Curr Probl Cancer 50 (2024) 101102

Contents lists available at ScienceDirect

Current Problems in Cancer

journal homepage: www.elsevier.com/locate/cpcancer

Prognostic significance of HER2 loss after HER2-targeted neoadjuvant treatment in patients with HER2-positive locally advanced breast cancer

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ARTICLE INFO

Keywords:
Breast cancer
Neoadjuvant treatment
HER2 expression loss
Disease-free survival

ABSTRACT

Loss of human epidermal growth factor receptor 2 (HER2) expression can be seen in almost 25–30 % patients after HER2 receptor directed neoadjuvant treatment. These patients have unclear clinical outcomes in previous studies. We aimed to investigate the importance of HER2 loss, additionally with predictive factors for the loss of HER2. This was a retrospective and multicenter study that included 272 HER2-positive BC patients with no pathological complete response who received neoadjuvant chemotherapy plus HER2-targeted treatments. The factors that may affect the loss of HER2 detected by immunohistochemistry(IHC) and the association with survival were analyzed.The rate of HER2 loss after neoadjuvant treatments(NAT) was 27.9 % ($n = 76$). Disease recurrence was observed in 18(23.7 %) patients with HER2 loss, while it was detected in 62 (31.7 %) patients without HER2 loss($p = 0.23$). Pre and post-NAT ER status, and post-NAT ki-67 status had a significant impact on disease-free survival(DFS) ($p = 0.0012$, $p = 0.004$, and $p = 0.04$, respectively).There were no significant association between DFS and loss of HER2 ($p = 0.64$) and dual anti-HER2 blockade ($p = 0.21$). Pre-NAT clinical stage (HR:1.65 $p = 0.013$), post-NAT LN status (HR:3.18, $p = 0.02$) and pre-NAT ER status (HR:0.24, $p = 0.041$) were significant independent prognostic factors for DFS while post-NAT residual disease in axillar tissue was an independent prognostic factor for OS (HR:1.54 $p = 0.019$). Moreover, age (<40 years vs ≥40 years) ($p = 0.031$) and tumor grade ($p = 0.004$) were predictive factors for HER2 loss. Our results showed that HER2 loss did not affect survivals. However, young age and being high grade tumor may predict HER2 loss.

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https://doi.org/10.1016/j.currproblcancer.2024.101102
Received 30 June 2023; Received in revised form 21 April 2024; Accepted 25 April 2024
Available online 11 May 2024

Introduction

Breast cancer (BC) is the most commonly diagnosed cancer and the second most common cause of cancer death in women.[1] Human epidermal growth factor receptor 2 (HER2) is a tyrosine-kinase receptor that is overexpressed in 15–20 % of BCs, associated with higher recurrence rates and worse oncologic outcomes.[2,3] Compared with other molecular subtypes of BC, HER2-positive patients achieve a pathologic complete response (pCR) to neoadjuvant therapy (NAT), even in the absence of HER2-targeted therapy.[4] The use of HER2-targeted therapy, further enhances the chemosensitivity of HER2-positive BC, increasing the pCR rate. Targeted therapeutics such as trastuzumab and pertuzumab given in the neoadjuvant setting, resulting in an improved rate of pCR from 40 % to 60 %.[5] This is a significant development in the care of patients with HER2-positive disease, as those with a pCR have been shown to have improved overall survival (OS) and disease-free survival (DFS).[5] However, patients without a pCR following NAT were associated with lower recurrence-free survival (RFS) and OS.[6]

Loss of HER2 can be seen at the rate of between 20–35 % in patients without pCR after neoadjuvant HER2-targeted treatment in residual tumors.[7,8] These patients have unclear clinical outcomes in previous studies. Two studies were associated with worse oncologic outcomes in patients with loss of HER2 after NAT,[9,10] although two studies demonstrated no significant difference in oncologic outcomes.[11,12] In addition, there is insufficient evidence about which adjuvant treatment is more beneficial in these patients. The biological basis for the loss of receptors and HER2 after NAT is not yet clearly understood.[7,13,14] However, tumor heterogeneity, genetic drift or the more aggressive tumor phenotype has been suggested as some of the reasons. However, technical errors may also partially explain the discrepancies. It allows direct assessment of the effects of treatment on tumor biology. In particular, examination of residual tumor biology may also enable a better understanding of the mechanisms.[13,14]

Our study aimed to evaluate the oncological outcomes of patients who experienced the loss of HER2 after NAT. Furthermore, we also investigated factors in predicting HER2 loss in patients with locally advanced HER2-positive BC.

Materials and methods

The study was a multicenter, retrospective study that included 422 women diagnosed with locally advanced HER2-positive BC, between September 2014 to June 2020. Each patient was discussed in a multidisciplinary council and decided to treat with NAT before curative surgery.

Clinicopathological factors such as age, tumor, node, metastasis (TNM) stage, date of diagnosis, tumor biomarkers status, grade, tumor size, and follow-up data were collected from patients' charts. Initial diagnostic pathology reports and final surgical pathology reports were evaluated and the patients who achieved pCR after neoadjuvant HER2-targeted treatment were excluded from the study. A consort diagram describing the flow of study patients is shown in Fig. 1. All pathological slides of initial biopsies and surgical specimens were re-evaluated to confirm the histopathological subtypes, HER2, hormone receptor (HR) and ki-67 status by the pathologist who was an expert in matters of breast pathology. HR, HER2 and ki-67 status were determined by immunohistochemistry (IHC). Tumors having a score of 3 (+) were considered as HER2-positive. Score 0 and 1 (+) were considered negative, and score 2 (+) was considered equivocal. Tumors scoring 2 (+) for HER2 expression were subsequently analyzed by fluorescence *in situ* hybridization (FISH) and were considered as HER2-positive if HER2 amplification was present in FISH. HER2 FISH positive result was defined as HER2/CEP17 ratio $\geq$2.0 or average HER2 copy number $\geq$6.0 signals per cell. Estrogen (ER) and progesterone receptor (PR) nuclear staining $\geq$ 1 % was accepted as ER and/or PR-positive by IHC evaluation according to the ASCO/CAP guidelines.

The majority of (n = 240, 88.2 %) patients received four cycles of epirubicin (75 mg/m^2) or doxorubicin (60 mg/m^2) with

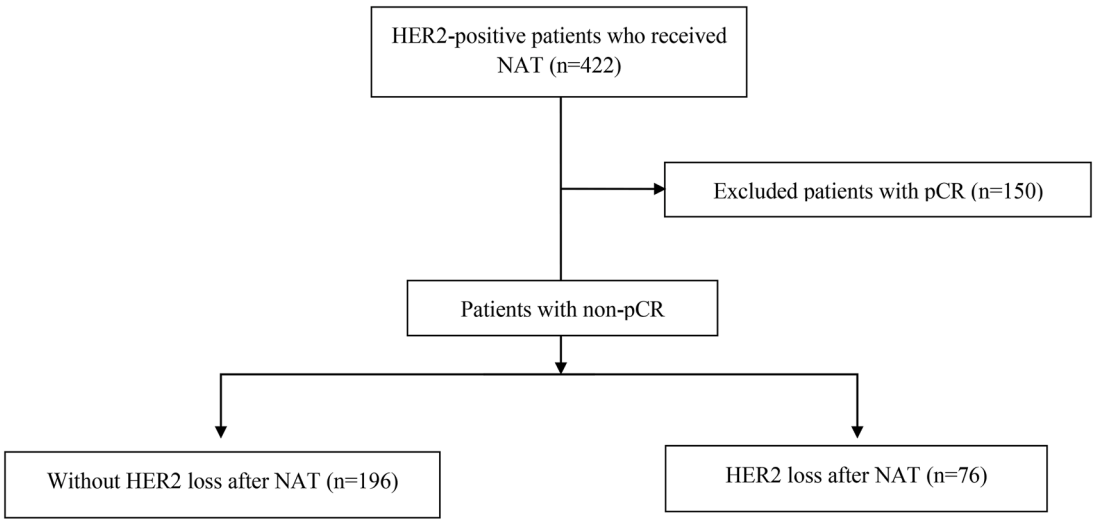

Fig. 1. Consort diagram. *NAT: Neoadjuvant treatment, pCR: pathologic complete response, HER2: Human epidermal growth factor receptor 2.

cyclophosphamide (600 mg/m^2) (AC/EC) every 3 weeks, followed by four cycles of docetaxel (100 mg/m^2) every three weeks or 12 cycles of weekly paclitaxel (80 mg/ m^2) with trastuzumab (trastuzumab 8 mg/kg loading dose, followed by 6 mg/kg). In addition, 32 patients (11.8 %) were treated with four cycles of AC/EC followed by taxanes plus trastuzumab plus pertuzumab (840 mg loading dose, followed by 420 mg; every 3 weeks). NAT regimens are listed in Table 1.

Written inform consent was obtained from all patients and the Local Ethics Committee of Istanbul Medipol University approved the study with the decision number E-10840098-772.02-2911.

Statistical analysis

Statistical analysis was done with SPSS 22.0 (SPSS Inc., Chicago, IL, USA) software. The relationship between clinicopathological factors and the loss of HER2 status was compared by the chi-squared test and fisher's exact test. Survival analysis and curves were performed according to the Kaplan-Meier method and compared by the log-rank test. DFS was defined as the time from curative breast surgery to disease progression or recurrence. OS was described as the time from diagnosis to the date of the patient's death or last known contact. Univariate and multivariate analyses of prognostic factors related to survival were performed by the Cox proportional hazards model. Multivariate p values were used to characterize the independence of these factors. Additional multivariate logistic regression analysis was performed to further evaluate all of the significant factors for predicting to loss of HER2. The 95 % confidence interval (CI) was used to quantify the relationship between survival time and each independent factor. All p values were two-sided in tests, and p values less than or equal to 0.05 were considered to be statistically significant.

Results

In this study, 272 women were included with median age 45 years (range 24–84). At the time of diagnosis, 101 patients (37.1 %) had stage II and 171 patients (62.9 %) had stage III disease. Thirty-two (11.8 %) patients received dual anti-HER2 therapy and a total of seventy-six patients (27.9 %) revealed HER2 loss after NAT. In the pre-NAT biopsy specimen, 55.2 % ($n = 42$) of patients with HER2 loss had HER2 IHC scores of 2+/FISH positive, while 44.8 % ($n = 34$) had HER2 IHC scores of 3+. Loss of HER2 receptor expression was higher in patients with HER2 IHC scores of 2+/FISH+ compared to those with HER2 IHC scores of 3+. In the post-NAT breast surgical specimen, 63.1 % ($n = 48$), 30.2 % ($n = 23$), and 6.7 % ($n = 5$) of patients had HER2 IHC scores of 0, 1+, and 2+/FISH-negative, respectively.

The baseline clinicopathological features were well matched and the association between the clinicopathologic features and HER2 loss are summarized in Table 2. When patients were categorized according to HER2 loss; age, disease stage at initial diagnosis, primary tumor size, initial ki-67 and PR status, post-NAT PR status and dual anti-HER2 blockade were not significantly differ between groups. Disease recurrence was observed in 18 (23.7 %) patients with HER2 loss, while it was detected in 62 (31.7 %) patients without HER2 loss. The difference between groups was not statistically significant ($p = 0.23$). Despite that, initial clinical nodal status, post-NAT ki-67 and ER status and pre-NAT ER status were significantly different between groups. In other words, pre and post-NAT ER positivity was observed in 105 (53.5 %) and 109 (55.6 %) patients without HER2 loss, while it was observed in 53 (69.7 %) and 57 (75 %) patients with HER2 loss, respectively ($p = 0.04$ and $p = 0.04$ respectively). Post-NAT ki-67 value was below 20 % in 65 (33.2 %) and 53 (69.7 %) patients without and with HER2 loss, respectively ($p = 0.002$). The clinically lymph node (LN) involvement at initial diagnosis and the rate of grade 1 tumor was statistically higher in patients without HER2 loss (79 % vs 63.1 %, $p = 0.033$ and 22.6 % vs 11.9 %, $p = 0.031$ respectively). Logistic regression analysis was performed in order to evaluate the significant factors that might predict the loss of HER2. It demonstrated that age (<40 years vs ≥ 40 years) (OR:2.6 CI 95 % 0.96–5.77, $p = 0.031$) and tumor grade (OR:0.36 CI 95 % 0.18–0.72, $p = 0.004$) were predictive factors for HER2 loss (Table 3). In other words, younger age (<40 years) and higher tumor grade may predict HER2 loss.

Of 146 (53.5 %) before NAT, ER-positive patients, ER loss was detected in 21 (7.7 %) patients after NAT. On the other hand, out of 90 (33.3 %) pre-NAT PR-positive patients, loss of PR was observed in 38 (13.9 %) post-NAT patients. Thus, there was a significant discordance in ER and PR status after NAT ($p < 0.001$ and $p < 0.001$ respectively) (Table 4).

At a median follow-up time of 66.6 months (range: 13.9–140.8 months), the median DFS was 34.2 months (range: 6.4–130.4) and the median OS was 44.3 months (range: 9.5–140.8). Patients with tumor size ≤3cm and initial early clinical stage of disease were significantly associated with longer DFS ($p = 0.038$ and $p = 0.0038$, respectively). The presence of nodal involvement after NAT was associated with worse DFS in the univariate analysis compared to those without nodal involvement after NAT. The median DFS were 62.1 months for those with nodal involvement and 98.4 months for those without, and this difference was statistically significant ($p = 0.003$). The IHC findings as pre and post-NAT ER status, and post-NAT ki-67 status had a significant impact on DFS ($p = 0.0012$, $p =$

Table 1
Neoadjuvant chemoterapy regimens.

Chemotherapy Regimens	$n = 272$
AC/EC + Docetaxel + Trastuzumab	125
AC/EC + Paclitaxel + Trastuzumab	115
AC/EC + Docetaxel + Trastuzumab + Pertuzumab	23
AC/EC + Paclitaxel + Trastuzumab + Pertuzumab	9

*EC: epirubicin and cyclophosphamide; AC: doxorubicin and cyclophosphamide

Table 2
The association of loss of HER2 with clinicopathological factor after NAT.

	Loss of HER2 Absent *n* (%)	Loss of HER2 Present *n* (%)	*p*
Age, years			0.23
<40	64 (33.2)	18 (23.7)	
>40	132 (66.8)	58 (76.3)	
Pre-NAT Clinical stage			0.22
IIA	33 (17.1)	11 (14.4)	
IIB	37 (18.8)	22 (28.9)	
IIIA	42 (21.4)	10 (13.4)	
IIIB	46 (23.4)	15 (19.7)	
IIIC	38 (19.3)	18 (23.6)	
Clinical lymph node			**0.033**
Negative	41 **(21.0)**	28 (36.9)	
Positive	155 **(79.0)**	48 (63.1)	
Tumor grade			**0.031**
Grade 1	15 (22.6)	9 (11.9)	
Grade 2	104 (53.0)	40 (52.6)	
Grade 3	77 (24.4)	27 (35.5)	
Tumor size			0.75
<3 cm	91 (46.5)	44 (57.8)	
>3 cm	105 (53.5)	32 (42.2)	
Initial Ki-67 index status			0.96
<20 %	51 (26.1)	23 (17.2)	
>20 %	145 (73.9)	63 (82.8)	
Post-NAT Ki-67 index status			**0.002**
<20 %	65 (33.2)	53 (69.7)	
>20 %	131 (66.8)	23 (30.3)	
Pre-NAT ER status			**0.04**
Positive	105 (53.5)	53 (69.7)	
Negative	81 (46.5)	23 (30.3)	
Pre-NAT PR status			0.054
Positive	81 (41.4)	41 (53.9)	
Negative	115 (58.6)	35 (46.1)	
Post-NAT ER status			**0.041**
Positive	109 (55.6)	57 **(75.0)**	
Negative	87 (44.4)	19 **(25.0)**	
Post-NAT PR status			0.09
Positive	81 (46.5)	40 (52.6)	
Negative	115 (53.5)	36 (47.4)	
Dual-anti-HER2			0.65
Absent	173 (88.2)	67 (88.2)	
Present	23 (11.8)	9 (11.8)	
Recurrence			0.23
Absent	134 (68.3)	58 (76.3)	
Present	62 (31.7)	18 (23.7)	

*HER2: Human epidermal growth factor receptor 2, ER: Estrogen receptor, PR: Progesterone receptor, NAT: Neoadjuvant treatment

Table 3
Predictive factors affecting the loss of HER2.

Factors	*p*	OR	95 % CI
Age (<40 vs. >40)	0.031	2.36	0.96–5.77
Tumor grade	0.004	0.36	0.18–0.72
Clinical lymph node (negative vs. positive)	0.26	0.72	0.41–1.27
Post-NAT lymph node (negative vs. positive)	0.14	1.74	0.82–3.69
Pre-NAT ER status	0.42	1.49	0.55–3.99
Post-NAT ER status	0.61	1.31	0.45–3.78
Initial Ki-67 index status (<20 % vs. >20 %)	0.80	1.11	0.46–2.66

*OR: Odds ratio, CI: confidence interval, ER: Estrogen receptor, PR: Progesterone receptor, NAT: Neoadjuvant treatment, HER2: Human epidermal growth factor receptor 2

0.004, $p = 0.04$ respectively). The univariate analysis showed no significant association between DFS and loss of HER2 ($p = 0.64$), dual anti-HER2 blockade ($p = 0.21$), pre and post-NAT PR status ($p = 0.13$, $p = 0.25$), initial ki-67 status ($p = 0.56$), tumor grade ($p = 0.52$), pre-NAT LN status ($p = 0.19$) and age ($p = 0.202$), respectively. Multivariate analysis indicated that pre-NAT clinical stage (HR:1.65 CI 95 %: 0.75–3.96 $p = 0.013$), post-NAT LN status (HR:3.18 CI 95 %: 1.19–6.46, $p = 0.02$) and pre-NAT ER status (HR: 0.24, CI 95 % 0.11–0.94, $p = 0.041$) were significant independent prognostic factors for DFS. Univariate and multivariate analysis for DFS was shown in Table 5. Furthermore, when examining patients based on the loss of HER2 and hormone receptors (ER and/or PR) for DFS and OS,

Table 4
HER2, ER and PR discordance before and after NAT.

	Pre-NAT positive *n* (%)	Post-NAT negative *n* (%)	Loss of IHC *n* (%)	*p*
ER				<0.001
Positive	146 (53.6)	21 (7.7)	21 (7.7)	
Negative	26 (9.6)	79 (29.1)		
PR				
Positive	90 (33.1)	38 (13.9)	38 (13.9)	<0.001
Negative	29 (10.7)	115 (42.3)		
HER2				
Positive	272 (100)	76 (27.9)	76 (27.9)	

*HER2: Human epidermal growth factor receptor 2, ER: Estrogen receptor, PR: Progesterone receptor, NAT: Neoadjuvant treatment, IHC: immunohistochemistry

the median DFS and OS durations for patients experiencing HER2 loss did not show significant discrepancies compared to those without HER2 loss (77.7 vs. 69.9 months, $p = 0.64$, and NR vs. 95 months, $p = 0.09$, respectively). In the group with PR loss, median DFS and OS times were similar compared with those without PR loss (97.8 vs. 77.7 months, $p = 0.25$ and 109.2 vs. 120.8 months, $p =$

Table 5
Univariate and multivariate analysis for disease-free survival (DFS).

	Median DFS (months)	Univariate *p* value	Multivariate *p* value	HR 95 % CI
Age, years		0.202		
<40	102.6			
>40	97.8			
Pre-NAT Clinical stage		**0.033**	**0.013**	**1.65**
IIA	97.8			**(0.75–3.96)**
IIB	98.4			
IIIA	86.3			
IIIB	80.1			
IIIC	77.7			
Clinical lymph node		0.19		
Negative	97.8			
Positive	85.4			
Post-NAT lymph node		**0.003**	**0.02**	**3.18**
Negative	98.4			**(1.19–6.46)**
Positive	62.1			
Tumor grade		0.52		
Grade 1	102.6			
Grade 2	97.3			
Grade 3	80.1			
Tumor size		**0.038**	0.28	0.58
<3 cm	100.1			(0.21–1.56)
>3 cm	64.9			
Initial Ki-67 index status		0.56		
<20 %	81.4			
>20 %	75.6			
Post-NAT Ki-67 index status		**0.04**	0.83	1.09
<20 %	90.8			(0.45–2.60)
>20 %	77.7			
Pre-NAT ER status		**0.012**	**0.041**	**0.24**
Positive	78.3			**(0.11–0.94)**
Negative	62.1			
Pre-NAT PR status		0.13		
Positive	97.8			
Negative	90.8			
Post-NAT ER status		**0.004**	0.12	0.44
Positive	97.8			(0.16–1.23)
Negative	45.6			
Post-NAT PR status		0.25		
Positive	97.8			
Negative	77.7			
Dual-anti-HER2		0.21		
Absent	97.8			
Present	91.8			
Loss of HER2		0.64		
Absent	77.7			
Present	69.9			

* HER2: Human epidermal growth factor receptor 2, ER: Estrogen receptor, PR: Progesterone receptor, NAT: Neoadjuvant treatment

0.92, respectively). However, patients with ER loss exhibited a significantly shorter median DFS duration compared to those without ER loss (45.6 vs. 97.8 months, $p = 0.004$, respectively), while OS remained similar (117.7 vs. 101.8 months, $p = 0.29$, respectively). Univariate analysis revealed no significant prognostic factors for either DFS or OS in the groups with loss of HER2 and loss of hormone receptors (p>0.05).

None of the pre and post-histopathologic findings had a significant impact on OS in univariate analysis. Patients who achieved a complete response in axillar tissue had significantly better survival outcomes rather than residual disease both in univariate (median OS 126.0 months vs 110.7 months, $p = 0.032$) and multivariate analysis (HR:1.54 CI 95 % 0.41–5.79, $p = 0.019$) (Table 6). HER2 status after NAT was not significantly associated with both DFS and OS (Figs. 2 and 3).

Discussion

NAT with anti-HER2 agents is the standard of care for locally advanced HER2-positive BC. The cause of HER2 loss followed by NAT in BC, and its clinical importance remain uncertain. The possible reasons might be an anti-HER2 treatment-related clonal selection or the heterogeneity of intra-tumoral HER2 expression. We evaluated the oncologic outcomes of patients with loss of HER2 after NAT in

Table 6
Univariate and multivariate analysis for overall survival (OS).

	Median OS (months)	Univariate *p* value	Multivariate *p* value	HR 95 % CI
Age, years		0.58		
<40	111.4			
>40	122.9			
Pre-NAT Clinical stage		0.41		
IIA	129.1			
IIB	120.4			
IIIA	118.0			
IIIB	NR			
IIIC	104.5			
Clinical lymph node		0.94		
Negative	120.6			
Positive	110.4			
Post-NAT lymph node		**0.032**	**0.019**	**1.54**
Negative	126.0			**(0.41–5.79)**
Positive	110.7			
Tumor grade		0.93		
Grade 1	NR			
Grade 2	104.3			
Grade 3	120.4			
Tumor size		0.72		
<3 cm	123.3			
>3 cm	102.2			
Initial Ki-67 index status		0.43		
<20 %	129.0			
>20 %	100.7			
Post-NAT Ki-67 index status		0.66		
<20 %	100.6			
>20 %	121.5			
Pre-NAT ER status		0.11	0.41	0.60
Positive	118.4			(0.17–2.06)
Negative	104.5			
Pre-NAT PR status		0.58		
Positive	122.2			
Negative	98.9			
Post-NAT ER status		0.29	0.80	1.16
Positive	101.8			(0.35–3.85)
Negative	117.7			
Post-NAT PR status		0.92		
Positive	109.2			
Negative	120.8			
Dual-anti-HER2		0.47		
Absent	120.7			
Present	NR			
Loss of HER2		0.09		
Absent	NR			
Present	95.0			
Recurrence		**<0.001**	**0.002**	**23.6**
Absent	137.3			**(3.80–38.5)**
Present	86.0			

*HER2: Human epidermal growth factor receptor 2, ER: Estrogen receptor, PR: Progesterone receptor, NAT: Neoadjuvant treatment

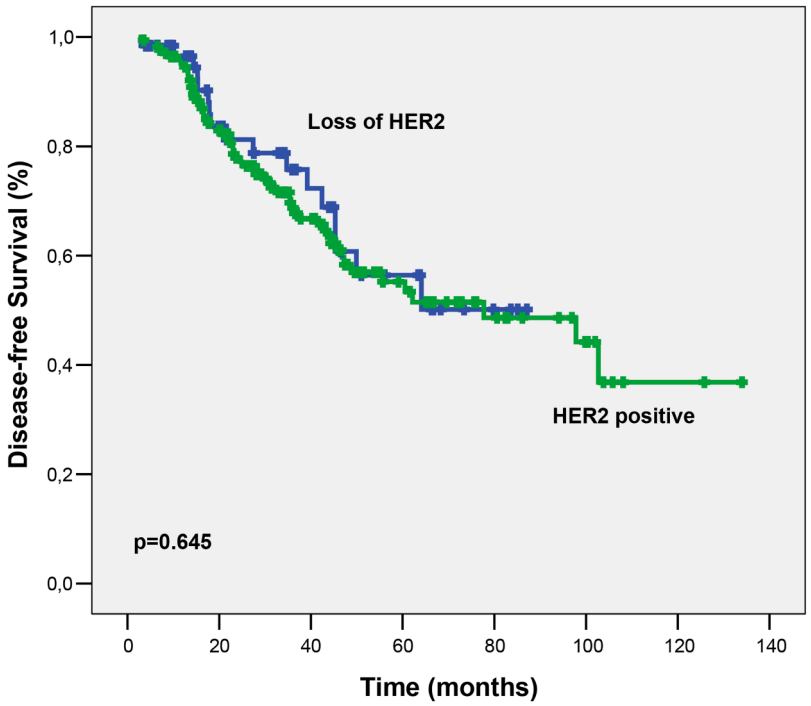

Fig. 2. There was no significant association between loss of HER2 overexpression and DFS ($p = 0.645$).

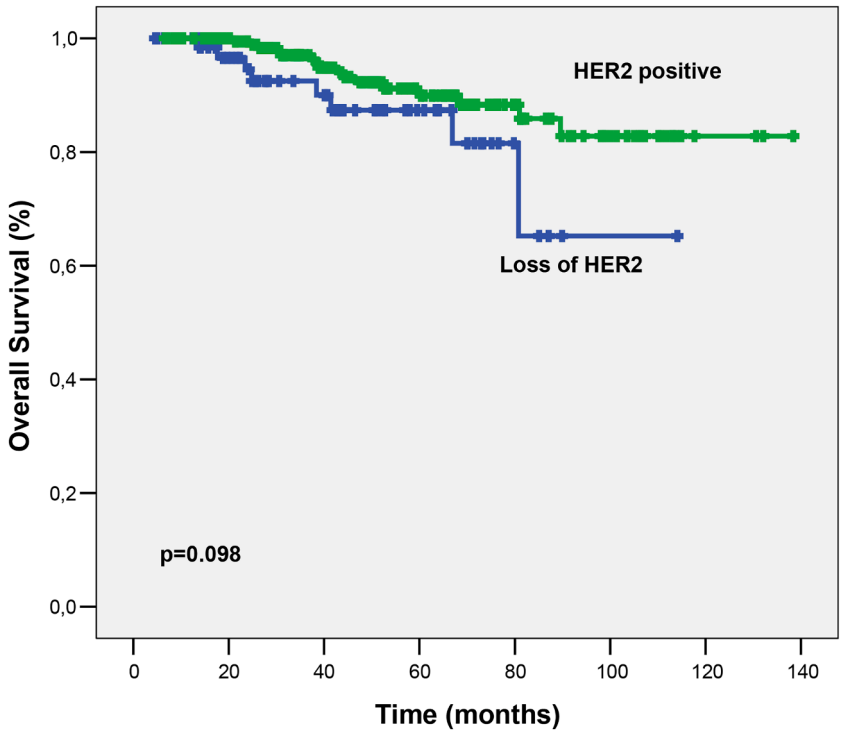

Fig. 3. There was no significant association between loss of HER2 overexpression and OS ($p = 0.098$).

our study.

In this study, 272 women who were discussed in the multidisciplinary BC council and decided to be treated with NAT were included and a total of 76 patients (27.9 %) revealed HER2 loss after NAT. This is the largest study to date that analyzed the outcomes and the

prognostic factors in BC patients with loss of HER2 expression after NAT. The rate of HER2 loss after NAT in our study was consistent with the results of a large retrospective cohort study from Niikura et al. with 21.755 patients.[7] This study revealed that in 2811 patients who were HER2-positive before NAT, 601 (21.4 %) patients had HER2 loss.

Prior studies regarding the oncologic outcomes of patients with HER2 loss following NAT have inconsistent results. In a multicenter study by Wetzel et al, loss of HER2 was observed in 34 (19 %) of 182 patients, and there was no statistically significant difference in 5-year RFS and OS in these patients.[12] Yoshida et al. reported loss of HER2 in 33 (33 %) of 99 HER2-positive patients, among these patients, showed that changes in HER2 status did not affect patients' prognosis.[11] Similarly, in our study, the significant impact of HER2 expression loss on DFS and OS could not be demonstrated.

Ignatov et al. reported that HER2 loss was significantly associated with a worse 5-year event-free survival (EFS) (74 % compared to 59 %).[15] However, in this study HER2 loss was only associated with anti-HER2 treatments. Thus, the EFS benefit could be related to anti-HER2 treatment not to HER2 loss.[15] Branco et al. demonstrated worse OS and RFS in patients who had HER2 loss, however, the sample size of this study was insufficient.[9] Wang et al. compared the outcomes between two treatment groups: chemotherapy plus trastuzumab versus chemotherapy alone. The pCR rate of 50 % and the rate of HER2 loss in residual tumors of 19 % were observed in the chemotherapy plus trastuzumab group.[16] They demonstrated the patients who experienced the loss of HER2 in residual tumor tissue had worse DFS. This may be due to the short follow-up period and the unknown number of patients receiving adjuvant therapy. In our study, all the patients received adjuvant anti-HER2 treatment regardless of HER2 loss. The better DFS and OS in our patients compared to the literature can be explained by the fact that all patients received adjuvant anti-HER2 therapy, regardless of HER2 loss.

The impact of ER and PR status on HER2 loss was demonstrated in many studies. Yoshida et al. showed a significant association between HER2 loss after NAT and lower nuclear grade ($p = 0.04$), ER-positivity ($p < 0.01$) and PR-positivity ($p < 0.01$) in patients with HER2-positive BC patients.[11] Ignatov et al. demonstrated a significant association between HER2-targeted treatment and HER2 loss; meanwhile, there was no significant association between HER2 loss and patient age, tumor size, tumor type, tumor grade and ki-67 expression, LN status, and the use of chemotherapy.[15] DFS benefit of the post-NAT expressions of ER ($p < 0.001$), PR ($p < 0.001$), and any receptor conversion ($p < 0.001$) and molecular subtypes ($p < 0.001$) were demonstrated in another study.[17] In our study, patients who had ER-positive and HER2-positive tumors before NAT more frequently showed HER2 loss than ER-negative tumors after NAT. Our findings were thus compatible with study of Yoshida et al.,[11] not with that of Ignatov et al.[15] The reason for this difference may be related to the heterogeneity of these tumors, such as ER-positive/HER2-positive tumors, are more resistant to chemotherapeutic agents.[18] The possible mechanism of crosstalk between ER and HER2 pathways might be associated with resistance to chemotherapy and changes in HER2 status, or different pCR rate after NAT according to ER status.[19] Because it is well known that ER-negative tumors show better pCR than ER-positive ones.[7]

Furthermore, we evaluated both pre and post-NAT IHC findings and, pre and post-NAT ER status, post-NAT ki-67 status had a significant impact on DFS ($p = 0.0012$, $p = 0.004$, and $p = 0.04$ respectively). Another contribution of our study was the logistic regression analysis which showed that; age (<40 years vs $\geq$ 40 years) ($p = 0.031$) and tumor grade ($p = 0.004$) were predictive factors for HER2 loss. In other words, younger age and higher tumor grade may predict HER2 loss. This is likely because the histological grade of the tumor reflects the degree of tumor cell differentiation. Well-differentiated tumors have tumor cell growth patterns and nuclear features similar to those of the normal mammary gland. When tumors begin to lose this differentiation, cells may develop larger nuclei, reach a higher proliferation rate, and lose HER2 status. The reasonable and well-accepted hypothesis for this phenomenon was that both HER loss and high histological grade indicate a tumor that has begun to lose some degree of differentiation. Similarly, women with breast cancer under the age of 40 have a more advanced disease and higher grade disease was significantly associated with an increased risk of breast cancer mortality. These phenomena are independent of each other and indicate a more aggressive tumor with a worse prognosis.[20] The addition of pertuzumab to trastuzumab in NAT improved survival in the NeoSphere study. The 5-year DFS rate was 86 % with dual anti-HER2 treatment in the NeoSphere study.[5] In our study dual blockage was not a significant prognostic factor for the DFS. This could be explained that we include the patients who did not achieve pCR after NAT.

The prognostic factors for DFS in patients who received NAT have been previously evaluated in some studies. Tumor size, stage, hormonal status, treatment modality, type of chemotherapy, pCR, and lymphovascular invasion (LVI) were significantly associated with the DFS and OS. Tumor size > 10 cm ($p = 0.001$), HR-negativity ($p = 0.001$), treatment modality ($p = 0.001$), lack of pCR ($p = 0.001$), and the presence of LVI ($p = 0.001$) was associated with poor OS rates.[21] Another study showed better survival outcomes with the patients who received a complete response after NAT in axillary tissue.[22] Similarly, to the literature in our study, multivariate analysis indicated that pre-NAT clinical stage ($p = 0.013$), post-NAT LN status ($p = 0.02$), and pre-NAT ER status ($p = 0.041$) were significant independent prognostic factors for DFS. Moreover, patients who achieved a complete response in axillar tissue had significantly better survival outcomes.

The major strength of this study is the inclusion of only patients with residual tumor after NAT and offering a large-scale real-world data on the prognostic significance of HER2 loss. However, certain limitations must be acknowledged. First, the data is retrospective and limited to the therapeutic options available for use in early HER2-positive BC within the study period. Second, relatively shorter follow-up time is another limitation given that a reliable survival outcome analysis necessitates a longer follow-up interval. Despite all these limitations, we believe that our study will contribute to the literature as it includes only the patients with residual tumor and analyzes the prognostic importance of HER2 loss and the predictive factors on HER2 loss.

Conclusions

In conclusion, in this real-world study, HER2 expression loss in BC patients post-NAT shows no significant association with survival. A positive initial clinical nodal status, lesser post-NAT ki-67 status, positive post-NAT ER status, positive pre-NAT ER status, younger

age and a higher tumor grade were significantly associated with HER2 loss. The use of dual anti-HER2 agents in NAT was not associated with a statistically significant loss of HER2 expression. Younger age and higher tumor grade were significant even on regression analysis to predict HER2 loss. Future studies will define the role of treatment modifications in the adjuvant setting based on levels of HER2 expression in the residual tumor post NAT.

Ethics approval

This is an observational study. Medipol University Ethics Committee has confirmed that no ethical approval is required.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

The authors declare that no funding were received during the preparation of this manuscript.

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