

# Predictive factors of pathological complete response in a Portuguese population with breast cancer treated with neoadjuvant chemotherapy

## Factores predictivos de resposta patológica completa numa população portuguesa com cancro da mama submetida a quimioterapia neoadjuvante

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### Abstract

**Overview and Aims:** Neoadjuvant chemotherapy is increasingly being used in the treatment of operable breast cancer. Our aim was to evaluate the factors that predict pathological complete response (pCR) after neoadjuvant chemotherapy in a Portuguese population of breast cancer patients.

**Study design:** Retrospective, cross-sectional study.

**Population:** Women diagnosed with breast cancer stage I-III treated with neoadjuvant chemotherapy and subsequent surgery in our institution during a 6-year period.

**Methods:** Clinical records were reviewed. Patient demographics and tumour characteristics were evaluated. Univariate and multivariate analysis were performed to evaluate tumour's clinical and immunohistochemical characteristics associated with pCR.

**Results:** A total of 164 patients enrolled the study. The overall rate of pCR was 17.7%. The rate was significantly higher in HER2-positive (non-luminal) tumours (66.7%) and lower in luminal A tumours (0%). The nuclear grade, estrogen receptor (ER), progesterone receptor (PR), HER2 status and Ki67 proliferation index significantly influenced the rate of pCR. However, multivariate analysis revealed that only negative ER and positive HER2 are significant predictors of pCR [respectively, ORadj 0.187 (95% CI 0.038-0.908; p=0.038) and ORadj 4.186 (95% CI 1.401-12.508; p=0.010)].

**Conclusion:** ER status and HER2 status are the most important predictors of pathological complete response. These findings should be considered in patient counselling, providing a more customized therapeutic approach..

**Keywords:** Breast cancer; Neoadjuvant therapy; Pathology; Immunohistochemistry.

### INTRODUCTION

Neoadjuvant chemotherapy (NAC), initially considered the standard of care for inoperable locally advanced breast cancer, has become more common for patients with operable disease<sup>1</sup>. The results of the NSABP B-18 trial showed that preoperative chemotherapy allows for a higher rate of breast conserving

surgery, especially in patients with tumours larger than 5 cm<sup>2,3</sup>. Also, it provides predictive information regarding the response of the primary tumour and involved lymph nodes to treatment<sup>2-5</sup>.

The interpretation of data from NAC trials is challenging due to the lack of a uniform definition for pathological complete response (pCR). According to the CTNeoBC consortium, pCR may be defined as the

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absence of any residual invasive cancer (ypT0/is ypN0) or any invasive and non-invasive tumour (ypT0 ypN0) following neoadjuvant therapy<sup>6</sup>. Eradication of the tumour from both the breast and lymph nodes was associated with improved event free survival (EFS) and overall survival (OS) compared to eradication of the tumour from the breast alone<sup>6</sup>.

Studies have shown that pCR is significantly correlated with improved survival. Patients achieving a pCR after NAC show a significantly improved disease free survival (DFS) and OS compared to those without pCR<sup>3,4,7-9</sup>. The strongest association between pCR and long-term outcome seems to be in patients with triple negative; hormone-receptor-positive, high-grade, and HER2-negative; and HER2-positive and hormone-receptor-negative tumours<sup>6,7,10</sup>. This association is more controversive for luminal B (HER2-negative or positive) and hormone-receptor-positive low grade tumours<sup>6,9-12</sup>.

The prognostic impact of pCR is well established. Defining clinical and immunohistological factors that better predict the achievement of pCR improves patient counseling and allows for a more tailored and individualized therapeutic approach.

We reviewed our single-institution experience of obtaining a pCR after NAC over a 6-year period and report for the first time the factors that predict pCR in a Portuguese population treated with NAC.

## METHODS

An observational retrospective single-centre study was conducted between January 2011 and December 2016 with patients diagnosed with breast cancer clinical stage I-III at Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal.

As eligibility criteria we included patients who had a histological diagnosis of breast cancer based on a core biopsy; had received neoadjuvant chemotherapy (NAC) and had undergone surgery after NAC. Inflammatory carcinomas, stage IV patients and patients whose pre- or post-NAC specimens were unavailable were excluded.

Tumour stage and T and N factors were stratified based on the American Joint Committee on Cancer (AJCC) Cancer Staging System, Eighth Edition<sup>13</sup>.

Patient characteristics were obtained through retrospective clinical records analysis and included: age at diagnosis, primary tumour classification (cT), clinical nodal status (cN), histological subtype, nuclear grade, immunohistochemical (IHC) evaluation of es-

trogen receptors (ER), progesterone receptors (PR), human epidermal growth factor receptor 2 (HER2) status, Ki67 proliferation marker, NAC regimen, type of surgery and pCR status. Clinicopathological classification was concluded based on ER, PR and HER2 status, as well as on Ki67 index: Luminal A-like (ER-positive, HER2-negative, Ki67 <20%, PR >20%); Luminal B-like HER2 positive (ER-positive, HER2-positive, any Ki67, any PR); Luminal B-like HER2 negative (ER-positive, HER2-negative and either Ki67 >20% or PR <20%); HER2-positive (non-luminal) (ER and PR absent, HER2-positive); triple negative (ER and PR <1%, HER2-negative)<sup>14,15</sup>. Tumours were considered ER-positive when ER  $\geq$  1% of tumour cell nuclei were immunoreactive as defined by the American Society of Clinical Oncology/ College of American Pathologists Guideline Recommendations<sup>15</sup>.

ER, PR, HER2 and Ki67 were evaluated by IHC analyses, performed on formalin-fixed paraffin-embedded tissue sections<sup>14,16</sup>. Tumours were considered HER2-positive when a score of 3+ was obtained on IHC and/or a ratio of greater than 2.0 was obtained on FISH analysis<sup>14</sup>.

All patients were submitted to a sequential regimen of anthracyclines and taxanes. In HER2-positive tumours, trastuzumab was started in association with the taxane part of the chemotherapy regimen and maintained for one year.

pCR was defined as absence of invasive cancer in the breast and axillary nodes (ypT0/is ypN0), as previously described<sup>6,7</sup>.

Statistical analysis was performed using SPSS v.23.0 (IBM Corp.). Univariate analysis was carried out to describe and compare the baseline characteristics tumours with or without pCR and to explore correlations between variables. Categorical variables were compared using the  $\chi^2$  test or the Fisher's exact test, according to the Cochran rules. Normality of continuous variables was assessed with Kolmogorov-Smirnov test, determining the use of independent samples Student's t-test when normality was assumed or independent samples Mann-Whitney U test as the non-parametric alternative. Multivariate logistic regression was performed to analyse the factors that were predictive of pCR in univariate analysis.

## RESULTS

A total of 164 patients were enrolled in the study. Pa-

<b>QUADRO I. PATIENT DEMOGRAPHICS AND TUMOR CHARACTERISTICS BEFORE NAC</b>					
<b>Characteristics</b>	<b>Entire cohort n=164</b>	<b>No pCR n=136</b>	<b>pCR n=28</b>	<b>p value*</b>	<b>Univariate OR (95% CI)</b>
Age at diagnosis (years)	52.4±11.1	52.0±11.3	54.1±9.7	0.366	
Post-menopause. % (n)	51.8 (85)	48.5 (65)	64.3 (18)	0.129	1.911 (0.822-4.444)
Multifocal tumor	27.4 (45)	27.4 (37)	28.6 (8)	0.900	1.059 (0.429-2.613)
Primary tumor stage (cT)					
cT1	9.1 (15)	8.1 (11)	14.3 (4)	0.300	1.894 (0.556-6.447)
cT2	51.2 (84)	51.5 (70)	53.6 (15)	0.839	1.088 (0.481-2.458)
cT3	25.6 (42)	26.5 (36)	21.4 (6)	0.578	0.758 (0.284-2.018)
cT4	13.5 (22)	14.0 (19)	10.7 (3)	0.645	0.739 (0.203-2.690)
Clinical nodal status (cN)					
cN+	79.3 (130)	81.6 (111)	67.9 (19)	0.102	0.475 (0.193-1.174)
Histologic type					
Invasive NST	89.0 (146)	88.2 (120)	92.9 (26)	0.532	1.733 (0.375-8.004)
Lobular invasive	7.3 (12)	8.8 (12)	0	0.103	0.816 (0.756-0.880)
Others	3.7 (6)	2.9 (4)	7.1 (2)	0.281	2.538 (0.442-14.589)
Nuclear grade	2.2±0.7	2.1±0.7	2.5±0.6	0.004	
Missing	3.0 (5)				
ER Status				0.001	0.256 (0.110-0.595)
Positive	72.0 (118)	77.2 (105)	46.4 (13)		
Negative	28.0 (46)	22.8 (31)	53.6 (15)		
PR Status				0.049	0.441 (0.193-1.008)
Positive	59.1 (97)	63.0 (85)	42.9 (12)		
Negative	40.6 (67)	37.0 (50)	57.6 (16)		
Missing	0.6 (1)				
HER2 Status				<0.001	4.882 (2.072-11.500)
Positive	25.0 (41)	19.1 (26)	53.6 (15)		
Negative	75.0 (123)	80.9 (110)	46.4 (13)		
Ki67 (%)	39.3±26.3	34.8±24.7	57.8±24.8	<0.001	
Missing	27.4 (45)				
Clinicopathologic classification					
Luminal A-like	13.4 (22)	22.9 (22)	0	0.011	0.763 (0.683-0.852)
Luminal B-like (HER2-positive)	17.7 (29)	16.2 (22)	24.9 (7)	0.265	1.727 (0.655-4.554)
Luminal B-like (HER2-negative)	22.6 (37)	28.3 (32)	18.5 (5)	0.299	0.575 (0.201-1.650)
HER2-positive (non-luminal)	7.3 (12)	2.9 (4)	28.6 (8)	<0.001	13.200 (3.637-47.909)
Triple negative	19.5 (32)	19.1 (26)	21.4 (6)	0.779	1.154 (0.425-3.133)
Sentinel node biopsy (n=51)				1.000	1.000 (0.253-3.945)
Positive	33.3 (17)	33.3 (13)	33.3 (4)		
Negative	66.7 (34)	66.7 (26)	66.7 (8)		
Breast surgery				0.353	1.582 (0.597-4.192)
Breast conserving surgery	28.7 (47)	30.1 (41)	21.4 (6)		
Mastectomy	71.3 (117)	69.9 (95)	78.6 (22)		

Values are mean±standard deviation or % (n); Invasive NST, Invasive carcinoma of no special type; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2. \*No pCR vs pCR. Chi-square test was used for categorical variables and Mann-Whitney U test was used for continuous variables comparing each location with all the others.

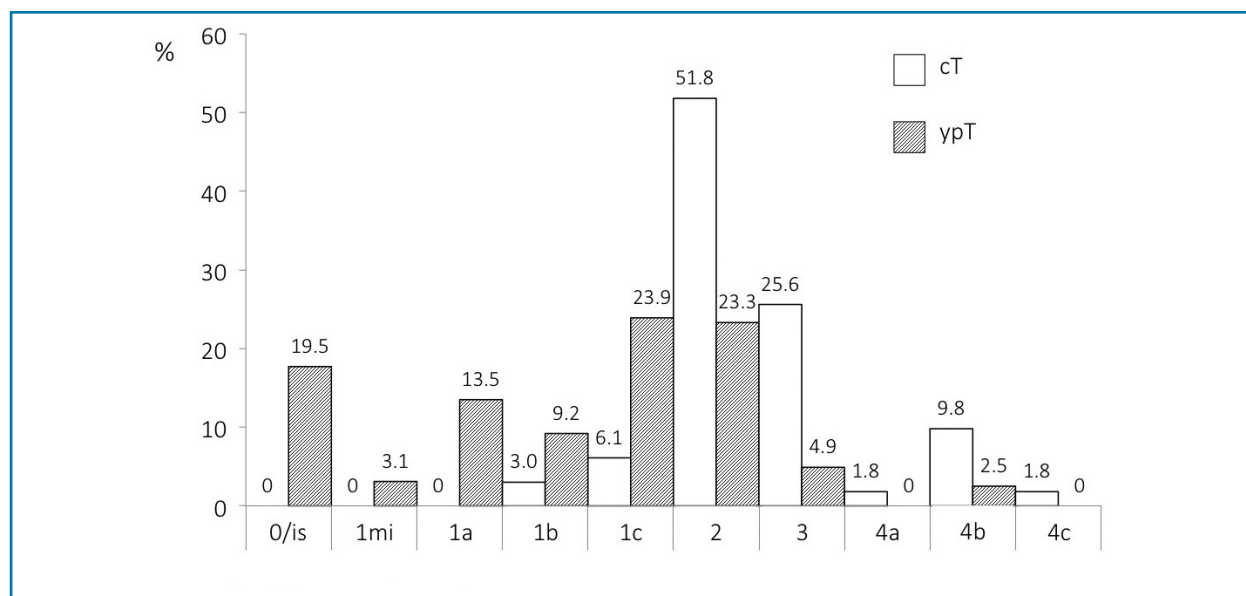


FIGURE 1. Tumour stage before and after NAC

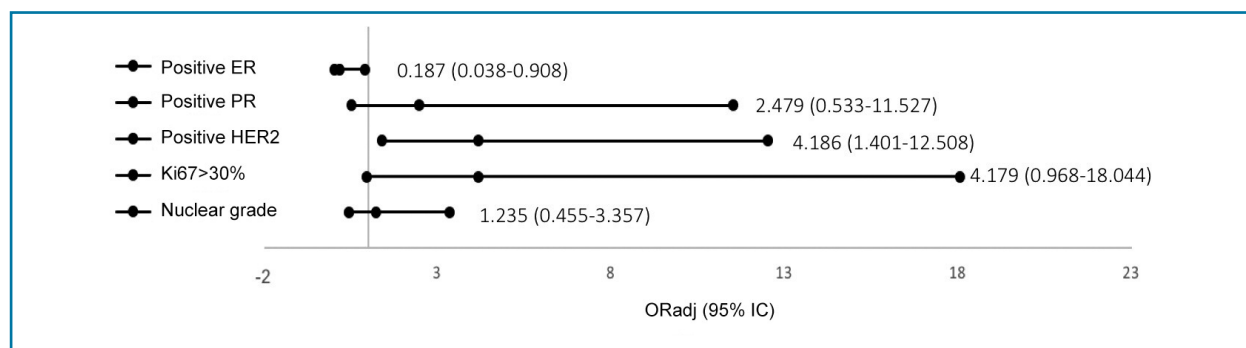


FIGURE 2. Factors that influence pCR

tient demographics and tumour characteristics are described in Table 1. The mean age at diagnosis was  $52.4 \pm 11.1$  years. The histological type invasive NST was the most frequent (89.0%, n=146) and most tumours were unifocal (72.6%, n=119). Tumour size classification before and after NAC is displayed in Figure 1. Most patients were cT2 or higher before NAC (90.8%, n=148), while the majority was ypT1 or lower after NAC (69.2%, n=114).

Most patients (79.3%, n=130) had cN+ nodal status before NAC. Sentinel node biopsy was performed in 31.1% (n=51), of which 44.9% (n=20) were positive for tumour deposits. Axillary lymphadenectomy was performed in 84.1% (n=138), of which 54.3% (n=75) presented metastasis. Nodal pCR was 43.1%.

The overall pCR was 17.7% (n=28). pCR by intrinsic subtype was as follows: Luminal A, 0%; Luminal B like HER2-negative, 13.5% (n=5); Luminal B like HER2-positive, 24.1% (n=7); HER2-positive (non luminal), 66.7% (n=8); Triple negative, 18.8% (n=6).

Univariate analysis (Table 1) revealed that nuclear grade, ER, PR and HER2 status and Ki67 significantly influenced the rate of pCR. However, regarding the clinicopathological classification, the rate of pCR was only significantly different for Luminal A-like and HER2-positive (non-luminal) tumours [respectively, OR 0.763 (95% CI 0.683-0.852; p=0.011) and OR 13.200 (95% CI 3.637-47.909; p<0.001)]. In our analysis, neither tumour histology, cT nor cN influenced the rate of pCR. The rate of pCR was also similar be-

tween patients submitted to mastectomy and breast conservative surgery [respectively, 18.8% (n=22) vs. 12.8% (n=6), OR= 1.582 (95%CI 0.597-4.192); p=0.353].

Multivariate analysis is displayed in Figure 2. Positive ER and HER2 status are significant predictors of pCR [respectively, ORadj 0.187 (95% CI 0.038-0.908; p=0.038) and ORadj 4.186 (95% CI 1.401-12.508; p=0.010)].

## DISCUSSION

The prognostic impact of the achievement of pCR is well recognised. Therefore, selecting patients who are most likely to benefit from NAC would be an important clinical step in their therapeutic approach. To our knowledge, this is the first study to perform such an analysis in a Portuguese population.

Our overall pCR rate was 17.7%. This result is similar to previous studies using the same pCR definition (ypT0/is N0). In a retrospective study by Choi *et al* (n=353) the pCR rate was 17.6%<sup>9</sup>. A pooled analysis from the Japan Breast Cancer Research Group (n=353) reported a pCR rate of 18.4%<sup>17</sup>. The meta-analysis published by the CTNeoBC group (n=13.125) revealed a pCR rate of 18%.<sup>6</sup> The Cochrane meta-analysis published in 2012 (n=1972) reported a pCR varying between 4.0% and 29.2%<sup>3</sup>. The German Breast Group and Arbeitsgemeinschaft Gynäkologische Onkologie-Breast Group (n=6377) reported a pCR rate of 19.8%<sup>11</sup>.

Similarly to the results of McFarland *et al* and Galvez *et al*, the only variables associated with pCR were the tumour's IHC features and not the patient's clinical characteristics (eg. age at diagnosis, menopause status, primary tumour size, tumour histology, tumour focality, clinical nodal status and sentinel node biopsy)<sup>8,18</sup>. Despite not reaching statistical significance, pCR was more prevalent in smaller tumours, which is in line with previous reports<sup>2,8</sup>. Some studies have reported non-lobular histology has a predictor of pCR. Besides, pCR patients with a lobular carcinoma do not seem to have a better long-term outcome when compared to non-pCR patients<sup>2,10</sup>. In our sample, none of the lobular tumours achieved pCR. However, our sample was underpowered to evaluate this difference.

As previously reported, univariate analysis revealed that nuclear grade, ER, PR and HER2 status and Ki67 significantly influenced the rate of pCR<sup>1,2,8,19</sup>. However,

multivariate analysis revealed that only negative ER and positive HER2 are significant predictors of pCR<sup>1,2,5,10</sup>. This is in line with the finding of a statistically significant difference in the pCR rate of subtypes Luminal A [OR 0.763 (95% CI 0.683-0.852); p=0.011] and HER 2-positive (non luminal) [OR 13.200 (3.637-47.909); p<0,001]. Similar findings have been reported previously<sup>2,18</sup>. ER-positive tumours (luminal A) are less responsive to chemotherapy and the prognostic significance of the achievement of pCR in these patients is yet to be cleared<sup>7</sup>. Highlighting the importance of targeted therapy, patients with HER2-positive tumours have experienced a significant prognostic improvement since the introduction of trastuzumab as a part of the primary treatment, especially when achieving pCR after NAC<sup>10</sup>. Similar to the results published by Asano *et al* but unlike previous studies, our study did not reveal significant differences regarding the pCR rate of triple negative tumours<sup>2,10,20</sup>. We hypothesize that this may be due to the small sample size of patients with triple negative tumours.

The finding of such a high rate mastectomy rate in the pCR group (78.6%, n=22) was surprising. However, in 4 patients, mastectomy was performed on patient demand. In the remaining 18 patients mastectomy was performed based on tumour size (large T2, T3 and T4 tumours), which was our Institutional approach during the study period.

The potential limitations of this study include its retrospective design and the limited number of patients and pCR events on the subgroups according to breast cancer subtypes.

## CONCLUSIONS

ER status and HER2 status were the most important predictors of pCR in our sample of Portuguese breast cancer patients treated with NAC. Patients with HER 2-positive (non luminal) breast cancer are most likely to achieve pCR while the benefit of NAC in Luminal A patients is yet to be established. In our sample triple negative tumours were not associated with pCR.

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