

Pathologic response and residual tumor cellularity after neo-adjuvant chemotherapy predict prognosis in breast cancer patients

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ABSTRACT

Introduction: Residual tumor cellularity (RTC) and pathologic complete response (pCR) after neo-adjuvant chemotherapy (NAC) are prognostic factors associated with improved outcomes in breast cancer (BC). However, the majority of patients achieve partial pathologic response (pPR) and no clear correlation between RTC patterns and outcomes was described. Our aims were to define predictive factors for pCR and compare different outcomes of patients with pCR or pPR and with different RTC patterns.

Materials and methods: Baseline and post-NAC demographics, clinicopathological characteristics, post-operative data, survival and recurrence status were recorded from our institutional database. A multivariable analysis was performed using a logistic regression model to identify independent predictors of pCR. Disease-free survival (DFS), distant disease-free survival (DDFS), and overall survival (OS) analyses were performed using the Kaplan-Meier method.

Results: Overall, of the 495 patients analyzed, 148 (29.9%) achieved pCR, 347 (70.1%) had pPR, and the median RTC was 40%. Multivariable analysis identified 3 independent factors predictive of pCR: tumor stage before NAC (cT1-2 84.5% versus cT3-4 15.5%), BC sub-type (HER2-positive 54.7% versus triple-negative 29.8% versus luminal-like 15.5%), and vascular invasion (absence 98.0% versus presence 2.0%). We found statistically significant longer DFS, DDFS, and OS in patients with pCR and with RTC <40%; no difference was observed in terms of OS between RTC <40% and RTC ≥40% groups.

Conclusions: Tumor stage before NAC, BC sub-type, and vascular invasion are significant and independent factors associated with pCR. Patients with pCR and with RTC <40% have longer DFS, DDFS, and OS compared with patients with pPR.

1. Introduction

Breast cancer (BC) is the most common cancer in women worldwide, and approximately 65% of women with BC are diagnosed with early-stage disease that is potentially curable with standard loco-regional and systemic treatments [1]. However, up to 30% of patients with BC experience disease recurrence, with either local or distant metastases

after surgery. Therefore, new treatment strategies aim to reduce the risk of recurrence and improve outcomes for these patients. Systemic treatment has historically been administered to patients with BC after surgery in an adjuvant setting [2]. Nowadays, however, the current indication recommends neo-adjuvant chemotherapy (NAC), as it has been shown to have several advantages when administered preoperatively. First, by downstaging the tumor burden, mastectomy can be

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avoided, and a better surgical outcome can be achieved. Second, tumor cellularity, defined as the percentage of invasive tumor cells to the primary tumor area, can be reduced to varying degrees in different BCs. In a large meta-analysis including more than 11,000 BC patients, Cortazar et al. [3] have confirmed the assumption that there is an association between the extent of tumor response to NAC and long-term outcomes. Residual tumor cellularity (RTC) after NAC is a histological measure of the response to primary systemic treatment and the evaluation of the extent of this response is an important prognostic factor for BC patients [4]. Indeed, achieving a pathologic complete response (pCR), has been associated with significant improvement in disease-free survival (DFS) and overall survival (OS) [5–7], and has therefore become as a surrogate end-point for long-term survival and primary aim in numerous clinical trials [3,8–10]. However, while only a minority of BC patients experience pCR after NAC [11], the majority of them, approximately 60–80%, achieve pathologic partial response (pPR) [4, 12–14]. Previous studies have attempted to classify the extent of RTC following NAC and its implication on oncological outcomes [8,15,16]; however, BC patients with different grades of pPR have been usually grouped and analyzed together with inconsistent results and unclear prognostic significance [17–20]. The primary aims of this study were to describe the clinical and therapeutic characteristics of BC patients treated with NAC, to identify independent predictive factors for pCR, and to compare the long-term oncological outcomes of patients achieving pCR or pPR. The secondary objective of this study was to measure the RTC of BC patients who failed to achieve a complete response after NAC and to compare the outcomes of groups of patients with different RTC in order to improve the prognostic information that can be obtained from the assessment of pPR.

2. Materials and methods

2.1. Study design and management of the patients

All the consecutive BC patients undergoing NAC at the Breast Unit of IRCCS Humanitas Research Hospital (Milan, Italy) between October 2006 and April 2020 and their corresponding post-operative pathology slides were reviewed. Baseline and post-NAC demographics, including age and menopausal status, and baseline and post-NAC clinical tumor and nodal stage, dimension, grade, sub-type, histotype, and vascular invasion were collected. Preoperative staging with bilateral breast and axillary ultrasound (US) was performed in all patients and preoperative diagnosis of invasive BC was based on a US-guided core needle biopsy (14-gauge). Before surgery, a metallic clip marker was placed in the original tumor area under US-guidance to enable identification of the primary BC at the time of surgery. Preoperative bilateral mammography, magnetic resonance imaging (MRI) of the breast, and total-body positron emission tomography (PET) were performed in the majority of patients. A multidisciplinary tumor board composed of breast surgeons, breast oncologists, breast pathologists, oncoplastic surgeons, radiotherapists, and radiologists discussed the management of every BC patient. Patients with cM1 disease were not excluded *a priori*; in fact, a limited subset of oligometastatic BC patients with a good performance status and no other comorbidities received preoperative chemotherapy with the intent of cytoreduction before surgery. Before surgery, to monitor response to systemic treatment and to estimate tumor size, all patients underwent post-NAC evaluation by one or a combination of the following examinations: bilateral breast and axillary US, MRI of the breast, or PET. All patients underwent either BCS or mastectomy. In patients who underwent BCS, an intraoperative radiograph of the specimen was obtained to correctly localize the tumor and metal clip marker and to assess the proximity of the lesion to the surgical margins. The entire resection specimen was then stained and sectioned at 2- to 3-mm intervals for pathologic evaluation of the surgical margins. Haematoxylin and eosin-stained pathology slides of the specimen obtained after surgery were reviewed by two different specialized breast

pathologists at our institution. The following exclusion criteria were used: excisional biopsy or debulking surgery as the first BC operation, patients with a previous diagnosis of BC or other previous or synchronous malignancies, male patients, unknown NAC regimen, disease progression during NAC, follow-up ≤ 12 months. Adjuvant taxane-based chemotherapy was administered to patients who did not achieve pCR and had not completed both anthracycline- and taxane-based therapy before surgery. Capecitabine and Trastuzumab emtansine (T-DM1) were administered to triple-negative and HER2-positive BC sub-types that did not achieve pCR, respectively. Follow-up was performed every six months with physical examination and routine biochemical tests including carcinoma antigen 15-3 level. Bilateral mammography, chest X-ray, breast, axilla, and abdominal US were performed annually. Each patient gave informed consent for operation and clinical data collection.

2.2. Definitions

Hormone receptor status was assessed by immunohistochemistry (IHC). Estrogen and progesterone receptor status was considered positive if expressed in $>1\%$ immune-reactive cells. HER2 status was assessed by IHC (0, 1+, 2+, or 3+ score) and by fluorescent in situ hybridization (FISH), the latter performed in all patients with a HER2 IHC score of 2+. HER2 overexpression positivity was defined according to ASCO-CAP guidelines [21] for a membrane staining IHC score of 3+ or 2+ with evidence of FISH amplification. HER2 IHC scores of 1+ and 0 defined a HER2-negative status. The absence of invasive and non-invasive (ypTis) residual disease in both breast and axillary lymph nodes was defined as pCR. The presence of any residual disease in the breast and/or lymph nodes was defined as pPR. After surgery, sections of the tumor cross-sectional area were reconstructed and the percentage of the primary tumor area composed of residual invasive tumor cells was defined as RTC. The period from the date of BC surgery to the appearance of any tumor, including loco-regional recurrence or distant metastasis, was defined as DFS. The time interval from the date of BC surgery to the date of detection of distant metastasis was defined as distant disease-free survival (DDFS). The time interval from BC treatment to death from any cause or to the date of last contact was defined as OS.

2.3. Statistical analysis

For descriptive statistics: demographic, radiological staging, tumor, surgical treatment, and post-operative data were collected from our prospectively maintained institutional database and presented as frequencies and percentages. Categorical variables were presented as the median and range, compared using the chi-square test, and retrospectively linked to the pathological outcomes of pCR or the presence of pPR. A multivariable analysis was performed using a logistic regression model to identify independent predictors of pCR. The multivariable analysis included any variable associated with the result in the univariate analysis (inclusion cut-off value $p < 0.10$). For patients who failed to achieve pCR, the percentage of RTC was calculated and compared with the pre-NAC pathology slides. Two different groups of patients with pPR were created: RTC $<40\%$ and RTC $\geq 40\%$. The Kaplan-Meier method was used to estimate the recurrence and survival probabilities and the log-rank test was used to compare different groups of BC patients who underwent NAC (pCR *versus* pPR, and RTC $<40\%$ *versus* RTC $\geq 40\%$). The last follow-up was updated to July 2, 2021. Statistical significance was set at $p < 0.050$. Data analyses and figures were performed with IBM SPSS 25.0 software.

3. Results

3.1. Characteristics of the patients and independent predictive factors of pathologic complete response

A total of 495 BC patients underwent NAC at the Breast Unit of IRCCS Humanitas Research Hospital (Milan, Italy). The mean age of the patients was 50 years (range, 20–87), and 256 (51.7%) patients were post-menopausal. Bilateral mammography, MRI of the breast, and PET were performed in 345 (69.7%), 161 (32.5%), and 303 (61.2%) patients, respectively. Before NAC, the median diameter of the tumor was 30 mm (range, 7–115), and 313 (63.2%) patients were affected by cT2 BC. The majority of patients undergoing NAC were affected by HER2-positive BC sub-type (40.4%), followed by luminal-like tumors (36.0%), and triple-negative sub-type (23.6%). Regarding the NAC treatment protocol, 69 (13.9%) patients received anthracycline only for 4 cycles (e.g., FEC, EC or AC: 600 mg/m² fluorouracil plus 600 mg/m² cyclophosphamide plus 90 mg/m² epirubicin or 60 mg/m² doxorubicin, administered every 3 weeks), and 426 (86.1%) patients received anthracycline and taxane (e.g., 90 mg/m² epirubicin or 60 mg/m² doxorubicin plus 600 mg/m² cyclophosphamide every 3 weeks for four cycles followed by 75 mg/m² docetaxel for four cycles or by 80 mg/m² paclitaxel for twelve cycles). Overall, 194 (39.2%) patients received docetaxel (75 mg/m²) or paclitaxel (80 mg/m²) in combination with anti-HER2 therapies, either Trastuzumab (loading dose of 8 mg/kg followed by 6 mg/kg in subsequent cycles) in 185 patients or Pertuzumab (loading dose of 840 mg followed by 420 mg in subsequent cycles) in the remaining patients, every three weeks. After NAC, the median diameter of the tumor was 7 mm (range, 0–110). A total of 250 (50.5%) patients underwent BCS, while 245 (49.5%) patients underwent mastectomy. Regarding axillary surgical treatment, 229 (46.3%) patients underwent sentinel lymph node biopsy (SLNB) without subsequent axillary lymph node dissection (ALND), and 258 (52.1%) patients underwent ALND either directly or after SLNB. Regarding post-operative treatment, adjuvant chemotherapy was administered to 66 (13.3%) patients, either a taxane-based regimen in 41 patients or Capecitabine in 25 patients. Adjuvant radiotherapy was performed in 401 (81.0%) patients. Endocrine treatment was administered to 281 (56.8%) patients, and T-DM1 to 44 (8.9%) patients. Characteristics of BC patients undergoing NAC are summarized in Table 1. A total of 148 (29.9%) patients achieved pCR, while 347 (70.1%) had residual tumor at the post-operative pathology slides. At multivariable analysis, three independent factors predicting pCR were identified. Tumor stage pre-NAC (cT1-2 84.5% versus cT3-4 15.5%, odds ratio (OR) = 0.119, 95% confidence interval (95% CI) = 0.048–0.189, *p* = 0.001), BC sub-type (HER2-positive 54.7% versus triple-negative 29.8% versus luminal-like 15.5%, OR = 2.178, 95% CI = 2.055–2.301, *p* = 0.001), and vascular invasion (absence 98.0% versus presence 2.0%, OR = 0.022, 95% CI = 0.004–0.090, *p* = 0.001). Univariate and multivariable analyses are summarized in Table 2.

3.2. Long-term oncological outcomes and prognosis

After a median follow-up of 43 months, 102 (/495, 20.6%) BC patients who underwent NAC developed recurrence; of these, 18 (/495, 3.6%) patients developed loco-regional recurrence only, 59 (/495, 11.9%) patients developed distant recurrence only, and 25 (/495, 5.1%) patients developed loco-regional recurrence associated with metastases. In the pCR group, 1 (/148, 0.6%) and 6 (/148, 4.1%) patients had loco-regional recurrence and distant metastases, respectively. In the pPR group, 17 (/347, 4.9%) and 78 (/347, 22.8%) had loco-regional recurrence and distant metastases, respectively. A total of 74 (/495, 15.0%) BC patients who underwent NAC died during the observation period; of these, 56 patients suffered a BC related death and 18 patients died for other reasons. Overall, 2 (/148, 1.4%) and 72 (/347, 20.8%) patients died in the pCR and pPR group, respectively. The DFS rate at 3-, 5-, and 10-years was 94.9% in patients who achieved pCR. The DFS rate at 3-, 5-

Table 1

Title: Characteristics of 495 breast cancer patients treated with neo-adjuvant chemotherapy.

Characteristics	Number (%) / Median (range)
Patients	
Age (years)	50 (20–87)
Post-menopausal	256 (51.7%)
Preoperative staging	
Mammography	345 (69.7%)
Breast and axillary US	495 (100%)
Breast biopsy	495 (100%)
Axillary biopsy	205 (41.4%)
MRI	161 (32.5%)
PET	303 (61.2%)
Dimension pre-NAC (mm)	30 (7–115)
Stage pre-NAC	
cT1	66 (13.3%)
cT2	313 (63.2%)
cT3	75 (15.2%)
cT4	41 (8.3%)
cN0	151 (30.5%)
cN1	344 (69.5%)
cM1	74 (15.0%)
NAC with anthracycline only	69 (13.9%)
NAC with anthracycline and taxanes	426 (86.1%)
Trastuzumab	185 (37.4%)
Pertuzumab	9 (1.8%)
Tumor	
Sub-type	
Luminal-like	178 (36.0%)
HER2-positive	200 (40.4%)
Triple negative	117 (23.6%)
Histotype	
Ductal	466 (94.1%)
Lobular	19 (3.8%)
Mucinous	7 (1.4%)
Papillary	2 (0.4%)
Apocrine	1 (0.3%)
Vascular invasion	114 (23.0%)
Single nodule	362 (73.1%)
pCR to NAC	148 (29.9%)
Dimension post-NAC (mm)	7 (0–250)
Stage post-NAC	
ypT0	160 (32.3%)
ypTis	37 (7.5%)
ypTmi	8 (1.6%)
ypT1a	24 (4.9%)
ypT1b	50 (10.1%)
ypT1c	85 (17.2%)
ypT2	94 (19.0%)
ypT3	24 (4.9%)
ypT4	13 (2.5%)
ypNx	8 (1.6%)
ypN0	302 (61.0%)
ypNi+	1 (0.3%)
ypNmi	16 (3.2%)
ypN1	75 (15.2%)
ypN2	56 (11.3%)
ypN3	37 (9.0%)
Surgical treatment	
BCS	250 (50.5%)
Mastectomy	245 (49.5%)
SLNB not followed by ALND	229 (46.3%)
SLNB followed by ALND	49 (9.9%)
ALND	209 (42.2%)
Post-operative treatment	
Taxanes	44 (8.9%)
Capecitabine	25 (5.1%)
Radiotherapy	401 (81.0%)
Endocrine	281 (56.8%)
T-DM1	44 (8.9%)

Footnotes: US: Ultrasound, MRI: Magnetic resonance imaging, PET: Positron emission tomography, NAC: Neo-adjuvant chemotherapy, pCR: Pathologic complete response, BCS: Breast-conserving surgery, SLNB: Sentinel lymph node biopsy, ALND: Axillary lymph node dissection, CHT: Chemotherapy, T-DM1: Trastuzumab emtansine.

Table 2

Title: Predictors of pathologic complete response in breast cancer patients undergoing neo-adjuvant chemotherapy.

Characteristics	pCR (No. 148) Tot. (%)	pPR (No. 347) Tot. (%)	Univariate analysis <i>p</i> -value	Multivariable analysis <i>p</i> -value OR (95% CI)
Demographic				
Age (years)				
≤50	75 (50.7%)	166 (47.8%)	0.564	-
	73 (49.3%)	181 (52.2%)	-	
Menopausal status				
Pre-menopausal	75 (50.7%)	164 (47.3%)	0.488	-
Post-menopausal	73 (49.3%)	183 (52.7%)	-	
Preoperative staging				
Dimension pre-NAC (mm)				
≤30	95 (64.2%)	214 (61.7%)	0.597	-
	53 (35.8%)	133 (38.3%)	-	
Stage pre-NAC				
cT1-2	125 (84.5%)	254 (73.2%)	<0.001 ^a	0.001 ^a 0.119 (0.048–0.189)
- cT3-4	23 (15.5%)	93 (26.8%)	-	-
NAC				
Anthracycline and taxanes	142 (96.0%)	284 (81.8%)	<0.001 ^a	0.221 0.956 (0.898–1.013)
Anthracycline only	6 (4.0%)	63 (18.2%)	-	-
Tumor				
Sub-type				
Luminal-like	23 (15.5%)	155 (44.7%)	<0.001 ^a	0.001 ^a 2.178 (2.055–2.301)
HER2-positive	81 (54.7%)	119 (34.3%)	-	-
Triple negative	44 (29.8%)	73 (21.0%)	-	-
Vascular invasion				
Yes	3 (2.0%)	111 (32.0%)	<0.001 ^a	0.001 ^a 0.022 (0.004–0.090)
No	145 (98.0%)	236 (68.0%)	-	-
Single nodule				
Yes	106 (71.6%)	256 (73.8%)	0.031 ^a	0.217 0.215 (0.152–1.277)
No	42 (28.4%)	91 (26.2%)	-	-

Footnotes: pCR: Pathologic complete response, pPR: Pathologic partial response, OR: Odds ratio, 95% CI: 95% Confidence interval, NAC: Neo-adjuvant chemotherapy, ^a: Statistically significant.

, and 10-years was 76.8%, 70.5%, 63.3%, in patients who achieved pPR. The DDFS rate at 3-, 5-, and 10-years was 95.6%, 93.3%, 93.3%, and 80.1%, 71.5%, 57.9%, in patients who achieved pCR or pPR, respectively. The OS rate at 3-, 5-, and 10-years was 98.2% in patients who achieved pCR. The OS rate at 3-, 5-, and 10-years was 87.9%, 79.6%, 64.9%, among patients who achieved pPR. Patients with BC who underwent NAC and achieved pCR had statistically significant longer DFS, DDFS, and OS ($p = < 0.001$). Differences of oncological outcomes between pCR and pPR groups are summarized in Table 3 and Fig. 1. Overall, 289 (/347, 83.3%) post-operative pathology slides were available for analysis in BC patients with residual disease and the median RTC was 40%. Two different groups of BC patients who underwent NAC with pPR were created (RTC <40% and RTC ≥40%) and their long-term oncological outcomes were compared. The DFS rate at 3-, 5-, and 10-years was 81.3%, 76.1%, 67.4% and 69.7%, 64.7%, 55.7%, in RTC <40% and RTC ≥40% patients, respectively. The DDFS rate at 3-, 5-, and 10-years was 83.7%, 78.4%, 62.3%, and 73.6%, 62.9%, 50.1%, in RTC

Table 3

Title: Comparison of disease-free, distant disease-free and overall survival in breast cancer patients with different pathologic response after neo-adjuvant chemotherapy.

Outcomes	pCR	pPR	<i>p</i> -value	RTC<40%	RTC≥40%	<i>p</i> -value
DFS rate						
3-year	94.9%	76.8%	<0.001 ^a	81.3%	69.7%	0.033 ^a
5-year	94.9%	70.5%		76.1%	64.7%	
10-year	94.9%	63.3%		67.4%	55.7%	
DDFS rate						
3-year	95.6%	80.1%	<0.001 ^a	83.7%	73.6%	0.015 ^a
5-year	93.3%	71.5%		78.4%	62.9%	
10-year	93.3%	57.9%		62.3%	50.1%	
OS rate						
3-year	98.2%	87.9%	<0.001 ^a	86.9%	86.9%	0.148
5-year	98.2%	79.6%		82.6%	76.4%	
10-year	98.2%	64.9%		69.0%	58.2%	

Footnotes: pCR: Pathologic complete response, pPR: Pathologic partial response, RTC: Residual tumor cellularity, DFS: Disease-free survival, DDFS: Distant disease-free survival, OS: Overall survival, ^a: Statistically significant.

<40% and RTC ≥40% patients, respectively. The OS rate at 3-, 5-, and 10-years was 86.9%, 82.6%, and 69.0% and 86.9%, 76.4%, 58.2%, in RTC <40% and RTC ≥40% patients, respectively. Patients with RTC <40% presented statistically significant better DFS and DDFS ($p = 0.033$, $p = 0.015$, respectively). However, no statistically significant difference in terms of OS was observed between RTC <40% and RTC ≥40% groups ($p = 0.148$). Long-term oncological outcomes between different groups of RTC are summarized in Table 3 and Fig. 2.

4. Discussion

This retrospective analysis emphasizes the fact that patients with BC treated with NAC and subsequent surgery who achieve pCR have better long-term oncological results in terms of DFS, DDFS, and OS compared to patients with pPR. These results are consistent with findings from other studies in which pCR has been associated with better oncological outcomes and has also emerged as a surrogate end-point for survival in numerous NAC clinical trials [3,9]. It is well known that response to NAC varies according to different BC characteristics and molecular sub-types. Interestingly, achieving pCR seems to be more strongly associated with the most aggressive biological variables, including: HER2-positive and triple-negative tumors [7,22]. A meta-analysis of 30 studies including 11,695 patients evaluating pCR after NAC found an overall pooled estimate of pCR of 19.2%; and when compared to luminal-like BC, the probability of achieving pCR was seven times higher for HER2-positive disease and five times higher for triple-negative disease [23]. In a recent retrospective analysis, Sasanpour et al. [24] showed that achieving a pCR in BC patients treated with NAC was associated with both OS and DFS; the presence of vascular invasion (OR = 0.22, 95% CI = 0.10–0.46, $p = 0.001$) remained associated with a worse response to NAC at multivariate analysis. Pu et al. [25] developed a nomogram based on multiple clinical and biological covariates to predict early response to NAC and showed that vascular invasion was associated with lower grades of response. Additionally, histological smaller tumor size remains a predictor of good prognostic outcome post-NAC [26,27]. In our study, small tumor dimension (cT1-2), absence of vascular invasion, and HER2-positive tumors were confirmed as independent predictors of pCR.

The presence of pPR after NAC may indicate relatively resistant tumor biology and has been used as a selection criterion in clinical trials [28]. Higher prognostic information by pathological response may improve the clinical and scientific knowledge from previous studies. This is because, pPR after NAC encompassed a broad spectrum of actual responses, from near pCR to frank resistance; therefore, dichotomization of response as pCR and pPR is too simplistic for those purposes. In order to do so, various classifications and grading systems for pathologic

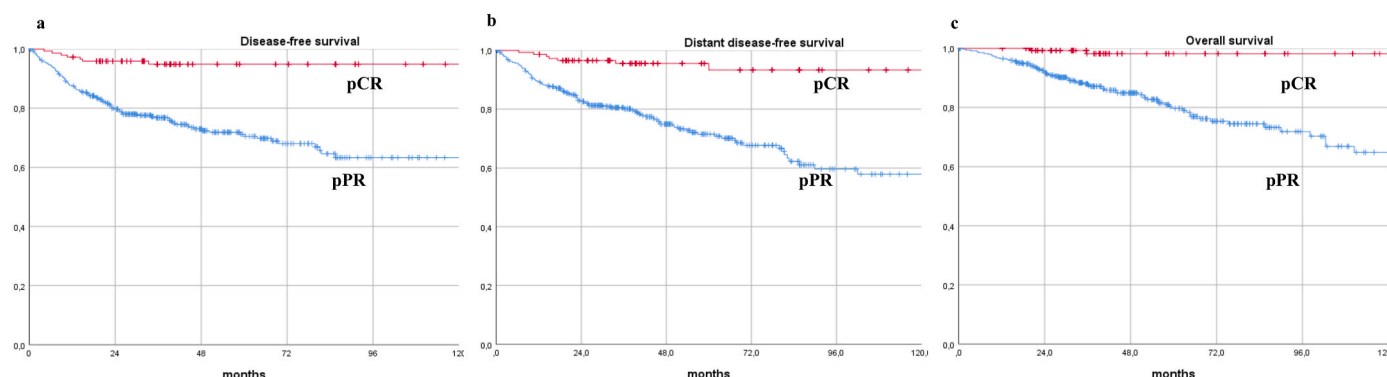


Fig. 1. (a–c) Title: Comparison of long-term oncological outcomes in breast cancer patients after neo-adjuvant chemotherapy (pathologic complete response *versus* pathologic partial response). This figure depicts the disease-free survival (a), distant disease-free survival (b), and overall survival (c) curves of breast cancer patients treated with neo-adjuvant chemotherapy and subsequent surgical operation who achieved pathologic complete response or with residual disease. Footnotes: pCR: Pathologic complete response, pPR: Pathologic partial response.

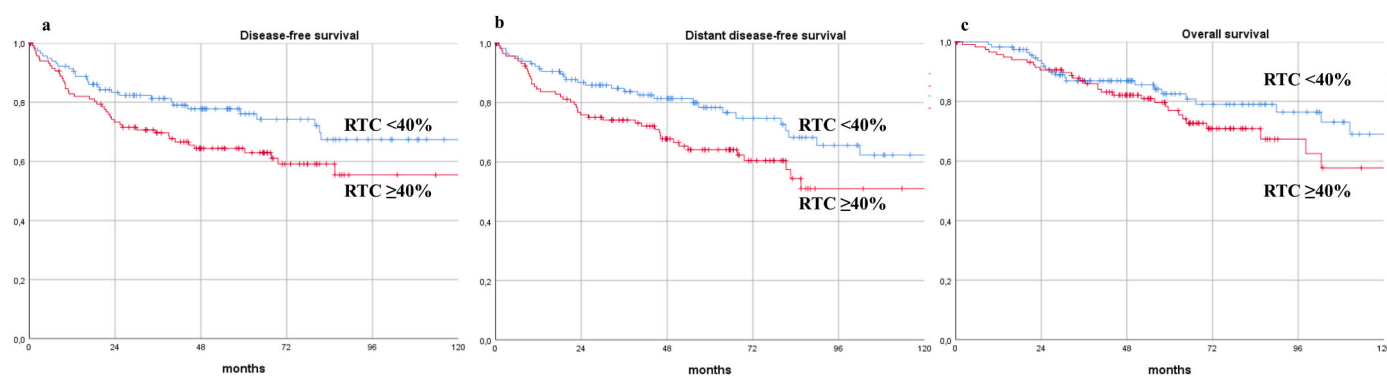


Fig. 2. (a–c) Title: Comparison of long-term oncological outcomes in breast cancer patients after neo-adjuvant chemotherapy and pathologic partial response with different residual tumor cellularity. This figure depicts the disease-free survival (a), distant disease-free survival (b), and overall survival (c) curves of breast cancer patients treated with neo-adjuvant chemotherapy with different patterns of residual tumor cellularity (<40% *versus* ≥40%). Footnotes: RTC: Residual tumor cellularity.

response after NAC have been proposed, but have been not used in clinical trials. Chevallier et al. [18] proposed a histopathological response system divided into four categories: 1, complete disappearance of the tumor both macroscopically and microscopically; 2, presence of *in-situ* carcinoma but no invasive tumor in the breast or lymph nodes; 3, invasive carcinoma with stromal modifications, such as sclerosis or fibrosis; 4, only minor changes in tumor appearance. Sataloff et al. [29] described a categorization system for NAC response: T-A, total or near-total therapeutic effect; T-B, subjectively >50% therapeutic effect; T-C, <50% therapeutic effect, but evident effect; T-D no evident effect or therapeutic benefit. Bonadonna et al. [30] described a system based on the change in BC: grade 1, some alterations to individual cells, but no overall decrease in cells number; grade 2, a slight loss of invasive cells but still high cellularity; grade 3, a significant loss of cells, up to 90%; grade 4, a marked reduction so that only small clusters of widely scattered cells are detected; grade 5, no invasive carcinoma detected. Smith et al. [31] recommended that BC histological characteristics post-NAC should be graded as: grade 1, no change or some alterations to individual malignant cells, but no reduction in total cell count; grade 2, a minor loss of tumor cells, but overall cellularity is still high (up to 30% loss); grade 3, between 30% and 90% reduction in tumor cells; grade 4, a marked disappearance of tumor cells such that only small clusters remain; grade 5, no malignant cells detectable. The same authors [8] validated the grading system on a consecutive series of 176 patients with large (≥4 cm) and locally advanced BC treated with NAC, surgery, radiotherapy, and tamoxifen, and found that there was a significant correlation between pathologic response according to the new grading

system and both OS and DSF. In our large series, we found that not all BC patients who fail to achieve pCR have the same prognosis; in fact, BC patients with a RTC <40% showed a statistically significant better DFS and DDFS.

No standard therapies exist for patients with residual disease after NAC and only few trials have explored this setting; therefore, the management of these patients still represents a clinical challenge [32]. The CREATE-X trial [33] was a multicenter, randomized, phase III clinical study that enrolled 910 patients with HER2-negative pPR to receive post-NAC treatment either with Capecitabine or without. The trial terminated early because the results of the *interim* analysis already met the primary end-point, considering the addition of adjuvant Capecitabine therapy was regarded as safe and effective in prolonging DFS and OS. In the KATHERINE trial, 1486 patients with pPR treated with anti-HER2 therapy were randomized to T-DM1 or continuation of Trastuzumab for 14 cycles. After a median follow-up of 41 months, 3-year DFS was 88.3% in the T-DM1 group *versus* 77.0% in the Trastuzumab arm ($p < 0.001$), approving T-DM1 for post-NAC treatment for patients with HER2-positive BC with pPR [28]. To sum up, the post-NAC setting is gaining increasing importance in the management of BC, prompting for an accurate patient selection that allows tailoring treatment according to the risk of recurrence [34].

Our study has some limitations. Firstly, it is a single-center analysis with a relatively short follow-up period, and it is subject to certain limitations because of its retrospective design based on observational data collected at a specific time point. Secondly, we defined pCR as the absence of invasive and non-invasive (ypTis) residual disease in both

breast and axilla; whereas, other authors [17] included patients with residual carcinoma *in-situ* in their definition of pCR, limiting the comparison of results. Moreover, 74 cM1 BC patients were included in the present analysis, representing a potential selection bias that might have affected our final results. Larger series of BC patients with longer observation period enrolled in multicenter clinical NAC trials are needed in order to validate our results.

5. Conclusions

In conclusion, our results show that tumor stage before NAC, BC subtype, and vascular invasion are significantly and independently associated with pCR among BC patients undergoing NAC. Patients who achieve pCR after NAC have a better prognosis compared to patients with residual disease in terms of DFS, DDFS, and OS. Measurement of RTC in BC patients with pPR improves the prognostic information that can be obtained from the assessment of pathologic response. Different patterns of residual disease play an important role in predicting the risk of subsequent loco-regional and distance recurrence, and patients with RTC <40% present significantly better DFS and DDFS.

Statement of ethics

The present study complied with the guidelines for human studies. The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The Institutional Review Board of our hospital approved this retrospective study. Each patient provided informed consent for operation and clinical data acquisition.

Declarations of competing interest

None.

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