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# Impact of HER2 Overexpression and Co-expression of Hormonal Receptors on Pathological Response after Neoadjuvant Chemotherapy in HER2-Positive Breast Cancer

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## Abstract

**Background:** HER2 overexpression in Breast Carcinomas (BC) has been associated with poorer prognosis and higher aggressiveness. The development of targeted therapies has changed the behavior of this disease. Targeted therapies became the optimal Neoadjuvant Chemotherapy (NAC), achieving high rates of complete Pathologic Response (pCR). The ASCO/CAP guidelines classify HER2 positive tumors either by immunohistochemistry (3+) and/or in situ hybridization (HER2 amplification), but no correlation with response is addressed. In this study, we asked whether all HER2-positive cases (IHC 2+/ISH+ vs. IHC 3+) show the same response to NAC aiming at pCR rates, and whether this can be influenced hormone receptors co-expression.

**Design:** A retrospective series of 207 HER2-positive BC treated with NAC at two institutions between 2011 and 2017 was reviewed. Age, histologic type, TNM staging, treatment received, and pathological response were evaluated. HER2 and hormone receptor assessment was performed according to ASCO/CAP guidelines. Bivariate comparative analysis for the categorized qualitative and quantitative variables and multivariate analysis using logistic regression were performed.

**Results:** Mean age was 53.9 years. pCR was observed in 116 cases (56%). 94,2% were carcinomas of no special type, 72 cases were non-luminal HER2 (34.8%) and 135 cases were Luminal B-HER2 positive (65%). Tumors with IHC 3+ presented a pCR rate of 60% compared to 29.6% of the IHC 2+/ ISH+ group (p=0.003), and the risk of achieving a pCR was three times higher in this first group (OR: 3.07 (1.24-7.59), p=0.015) (Table 1). Furthermore, non-luminal HER2 tumors presented 75% pCR (54/72) compared to 45.9% of luminal HER2-positive tumors (62/135) (p=0.0001). No differences in pCR rates were observed depending on Ki67 (p=0.179) or type of surgery or anti-HER2 treatment received (p=0.503).

**Conclusion:** Overall, not all tumors classified as "HER2 positive" respond equally to NAC. pCR rates after NAC were higher in tumors with complete immunohistochemical overexpression (IHC 3+) and without hormone receptor co-expression (HER2 positive (non-Luminal). In addition, progesterone receptor co-expression was associated with lower pCR.

**Keywords: Breast cancer; HER2; Neoadjuvant chemotherapy; Pathological complete response**

## Introduction

Breast cancer remains one of the most important health problems worldwide, with more than 2 million cases diagnosed in 2020 and nearly 685,000 deaths (Global Cancer Statistics, 2020). There are numerous factors that play a role in the outcome of the disease, especially the presence of biomarkers that also determine the therapeutic approach, as they are predictive factors for response to specific treatments. Among these factors, the role of Human Epidermal Membrane Receptor 2 (HER2), which is amplified in 15% to 25% of breast cancers, should be highlighted [1,2]. Its overexpression has been associated with poorer prognosis and greater biological aggressiveness, although the development of targeted therapies has altered the behavior of this disease [1,3-5].

In addition to advances in specific therapies in the treatment of breast cancer, the order of application of the various therapeutic modalities has changed, as it has in other solid tumors. Initially, this was due to the inability or failure to provide adequate locoregional treatment at the beginning, for inoperable or unresectable tumors where initial chemotherapy was administered as the only option. Later, Neoadjuvant Systemic Therapy (NST) began to be administered in order to perform conservative surgery, and eventually it became a usual therapy, allowing *in vivo* verification of tumor sensitivity [6,7]. In this sense, NST has not shown prognostic differences with adjuvant therapy in terms of overall survival [8], and the presence of a pathological Complete Response (pCR) after its administration is even considered a prognostic surrogate marker for survival [8].

For HER2-positive tumors, trastuzumab became the optimal NST, achieving high rates of pCR [9,10]. Subsequently, dual blockade with trastuzumab and pertuzumab has improved response in this NST modality [11,12]. However, despite the positive results, not all tumors respond equally, and an explanation for these different behaviors is still needed.

Although the ASCO/CAP guidelines describe the recommendations for a correct HER2 evaluation [13], they end up classifying tumors as HER2-positive or negative without explaining the different responses to treatment that can be derived from this classification. Some studies have already pointed out that the evaluation of HER2 by immunohistochemistry or *in situ* Hybridization (ISH) shows different responses to treatment [14,15], and tumor heterogeneity could also explain these discrepancies. Thus, some series have shown that patients with homogeneous tumors had 76% pCR compared with 26% of those with heterogeneity [16].

It has also been reported that co-expression of hormone receptors in HER2-positive tumors may influence the degree of response to NST [17,18] although with inconclusive results in other series [19].

In this study, we addressed the question of whether all HER2-positive cases (2+ ISH + *vs.* 3+) show the same type of response to NST with HER2-targeted therapy, aiming at pCR rates, and whether this can also be influenced by the co-expression of other receptors of known prognostic and predictive relevance, such as hormone receptors.

## Material and Methods

### Clinical features

Retrospective and multicenter observational study approved by the institutional Ethics Committee that included 207 cases of HER2-positive breast cancers treated with NST with HER2-targeted therapy. The study was conducted in two institutions (H.U. Virgen de la Arrixaca in Murcia and H.U. Vall d'Hebron in Barcelona) between 2011 and 2017. Clinical data analyzed included age, histological type (invasive carcinoma of no special type, Invasive lobular carcinoma or others), TNM staging (based on the 8$^{th}$ edition of the American Joint Committee on Cancer), type of surgery (radical or conservative), type of treatment (trastuzumab, trastuzumab-pertuzumab, trastuzumab-lapatinib, or trastuzumab-neratinib), and degree of response (complete or partial) in both breast and axilla. Axillary involvement prior to NST was confirmed by Fine Needle Aspiration (FNA) and/or Core Needle Biopsy (CNB) on clinically suspicious lymph nodes.

pCR was defined as the absence of invasive residual disease in both the breast and axilla, accepting the presence of an intraductal carcinoma (ypT0/is ypN0). Response to treatment in the breast and axilla was also analyzed independently of tumor characteristics. In the latter, cases with evidence of axillary disease before systemic treatment were considered.

### Biomarker study

HER2 and hormone receptor (estrogen and progesterone) evaluation were performed according to ASCO/CAP guidelines recommendations [13,20]. Those cases with an HER2 immunohistochemical result of 3+ or 2+ with amplification after ISH evaluation (VENTANA HER2 Dual ISH DNA Probe Cocktail Assay, Ventana USA) were classified as HER2 positive and included in the study. The cutoff point for classifying a tumor as positive for hormone receptors, both estrogen and progesterone, was 1% [20]. Ki67 assay was added, with a cutoff point of 20% defined to consider the tumor as "highly proliferative".

### Statistical analysis

A descriptive study of the variables and a comparative analysis were performed for pCR as the dependent variable, both for the degree of HER2 overexpression and for the hormone receptors co-expression and their combination. Adjustment for normal distribution was examined using the Kolmogorov test. Bivariate comparative analysis for the categorized qualitative and quantitative variables was performed by contingency table analysis with residual analysis using Pearson's Chi-square test. Multivariate analysis using logistic regression was also performed with the variables that showed significant differences in the bivariate analysis. A level of $p<0.05$ was considered statistically significant.

## Results

pCR was observed in 116 cases of the global series (56%), in 60.4% if just the primary tumor was considered and 70.1% for axillary disease in cases with lymph node involvement.

The mean age at presentation was 53.9 years [26 to 87 years]. In terms of histological type, most cases were carcinomas of no special type (94.2%) and regarding the immunohistochemical profile, 72 cases were non-luminal HER2 (34.8%) and 135 cases were luminal B-HER2 positive (65%). In 134 cases (64.7%), axillary disease was detected at diagnosis (cN+). HER2 2+/ISH amplified was detected in 27 cases (13%), while HER2 3+ was observed in 180 cases (87%). 172 (83.9%) showed a high proliferation index compared to 33 (16.1%) in which Ki67 was less than 20%. Regarding surgical technique, 110 cases were treated with conservative surgery (53.2%) and 97 cases with radical surgery (46.8%). The other clinicopathological characteristics

**Table 1:** Clinicopathological features.

| Age | 53.86 ± 13.01 | (26-87) |
|---|---|---|
| **Histological subtype** | | |
| Carcinoma NST | 195 | 94.20% |
| ILC | 6 | 2.90% |
| Others | 6 | 2.90% |
| **TNM** | | |
| **cT** | | |
| 1 | 20 | 9.70% |
| 2 | 131 | 63.30% |
| 3 | 33 | 15.90% |
| 4 | 23 | 11.10% |
| **cN** | | |
| 0 | 73 | 35.30% |
| 1 | 107 | 51.70% |
| 2 | 27 | 13.00% |
| **ypT** | | |
| 0 | 109 | 52.90% |
| 1 | 46 | 22.30% |
| 2 | 52 | 25.20% |
| **ypN** | | |
| 0 | 169 | 81.60% |
| 1 | 26 | 12.60% |
| 2 | 12 | 5.80% |
| **Type of surgery** | | |
| Conservative | 110 | 53.2 |
| Radical | 97 | 46.8 |
| **Her2neu** | | |
| 2+ | 27 | 13% |
| 3+ | 180 | 87% |
| **IHC profile** | | |
| HER2 positive (no Luminal) | 72 | 34.80% |
| Luminal B-HER2 positive | 135 | 65.20% |
| **ER** | | |
| <1% | 67 | 32.40% |
| ≥ 1% | 140 | 67.60% |
| **PR** | | |
| <1% | 115 | 55.60% |
| ≥ 1% | 92 | 44.40% |
| **Ki67** | | |
| <20% | 33 | 16.10% |
| ≥ 20% | 172 | 83.90% |
| **Chemotherapy** | | |
| Trastuzumab + Pertuzumab | 119 | 57.50% |
| Trastuzumab | 70 | 33.80% |
| Trastuzumab + Lapatinib | 13 | 6.30% |
| Trastuzumab + Neratinib | 5 | 2.40% |
| Pathologic complete response | 116 | 56% |
| Pathologic complete response in the breast | 125 | 60.40% |
| Pathologic complete response in the axilla* | 94 | 70.10% |

*: assessed on 134 cases with axillary involvement

**Table 2:** Univariate and multivariate analysis of the complete pathological response in the general series.

| n=207 | NO pCR | pCR | p | p - OR (IC 95%) |
|---|---|---|---|---|
| **Age** | 55.08 ± 14.23 | 52.91 ± 11.95 | 0.234 | |
| **Histological subtype** | | | | |
| Carcinoma NST (n=195) | 83 (42.6%) | 112 (57.4%) | 0.264 | |
| ILC (n=6) | 4 (66.7%) | 2 (33.3%) | | |
| Others (n=6) | 4 (66.7%) | 2 (33.3%) | | |
| **cT** | | | | |
| 1 (n=20) | 8 (40%) | 12 (60%) | | |
| 2 (n=131) | 58 (44.3%) | 73 (55.7%) | 0.983 | |
| 3 (n=33) | 15 (45.5%) | 18 (54.5%) | | |
| 4 (n=23) | 10 (43.5%) | 13 (56.5%) | | |
| **cN** | | | | |
| 0 (n=73) | 35 (47.9%) | 38 (52.0%) | | |
| 1 (n=107) | 48 (44.8%) | 59 (55.1%) | 0.575 | |
| 2 (n=27) | 9 (33.3%) | 18 (66.6%) | | |
| **Type of surgery** | | | | |
| Conservative (n=109) | 45 (41.3%) | 64 (58.7%) | 0.512 | |
| Radical (n=96) | 44 (45.8%) | 52 (54.2%) | | |
| **Her2neu** | | | | 0.01 |
| 2+ (n=27) | **19 (70.4%)** | 8 (29.6%) | **0.003** | OR: 3.07 |
| 3+ (n=180) | 72 (40.0%) | **108 (60.0%)** | | (1.24-7.59) |
| **IHC profile** | | | | 0.0001 |
| HER2 positive (no Luminal) (n=72) | 18 (25.0%) | **54 (75.0%)** | **0.0001** | OR: 0.30 |
| Lunimal B-HER2 positive (n=135) | **73 (54.1%)** | 62 (45.9%) | | (0.16-0.57) |
| **ER** | | | | |
| <1% (n=67) | 18 (26.9%) | **49 (73.1%)** | **0.001** | |
| ≥ 1% (n=140) | **73 (52.1%)** | 67 (47.9%) | | |
| **PR** | | | | |
| <1% (n=115) | 37 (32.2%) | **78 (67.8%)** | **0.0001** | |
| ≥ 1% (n=92) | **54 (58.7%)** | 38 (41.3%) | | |
| **Hormone receptors** | | | | |
| Negative (n=65) | 18 (27.7%) | **47 (72.3%)** | **0.001** | |
| Positive (n=142) | **73 (51.4%)** | 69 (48.6%) | | |
| **Ki67** | | | | |
| <20% (n=33) | 18 (54.5%) | 15 (45.5%) | 0.179 | |
| 20% (n=172) | 72 (41.9%) | 100 (58.1%) | | |
| **Chemotherapy** | | | | |
| T+P (n=119) | 48 (40.3%) | 71 (59.7%) | | |
| T (n=70) | 35 (50.0%) | 35 (50.0%) | 0.503 | |
| T+L (n=13) | 5 (38.5%) | 8 (61.5%) | | |
| T+N (n=5) | 3 (60.0%) | 2 (40.0%) | | |

as well as the treatment and type of response are shown in Table 1.

In terms of response to primary systemic treatment, HER2 3+ tumors, as shown in Table 2, had a pCR rate of 60% (108/180) compared to 29.6% of HER2 2+/ISH amplified cases (8/27) (p=0.003), and the risk of achieving a pCR was three times higher in this first group of patients (OR: 3.07 (1.24-7.59), p=0.015). In turn, HER2- non-luminal tumors presented 75% pCR (54/72) compared

**Table 3:** Univariate and multivariate analysis of the complete pathological response in the breast.

| n=207 | NO pCR | pCR | p | p - OR (IC 95%) |
|---|---|---|---|---|
| **Age** | 55.70 ± 14.51 | 52.66 ± 11.84 | 0.101 | |
| **Histological subtype** | | | | |
| Carcinoma NST (n=195) | 74 (37.9%) | 121 (62.1%) | 0.142 | |
| ILC (n=6) | 4 (66.7%) | 2 (33.3%) | | |
| Others (n=6) | 4(66.7%) | 2 (33.3%) | | |
| **cT** | | | | |
| 1 (n=20) | 6 (30%) | 14 (70%) | | |
| 2 (n=131) | 55 (42%) | 76 (58%) | 0.728 | |
| 3 (n=33) | 13 (39.4%) | 20 (60.6%) | | |
| 4 (n=23) | 8 (34.8%) | 15 (65.2%) | | |
| **cN** | | | | |
| 0 (n=73) | 35 (47.9%) | 38 (52.0%) | | |
| 1 (n=107) | 40 (37.3%) | 67 (62.6%) | 0.282 | |
| 2 (n=27) | 8 (29.6%) | 19 (70.3%) | | |
| **Type of surgery** | | | | |
| Conservative (n=109) | 41 (37.6%) | 68 (62.4%) | 0.554 | |
| Radical (n=96) | 40 (41.7%) | 56 (58.3%) | | |
| **Her2neu** | | | | 0.006 |
| 2+ (n=27) | **19 (70.4%)** | 8 (29.6%) | **0.0001** | OR: 3.59 |
| 3+ (n=180) | 63 (35.0%) | **117 (65.0%)** | | (1.43-9.00) |
| **IHC profile** | | | | 0.04 |
| HER2 positive (no Luminal) (n=72) | 16 (22.2%) | **56 (77.8%)** | **0.0001** | OR:0.46 |
| Luminal B-HER2 positive (n=135) | **66 (48.9%)** | 69 (51.1%) | | (0.21-0.99) |
| **ER** | | | | |
| <1% (n=67) | 16 (23.9%) | **51 (76.1%)** | **0.001** | |
| ≥ 1% (n=140) | **66 (47.1%)** | 74 (52.9%) | | |
| **PR** | | | | 0.05 |
| <1% (n=115) | 32 (27.8%) | **83 (72.2%)** | **0.0001** | OR: 0.51 |
| ≥ 1% (n=92) | **50 (54.3%)** | 42 (45.7%) | | (0.26-1.02) |
| **Hormone receptors** | | | | |
| Negative (n=65) | 16 (24.6%) | **49 (75.4%)** | **0.003** | |
| Positive (n=142) | **66 (46.5%)** | 76 (53.5%) | | |
| **Ki67** | | | | |
| <20% (n=33) | 14 (42.4%) | 19 (57.6%) | 0.709 | |
| ≥ 20% (n=172) | 67 (39.0%) | 105 (61.0%) | | |
| **Chemotherapy** | | | | |
| T+P (n=119) | 43 (36.1%) | 76 (63.9%) | | |
| T (n=70) | 31 (44.3%) | 39 (55.7%) | 0.549 | |
| T+L (n=13) | 5 (38.5%) | 8 (61.5%) | | |
| T+N (n=5) | 3 (60.0%) | 2 (40.0%) | | |

**Table 4:** Univariate and multivariate analysis of the complete pathological response in the axilla.

| n=134 | NO pCR | pCR | p | p - OR (IC 95%) |
|---|---|---|---|---|
| **Age** | 56.15 ± 14.61 | 53.49 ± 12.57 | 0.288 | |
| **Histological subtype** | | | | |
| Carcinoma NST (n=126) | 37 (29.4%) | 89 (70.6%) | 0.659 | |
| ILC (n=4) | 1 (25.0%) | 3 (75.0%) | | |
| Others (n=4) | 2 (50.0%) | 2 (50.0%) | | |
| **cT** | | | | |
| 1 (n=13) | 3 (23.1%) | 10 (76.9%) | | |
| 2 (n=72) | 22 (30.6%) | 50 (69.4%) | 0.931 | |
| 3 (n=28) | 8 (28.6%) | 20 (71.4%) | | |
| 4 (n=21) | 7 (33.3%) | 14 (66.7%) | | |
| **cN** | | | | |
| 1 (n=107) | 34 (31.7%) | 74 (69.2%) | 0.419 | |
| 2 (n=27) | 7 (25.9%) | 20 (74.1%) | | |
| **Type of surgery** | | | | |
| Conservative (n=58) | 13 (22.4%) | 45 (77.6%) | 0.124 | |
| Radical (n=75) | 26 (34.7%) | 49 (65.3%) | | |
| **Her2neu** | | | | |
| 2+ (n=16) | 7 (43.8%) | 33 (28.0%) | 0.195 | |
| 3+ (n=118) | 33 (28.0%) | 85 (72.0%) | | |
| **IHC profile** | | | | 0.001 |
| HER2 positive (No Luminal) (n=49) | 5 (10.2%) | **44 (89.8%)** | **0.0001** | OR: 0.16 |
| Luminal B-HER2 positive (n=85) | **35 (41.2%)** | 50 (58.8%) | | (0.058-0.45) |
| **ER** | | | | |
| <1% (n=45) | 5 (11.1%) | **40 (88.9%)** | **0.001** | |
| ≥ 1% (n=89) | **35 (39.3%)** | 54 (60.7%) | | |
| **PR** | | | | |
| <1% (n=78) | 17 (21.8%) | **61 (78.2%)** | **0.016** | |
| ≥ 1% (n=56) | **23 (41.1%)** | 33 (58.9%) | | |
| **Hormone receptors** | | | | |
| Negative (n=44) | 5 (11.4%) | **39 (88.6%)** | **0.001** | |
| Positive (n=90) | **35 (38.9%)** | 55 (61.1%) | | |
| **Ki67** | | | | |
| <20% (n=20) | 9 (45.0%) | 11 (55.0%) | 0.1 | |
| ≥ 20% (n=112) | 30 (26.8%) | 82 (73.2%) | | |
| **Chemotherapy** | | | | |
| T+P (n=71) | 20 (28.2%) | 51 (71.8%) | | |
| T (n=54) | 16 (29.6%) | 38 (70.4%) | 0.51 | |
| T+L (n=5) | 3 (60.0%) | 2 (40.0%) | | |
| T+N (n=4) | 1 (25.0%) | 3 (75.0%) | | |

to 45.9% of luminal HER2-positive tumors (62/135) (p=0.0001). No differences in pCR rates were observed depending on Ki67 (p=0.179) or type of surgery or anti-HER2 treatment received (p=0.503).

This pattern of response was also observed in the separate analysis according to the tumor site (breast or axilla) (Table 3) so that no residual invasive disease was detected in breast in 65% of HER2 3+ tumors (p=0.0001) and in 77.8% of HER2 2 positive-non-luminal (p=0.0001). This significance persisted in multivariate analysis, both for HER2 3+ tumors (p=0.006), with an OR of 3.59 (95% CI: 1.43-9.00), and for non-luminal HER 2 (p=0.048), with an OR of 0.46 (95% CI: 0.21-0.99). Non-expression of progesterone receptors showed an important relationship with complete pathological response (p=0.05), with an OR of 0.51 (95% CI: 0.26-1.20).

**Table 5:** Comparative stratified analysis of the general complete pathological response, in the breast and in the axilla, depending on the co-expression of hormonal receptors and the degree of HER2 overexpression.

| GENERAL | | NO pCR | pCR | p |
|---|---|---|---|---|
| 2+ (n=27) | Hormone receptors negative (n=6) | 3 (50.0%) | 3 (50.0%) | 0.215 |
| | Hormone receptors positive (n=21) | 16 (76.2%) | 5 (23.8%) | |
| 3+ (n=180) | Hormone receptors negative (n=61) | 15 (24.6%) | **46 (75.4%)** | **0.003** |
| | Hormone receptors positive (n=119) | **57 (47.9%)** | 62 (52.1%) | |
| **IN THE BREAST** | | | | |
| 2+ (n=27) | Hormone receptors negative (n=6) | 3 (50.0%) | 3 (50.0%) | 0.215 |
| | Hormone receptors positive (n=21) | 16 (76.2%) | 5 (23.8%) | |
| 3+ (n=180) | Hormone receptors negative (n=61) | 13 (21.3%) | **48 (78.7%)** | **0.006** |
| | Hormone receptors positive (n=119) | **50 (42.0%)** | 69 (58.0%) | |
| **IN THE AXILLA** | | | | |
| 2+ (n=16) | Hormone receptors negative (n=4) | 0 (0.0%) | **4 (100%)** | **0.04** |
| | Hormone receptors positive (n=12) | **7 (58.3%)** | 5 (41.7%) | |
| 3+ (n=118) | Hormone receptors negative (n=41) | 5 (12.2%) | **36 (87.8%)** | **0.005** |
| | Hormone receptors positive (n=77) | **28 (36.4%)** | 49 (63.6%) | |

**Table 6:** Relationship between HER2 overexpression and hormone receptors co-expression

| | HER2 non-Luminal (n=72) | Luminal HER2 (n=135) | p |
|---|---|---|---|
| HER 2+ (n=27) | 5 (18.5%) | **22 (81.5%)** | 0.05 |
| HER 3+ (n=180) | **67 (37.2%)** | 113 (62.8%) | |

Table 4 shows the analysis of axillary response, and although no significant differences were found for the relationship with HER2 3+ profile (p=0.195), a significant relationship (p=0.0001) was observed between pathological response and HER2 non-luminal profile (89.9%), which persisted in multivariate analysis (p=0.001), with an OR of 0.16 (95% CI: 0.058-0.45).

Combined stratified analysis for the relationship between pCR as a function of hormone receptor co-expression in HER2 3+ and HER2 2+ cases showed a significant relationship between pCR and HER2 non-luminal profile, both in the general series (75.4%, p=0.003), as when considering separately the response in the breast (78.7%, p=0.006) and axilla (87.8%, p=0.005) (Table 5). In the case of complete response in the axilla, this was even observed in all Her2 2+ cases in the absence of hormone receptor co-expression (p=0.042).

Finally, when examining the possible relationship between tumors with and without hormone receptor co-expression and HER2 overexpression, an important relationship (p=0.05) was found between HER2 2+ tumors and those with hormone receptor co-expression (81.5%) (Table 6).

## Discussion

The most important prognostic factor in breast cancer treated with primary systemic therapy, both for recurrence and survival, is pCR [21]. This means that tumors with a higher rate of pCR present a longer survival and that it is considered a surrogate marker of outcome [7]. With the application of primary therapy with dual blockade (trastuzumab and pertuzumab, pCR rates of 46% to 66% have been achieved for tumors with HER2 overexpression [11,22].

ASCO and CAP jointly publish since 2013 recommendations for the assessment of HER2 in breast cancer [13,23,24]. According to these guidelines, there are different definitions of a HER2 positive tumor, either by immunohistochemistry (complete and intense membrane staining in >10% of neoplastic cells) and/or by *in situ* hybridization (demonstrating amplification of the *Her2neu* gene) [13]. This concept is essential, not only when it comes to indicating the treatment, but also when it comes to predict the degree of pathological response, since different types of response have been described according to the type of "positivity" of HER2. In this sense, the ToGA study already demonstrated that, in gastric carcinoma, the response to treatment did not depend only on HER2 positivity defined exclusively by gene amplification but also on immunohistochemical expression [25].

In breast cancer, several studies have focused on the relationship between the type of response and the overexpression of HER2. Zhao et al. [14] demonstrated how tumors with an immunohistochemical HER2 3+ pattern and those HER2 2+ amplified (assessed by FISH), although they both presented high rates of pCR, the response rate was lower for the latter. Krystel-Whittemore et al. [15], in 560 cases, found a pCR of 92% *vs.* 29% when comparing tumors with protein overexpression and cases with gene amplification without immunohistochemical overexpression. In this aspect, the results of our work are aligned, as 70.4% of the HER2 2+/ISH + tumors did not achieve a pCR. Furthermore, this was observed both for the global series and for breast response, but not in the case of lymph node, where the differences in axillary pCR were not statistically significant depending on the overexpression of HER2. This could be consistent with a greater cellular heterogeneity in tumors spread to the lymph nodes, with a theoretically greater chemoresistance in these cases.

Also, in a recent study on 173 tumors [26], Meisel et al. found that the degree of HER2 overexpression was a variable related to pCR (71.4% in HER2 3+ *vs.* 28.6% in HER2 1+/2+), although in this case the pCR definition included the RCB-I in addition to the pCR [26].

Another relevant aspect in the response of these tumors to neoadjuvant treatment is the interference that the co-expression of hormonal receptors may have on it. In the definition of the different

tumor types of invasive breast cancer, two types of HER2 tumors are differentiated depending on this fact [27]: Non-luminal HER2 tumors (without estrogen or progesterone receptor co-expression) and HER2-luminal tumors (in which HER2 overexpression is accompanied by estrogen receptor expression). This distinction has prognostic and predictive implications. The interaction between hormone receptor pathways and HER2 pathways has been extensively described previously, which could explain different response patterns [28]. Thus, resistance to hormonal treatment has been reported in HER2-luminal tumors compared to luminal [29,30] due to the activation of hormonal receptors that is produced by the interaction that the activation of HER2 supposes, even with active hormonal treatment [31] and that, according to other series, can be partially reversed with treatment with HER2 inhibitors (trastuzumab) and improve prognosis [32,33].

The influence of the co-expression of hormonal receptors, in the response to primary systemic therapy in HER2 tumors, is shown in the series of Meisel JL et al. [26], in which the presence of both estrogen and progesterone receptors is related to a lower pCR. In fact, the multivariate analysis shows that the presence of progesterone receptors had a slight negative influence (OR: 0.99) on the pCR. Also, Hou Y et al. [16] found that the expression of progesterone receptors is related in the multivariate analysis with a worse pCR (OR: 0.21) although this was not the case the presence of estrogen receptors. Myers et al. also demonstrated higher rates of pCR according to the tumor subtype, being lower in Luminal cases [34]. In the results of the present study, the negative effect of the co-expression of hormonal receptors with pCR is confirmed, both in relation to the response in the breast and in the axilla. In this sense, the results coincide in the deleterious effect produced by the presence of progesterone receptors in the pCR of breast disease (not in the axilla), reducing the pCR rate by half (OR: 0.51) when it is expressed.

Thus, the co-expression of hormonal receptors and HER2 carry peculiar prognostic properties to the patients. First, and like it has previously been demonstrated by other authors [17,26,35] co-expression leads to a significant decrease in the rate of pCR, both in the breast and axilla. However, some series have been reported in which a better prognosis is observed, at least during early follow-up (first 4 to 5 years) in triple positive cases [36]. In addition to its relationship with the response, an interesting aspect is that the analysis of the tumors characteristics shows that there is a greater relationship between tumors with lower overexpression of HER2 (HER2 2+/ ISH +) and the co-expression of hormonal receptors.

Regarding other variables related to pCR that have been described in the literature, such as histological grade and double blockade (14), Ki67 proliferation index and tumor [26], no significant differences were found in the present series.

Finally, it should be noted that, despite the limitations of being a retrospective study with a limited series of cases, the results of this study also endorse the differences that exist for complete response in the breast and in the axillary nodes. As in other studies, different response rates are evidenced in both locations, as well as greater heterogeneity in the degree of response in axillary disease that depends, among other variables, on the type of tumor [37].

Our study highlights that, globally, not all tumors considered as "HER2 positive" show the same response to neoadjuvant treatment, and pCR rates is greater in tumors with complete immunohistochemical overexpression (IHC 3+) and without hormone receptor co-expression (non-luminal HER2 +). Furthermore, the co-expression of progesterone receptors is related to a lower pCR in the breast. Regarding the response of axillary lymph nodes, the degree of overexpression of HER2 does not seem to play a role, while the co-expression of hormone receptors leads to a lower rate of axillary pCR. These results are aligned with those published in other series, but long-term follow-up is mandatory to confirm if these differences are maintained in the patient outcome.

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