, Perineural invasion; RCB, Residual cancer burden; SD, Standard deviation; Ki67 expression ≥40% was considered as high.

findings of the current study indicate that sociodemographic and clinicopathological factors, response rates to neoadjuvant chemotherapy and survival outcomes do not differ significantly across TNBC subgroups stratified by varying levels of HER2 expression.

The HER2 subgroups displayed a close proportional distribution, with a predominant representation of younger, non-white women, and those with poorly differentiated stage III disease. These patients also exhibited a high prevalence rate of obesity, higher Ki67 score, as well as LVI and PNI, all of which are traditional prognostic factors for poorer outcomes in breast cancer patients.^{23,24} Additionally, RCB, an outcome used to determine the volume of residual disease after NACT with well-known strong impact on patient survival, displayed similar proportions among HER2 subgroups.²⁵ That said, the pattern of response to NACT in patients with TNBC might be independent of HER2 expression status by IHC. Notably, the systemic and surgical interventions provided to the patients demonstrated a remarkable degree of consistency across the HER2 subgroups, with the majority receiving the NACT regimen AC-T, as well as a radical mastectomy and axillary dissection.

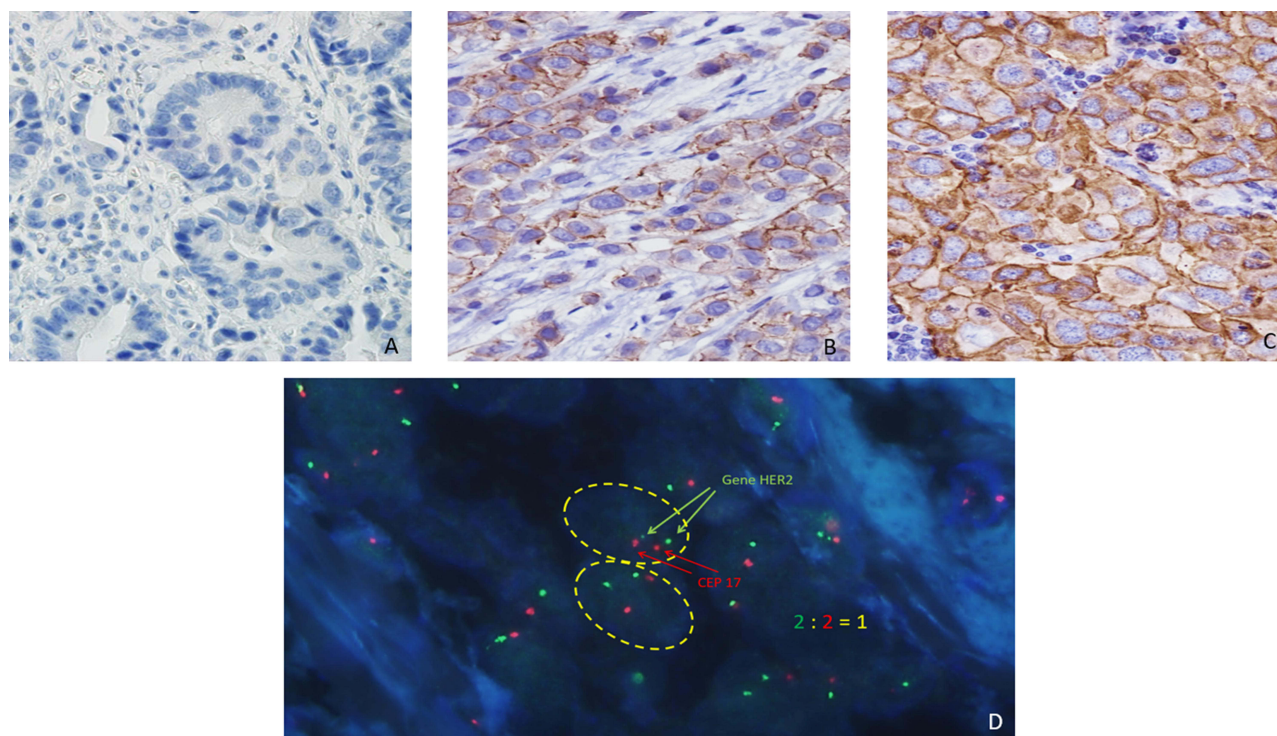


Figure 2 This figure displays exemplary images of HER2 status using Immunohistochemistry (IHC) and in situ hybridization (ISH).

Notes: Representative photomicrographs of immunohistochemistry (IHC) and in situ hybridization (ISH) staining for HER2 expression. **(A)** Negative HER2 IHC score (0) at ×40 magnification. **(B)** Low HER2 IHC score (1+) at ×40 magnification. **(C)** Intermediate HER2 IHC score (2+) at ×40 magnification. **(D)** Negative HER2 status by ISH, shown by a HER2/CEP17 ratio of 1, with red signals indicating the HER2 gene and green signals indicating the chromosome 17 enumeration probe (CEP17), at ×600 magnification.

In a large cohort study of 15,054 patients diagnosed with metastatic breast cancer without HER2 overexpression, Calbiac et al²⁶ found that patients with TNBC had a smaller proportion of the HER2-low subgroup (21%) as compared to the group, suggesting a slightly better overall survival (OS) compared to patients with HER2-zero tumors. However, there was no significant difference in progression-free survival (PFS) between these subgroups. In another pooled

Table 2 Treatment Strategies by HER2 Status

Characteristic	HER2 Status			Total (%)	Crude p-value
	IHC 0 (%)	IHC 1+ (%)	IHC 2+ (%)		
NACT regimen					
FAC	3 (3.8)	2 (2.7)	1 (5.9)	6 (3.5)	0.869
AC	1 (1.2)	0 (0.0)	0 (0.0)	1 (0.6)	-
AC-T	54 (67.5)	51 (69.9)	10 (58.8)	115 (67.6)	-
FAC-T	18 (22.5)	19 (26.0)	5 (29.4)	42 (24.7)	-
Other	4 (5.0)	1 (1.4)	1 (5.9)	6 (3.5)	-
Type of breast surgery					
Radical mastectomy	78 (97.5)	70 (95.9)	16 (94.1)	164 (96.5)	0.742
Conservative mastectomy	2 (2.5)	3 (4.1)	1 (5.9)	6 (3.5)	-
Type of axillary dissection					
Axillary lymph node dissection	71 (88.8)	60 (82.2)	15 (88.2)	146 (85.9)	0.177
Sentinel lymph node biopsy	2 (2.5)	7 (9.6)	1 (5.9)	10 (5.9)	-

Abbreviations: IHC, Immunohistochemistry; SD, Standard deviation; NACT, neoadjuvant chemotherapy; FAC, doxorubicin/cyclophosphamide/fluorouracil; AC, doxorubicin/cyclophosphamide; AC-T, doxorubicin/cyclophosphamide followed by taxane; FAC-T, doxorubicin/cyclophosphamide/fluorouracil followed by taxane.

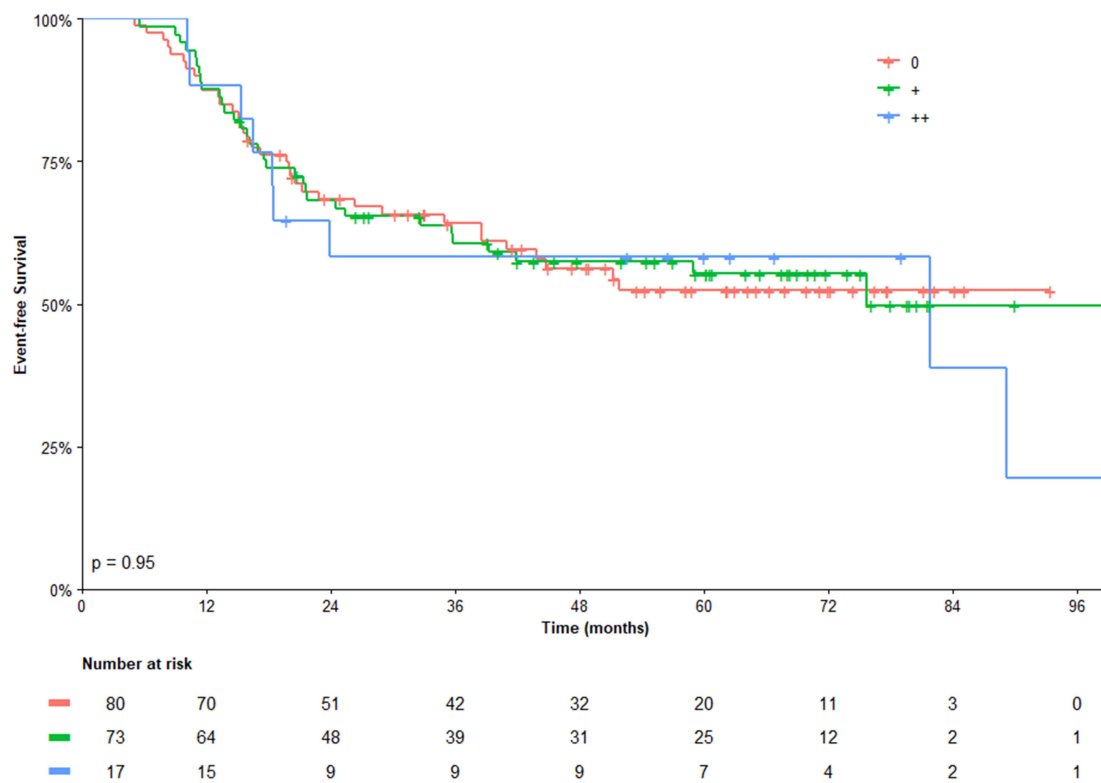


Figure 3 Kaplan-Meier estimates of event-free survival stratified by HER2 immunohistochemistry subgroups.

analysis of prospective individual patient data on 2310 women with early breast cancer, Denkert et al¹¹ reported that among patients with TNBC, those who were HER2-low (36% of patients) had no significant difference in pathologic complete response when compared to their HER2-zero counterparts. However, the HER2-low subgroup exhibited significantly higher 3-year disease-free survival and OS. Furthermore, Agostinetti et al¹² reported in their study on 74 patients with TNBC and HER2-low status, where intrinsic PAM50 subtypes were characterized, that HER2-enriched tumors were more prevalent, accounting for 13.7% of cases.

In a cohort study by Schettini et al¹³ that analyzed molecular subtypes using PAM50 in patients with metastatic breast cancer, it was found that 36% of TNBC cases were HER2-low. The study also emphasized that there were no significant differences in OS and distribution of intrinsic molecular subtypes among the HER2 subgroups and highlighted the suboptimal reproducibility of HER2-low among pathologists. Moreover, a Chinese cohort study of 1433 patients with non-overexpressed HER2 metastatic breast cancer reported a prevalence of 43.1% for the HER2-low subtype, which was associated with significantly longer survival compared to patients with HER2-zero tumors.⁸ Additionally, results from the Austrian AGMT_MBC-Registry on metastatic breast cancer also indicated that low HER2 expression did not significantly affect OS as compared to HER2-zero expression.⁹

An intriguing proposal to expand the concept of HER2 status in breast cancer as a continuum spectrum has emerged, leading to the possibility of HER2 targeting in HER2 “ultra-low” subgroups (ie tumors with a score of 0, exhibiting incomplete and faint staining in $\leq 10\%$ of tumor cells) due to increasing knowledge about intra-tumor heterogeneity phenomena.²¹ This unique phenotype could be the reason for some favorable evidence on treatment response in HER2-zero breast cancer.¹¹ Given the limitations of IHC for HER2 testing, more precise and reliable methods based on artificial intelligence (AI) have been evaluated.^{27,28} Gustavson et al²⁹ proposed a novel HER2 Quantitative Continuous Score using deep learning-based image analysis, which has the potential to improve the prediction of patient outcomes with T-DXd.

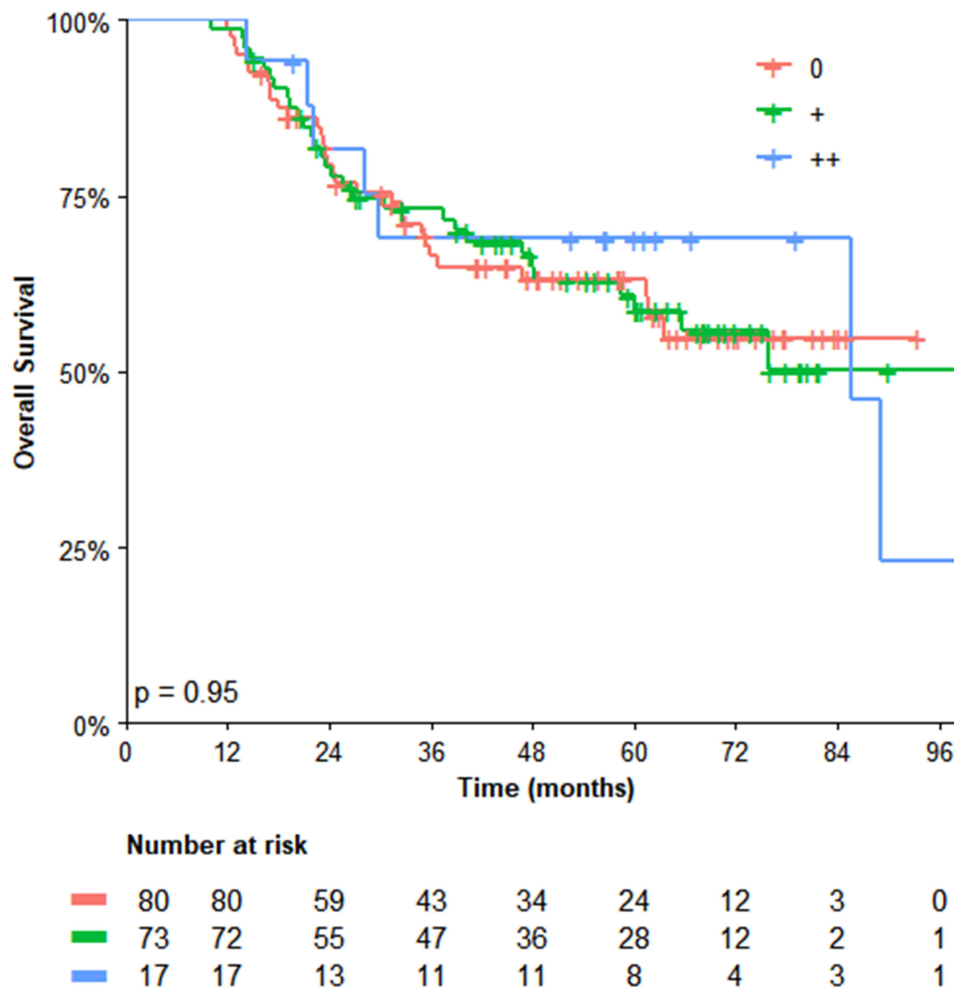


Figure 4 Kaplan-Meier estimates of overall survival stratified by HER2 immunohistochemistry subgroups.

Recently, the HER2Complete[®] program, a product of AI-based software, has gained popularity in the digital pathology community for its ability to detect HER2 expression levels in HER2-zero and HER2-low cases in an objective and reproducible manner. Despite not yet being approved for diagnostic procedures, it has received United Kingdom Conformity Assessed (UKCA) and In Vitro Diagnostic (IVD) CE marking.³⁰ Additionally, Moutafi et al³¹ developed a quantitative immunofluorescence coupled with a standardized mass spectrometry HER2 array that measures absolute amounts of HER2 protein on conventional histology sections. Kennedy et al³² reported promising results with immunoaffinity enrichment paired with multiple reaction monitoring-mass spectrometry (immuno-MRM-MS) as it showed higher concordance with predicate assays, especially at low HER2 expression levels. Xu et al³³ suggested that molecular methods like mRNA could be useful in accurately defining HER2-low cancer for treatment decision-making due to its wider dynamic range.

The robustness of this study primarily rests on its meticulous patient selection process, which facilitated a precise evaluation of distinct HER2 subgroups in the early-stage TNBC scenario. Specifically, only patients who received NACT followed by primary surgery were eligible for inclusion. Additionally, the analysis of HER2 status was centralized within the Department of Pathology of INCA, which conferred greater reliability to the results. To enhance the rigor of the study, experienced pathologists, who were unaware of the clinical data, performed a blinded double-check of the core biopsies.

The present study is subject to certain limitations, most notably its retrospective nature, which could result in some confounding factors not being taken into account and hence weakening the analysis. Furthermore, the single-center

design of the study raises the possibility of regional differences and specific patient characteristics in the cohort that may have impacted the results. Additionally, due to the unavailability of adequate samples, gene expression profiling was not conducted. Finally, the assessment of core biopsy analysis might have been influenced by intratumoral heterogeneity.

Conclusion

By restricting the patient cohort to a defined subset of individuals diagnosed with early-stage TNBC and analyzing the HER2 subtypes based on IHC, the current study humbly proposes that the HER2-low subgroup does not exhibit distinct clinical, sociodemographic, or clinical pathological features compared to the HER2-zero subgroup. Nevertheless, additional research using AI-based methods capable of providing objective and quantitative assessment to improve the identification of HER2-low status should be given high priority, given the potential for significant benefit from novel anti-HER2 ADCs in this subgroup.

Data Sharing Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethical Approval

The current investigation was subjected to ethical scrutiny and received approval from the Ethics in Human Research Committee of INCA, Rio de Janeiro, Brazil, with registration number CAAE 61675516.9.0000.5274, adhering to the principles of Good Clinical Practice guidelines.

Informed Consent

Considering the retrospective nature of this observational study, which involved the use of anonymized data for analysis, the Institutional Review Board (IRB), also known as the Ethics in Human Research Committee of the National Cancer Institute (Comitê de Ética em Pesquisa do Instituto Nacional de Câncer; CEP-INCA), deemed it appropriate to waive the requirement for informed consent from participants.

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Disclosure

The authors declare that they have no conflicts of interest.

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