Original Article

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Assessment of the Predictive Role of Ki-67 in Breast Cancer Patients' Responses to Neoadjuvant Chemotherapy

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ABSTRACT

Objective: Neoadjuvant chemotherapy (NAC) in breast cancer (BC) is being considered for a broader range of cases, including locally advanced tumors and situations where downstaging could reduce extensive surgery. Several trials have explored predictive markers of pathological complete response (pCR). The role of Ki-67 as a predictor of pCR has been demonstrated in studies. However, the cut-off remains vague, given the lack of standardization of measurement methods. The aim of our study was to evaluate the predictive value of Ki-67 in response to NAC and to identify the cut-off values that exhibit the strongest correlation with best response.

Materials and Methods: This retrospective study included 187 patients who had undergone surgery following NAC for BC at the CHU Souss Massa of Agadir between January 2020 and January 2023. Logistic regression was used to assess the correlation between Ki-67 and patients' characteristics. Optimal Ki-67 cutoff was identified by receiver operating characteristic curve. Kaplan-Meier curves were used to assess disease-free survival (DFS), and survival comparisons were assessed with the log-rank test.

Results: The median age was 51.8±10.7 years and 51.4% of tumors were smaller than 5 cm. Node invasion was found in 55.4%. Luminal B subtype was found in 49.7%, followed by human epidermal growth factor receptor-2 (HER-2)-positive in 27.4%, triple-negative in 14.3% and Luminal A in 8.6%. pCR occurred in 40% of patients overall. Subgroup analysis revealed a significant association between pCR and tumor size (p<0.001), lymph node involvement (p<0.001), grade 2 (p<0.001), vascular invasion (p<0.001), and positive HER-2 status (p = 0.022). In statistical analysis, pathological responses were improved in patients with Ki-67 >35% (p<0.001). DFS was 98.8% at 12 months. No statistical difference was found in DFS according to Ki-67 values and pCR status.

Conclusion: Our results indicate that Ki-67 is a predictive marker for response in the neoadjuvant setting in BC patients. Our study showed that a Ki-67 cut-off >35% predicts a better pCR rate in response to NAC. However, this cutoff value remains controversial due to the absence of a standard method of measurement, with inter- and intra-observer variability. It would be necessary to validate this cutoff in other studies.

Keywords: Breast cancer; Ki-67; neoadjuvant chemotherapy

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Key Points

- Breast cancer
- Neoadjuvant chemotherapy
- Ki-67

Introduction

Neoadjuvant chemotherapy (NAC) in breast cancer is being considered for a broader range of cases, including locally advanced tumors and situations where downstaging may facilitate less extensive surgery. This approach allows for tailored treatment based on the tumor response before surgery. In numerous neoadjuvant analyses, patients who attained a pathological complete response (pCR) demonstrated a more favorable survival outcome (1). Several trials have explored clinical, biological and histological markers to predict pCR in breast cancer.

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Various factors have been recognized as predictors of pCR such as age, menopausal status, tumor stage, nuclear grade, lymphatic invasion, genomic signature, molecular subtype and Ki-67 value (2, 3). Ki-67 is a protein marker used to measure cell proliferation. It is expressed in the active phases of the cell cycle (G1, S, G2 and M), but not in the quiescent phase (G0) (4). The strong correlation between Ki67 and pCR has been demonstrated in numerous studies (5). However, the cut-off remains vague and inaccurate, given the lack of standardization and variability of measurement methods (6). To the best of our knowledge, only a few studies in a Moroccan population have been reported to date. The specific aim of this study was to analyze the potential role of Ki-67 in patients receiving NAC for breast cancer. In addition, we sought to identify the cut-off values of Ki-67 that exhibited the strongest correlation with the best response to NAC.

Materials and Methods

Patients

This retrospective study included 187 patients who had undergone surgery following NAC for breast cancer at the Regional Oncology Center in Agadir between January 2020 and January 2023. For inclusion in the study, patients were required to be at least 18 years old. They were enrolled only if they had completed NAC, followed by surgery. The patient selection process is shown in Figure 1.

The inclusion criteria for NAC administration were: Confirmed invasive breast cancer, from stage T2 and or lymph node involvement for human epidermal growth factor receptor-2 (HER-2)-positive or triple-negative tumors, and T4 for luminal tumors. The patient's overall health status and ability to tolerate chemotherapy were taken into consideration.

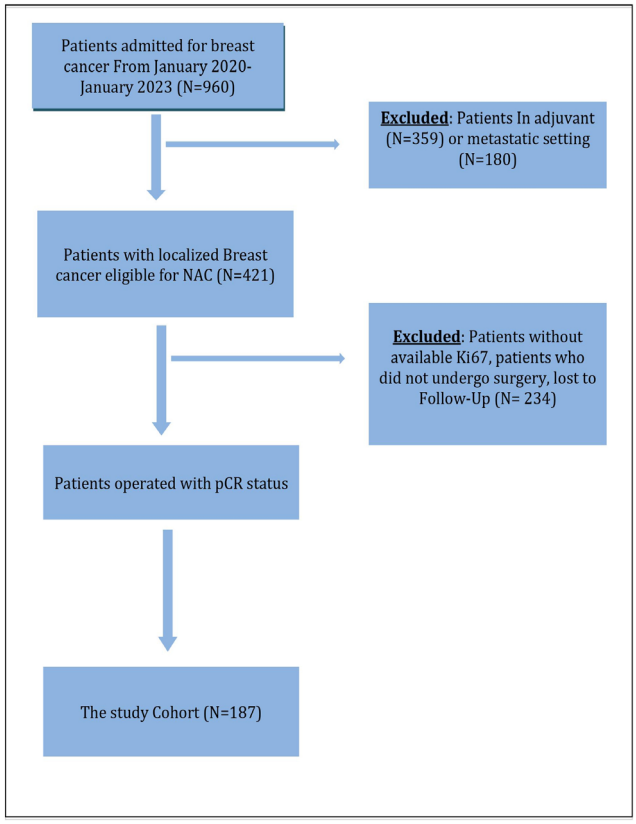

Figure 1. Patient selection

The study adhered to the principles outlined in the Declaration of Helsinki (1964) and received approval from the Local Ethics Committee (CHU Souss Massa, Biomed Laboratory, Faculty of Medicine and Pharmacy of Agadir, University Ibn Zohr Agadir, approval number: 25_01_2023, date: 25.01.2023).

Clinical Data

Patients' characteristics were selected from a database containing archived medical records. They included: Patients' age, menopausal status, disease stage, chemotherapy protocol, surgical treatment, histological results, tumor grade, molecular sub-type, and Ki-67 value. A staging assessment was carried out on all patients, which included thoraco-abdomino-pelvic computed tomography and bone scintigraphy.

Histopathological Data

Histological parameters of the tumor included the histological type, histological grade, estrogen receptor (ER) status, progesterone receptor (PR) status, HER-2 status and Ki-67 expression level, obtained from the original pathology reports. Molecular subtypes were classified according to the Saint Gallen recommendations from 2013 into four groups: Luminal-A, Luminal-B, HER-2+, and triple negative.

Molecular profiling was carried out using monoclonal mouse antibodies (for ER: ID5, 1:50 dilution; Dako, Glostrup, Denmark), (for PR (PgR636, 1:100 dilution; Dako) and HER-2 protein (CB11, 1:100 dilution; NeoMarker, Fremont, USA). Hormone receptor positivity was defined by an ER and PR cut-off value of 1%. Hormone-negative status was determined by the absence of ER and PR expression by the tumor. HER2 status was assessed using the Hercep test by immunohistochemistry (IHC). Positive results were defined as either 3+ expressions on IHC or 2+ expressions on IHC and positive results in fluorescent in situ hybridization. The Ki-67 value was evaluated by automated quantitative analysis, using a monoclonal antibody (MIB-1, 1:400 dilution; Dako, Denmark). The process typically included digitization of tissue slides byscanning to create high-resolution (×40 objectives) digital images. The images were then analyzed by computer algorithms to detect and quantify Ki-67-positive nuclei within the tissue. The percentage of Ki-67-positive cells is calculated and reported.

Treatment

Anthracyclines-based treatment followed by taxanes was administered to all patients in our study. NAC typically involved administering three to four cycles of anthracyclines as part of the standard regimen (AC60: Doxorubicin 60 mg/m² and cyclophosphamide 600 mg/m², or EC100: Epirubicin 100 mg/m² and cyclophosphamide 500 mg/m² every three weeks). The taxane-based regimens used were as follows: Weekly paclitaxel 80 mg/m² or triweekly docetaxel 100 mg/m².

For patients with HER2-positive breast cancer, anti-HER-2 targeted therapies were combined with taxanes: Either a dual HER-2 blocking therapy with pertuzumab 840 mg intravenously, followed by 420 mg and trastuzumab 600 mg subcutaneously or trastuzumab 600 mg subcutaneously every three weeks. In triple-negative breast cancer, patients received the dose-dense protocol: AC followed by weekly paclitaxel and carboplatin: Doxorubicin 60 mg/m² and cyclophosphamide 600 mg/m² every 2 weeks, followed by paclitaxel 80 mg/m² on days 1, 8 and 15, and carboplatin AUC5 (area under the curve) every 3 weeks.

The patients underwent surgery after completing NAC with a median interval of 24.7±3.68 days. All patients underwent mastectomy or breast-conserving surgery with axillary lymph node dissection. The choice of surgical procedure was discussed in a multidisciplinary consultation meeting, and it was determined by the initial stage, the optimal cosmetic results, and the patient's choice.

PCR Assessment

The pathological response to chemotherapy was assessed by analyzing surgical specimens taken from the tumor. The pCR was defined as the absence of invasive residues in the breast or nodes (ypT0 and ypN0). The pCR analysis was performed using the Sataloff and Chevalier classifications. At the time of the study, Residual Cancer Burden (RCB) classification was not used in the Moroccan centers.

Statistical Analysis

Data was collected in an Excel database. Statistical analysis was performed using Jamovi software (https://www.jamovi.org), $p<0.05$ was considered to be statistically significant.

The optimal cut-off value for Ki-67 percentage was assessed by receiver operating characteristic (ROC) curve analysis and area under the curve. The cut-off value refers to the value corresponding to maximum sensitivity and minimum 1-Specificity. The cut-off value for Ki-67 used in our series to define either high or low classification was 35%.

Student's t-test was employed for analyzing quantitative characteristics, while Pearson's chi-square test was used to contrast categorical variables. Logistic regression methods were adopted to approximate the risk ratio of achieving pCR according to the baseline factors.

Disease-free survival (DFS) was characterized as the time between surgery and relapse. Kaplan-Meier curves were used to assess DFS, and differences between survival curves were assessed with the log-rank test.

Results

Clinical and Histological Characteristics

A total of 187 patients were included in the study. The average age of patients was 518±10.7 years. Of these, 75 (40%) were premenopausal at diagnosis, and 112 were menopausal (60%). In 96 (51.4%) the tumor size <5 cm, while 91 patients (48.6%) had a tumor size >5 cm. Lymph node involvement was documented among 104 patients (55.4%). The mean CA 15-3 value was 18.6±10 U/mL. When diagnosed, 137 patients were classified as stage II (73,1%), and 50 patients were classified as stage III (26.8%) (Table 1).

The distribution of patients according to histological type was: Invasive ductal carcinoma in 171 (91.4%) and invasive lobular carcinoma in 16 (8.6%). The histological prognosis grade revealed a predominance of grade 3 in 64.4% of cases (120) and grade 2 in 35.4% of cases (67). Among the 187 patients, there were 16 patients (8.6%) with the luminal A subtype, 93 (49.7%) patients with the luminal B subtype, 51 patients (27.4%) with the HER-2 subtype, and 27 patients (14.3%) with the triple negative type. With the study cut-off value of Ki-67 >35%, 113 patients (60.2%) were considered to have a high level of Ki-67 (Table 1).

After NAC, 75 patients (40%) showed a clinical complete response (cCR), 85 patients (45.7%) showed a clinical partial response (cPR), and 27 patients (14.3%) showed clinical stable disease (cSD).

The complete absence of residual invasive carcinoma cells in the breast was confirmed by histological examination in 75 patients (40%), however, 115 (60%) patients had residual carcinoma cells in the breast or in the resected lymph nodes (Table 1).

Analysis of the Relationships Between the pCR Rate and Ki-67

As a result of a univariate analysis, clinical and pathological responses to NAC were significantly improved when Ki-67 levels were high >35% ($p<0.001$). Furthermore, a better rate of pCR was significantly associated with tumor size <5 cm ($p<0.001$), lymph node invasion ($p<0.001$), nuclear grade 2 ($p<0.001$), vascular invasion ($p<0.001$), hormone receptor positive subgroup ($p<0.001$) and HER-2-positive subgroup ($p = 0.022$) (Table 2).

Study of the Correlation Between pCR and Clinico-Pathological Factors

The multivariate logistic regression analysis of the correlation between pCR and clinicopathological factors was performed using tumor diameter, tumor grade, lymph node invasion, vascular invasion, molecular subtype (HR, HER-2), and Ki-67 expression level. It showed significant correlations between pCR and Ki-67 expression >35% ($p<0.001$), tumor size <5cm ($p<0.001$), HER2 positive status ($p = 0.023$), and lymph node invasion ($p<0.001$) (Table 3).

Progression-Free Survival

All patients were monitored until January 2024. Six (3.4%) patients presented with a local or metastatic relapse of the disease. The average

Table 1. Patient characteristics

		Value (%)
Age, years	Mean ± SD	51.8±10.7
Menopausal status	Premenopausal	75 (40%)
	Postmenopausal	112 (60%)
Tumor size (cm)	<5 cm	96 (51.4%)
	>5 cm	91 (48.6%)
Nodal status	Negative	96 (55.4%)
	Positive	91 (48.6%)
Histological type	Invasive ductal carcinoma	171 (91.4%)
	Invasive lobular carcinoma	16 (8.6%)
Nuclear grade SBR	Grade 2	67 (35.4%)
	Grade 3	120 (64.4%)
Vascular invasion	Positive	71 (38.3%)
	Negative	116 (61.7%)
Tumor subtype	Luminal A	16 (8.6%)
	Luminal B	93 (49.7%)
	HER-2 overexpression	51 (27.4%)
	Triple negative	27 (14.3%)
Ki-67 expression	≥35%	113 (60.2%)
	<35%	74 (39.4%)
Response to NCT	Non pCR	112 (60%)
	pCR	75 (40%)

SD: Standard deviation; SBR: Scarff-Bloom-Richardson; HER-2: Human epidermal growth factor receptor-2; pCR: Pathological complete response; NCT: Neoadjuvant chemotherapy

DFS period was 51.1 months (49.5-52.6) (Figure 2). No significant difference was found when comparing DFS in terms of pCR status or Ki-67 cut-off (Figures 3 and 4).

Discussion and Conclusion

Antigen Ki-67, also known as Ki-67 or Marker of Proliferation Ki-67 (MKI67), is a nuclear antigen and is closely associated with increased proliferation and a poorer prognosis in breast cancer. (7) The cellular expression level of Ki-67 can be detected using IHC and immunofluorescence (IF) methods. IHC is more frequently used (8). Measurements are conducted using various antibodies, such as mouse or rabbit monoclonal antibodies (MM1, MIB-1, SP-6) (9). The manual counting of at least 500–1000 malignant invasive cells, as proposed by the International Ki-67 in Breast Cancer Working Group, is frequently used to assess Ki-67 (10). However, counting this number of cells is a substantial, labor-intensive, and time-consuming task for histopathologists and poses challenges in terms of reproducibility (11). The evaluation and measurement methods for Ki-67 are variable, leading to inconsistencies in results. In a study by Chung et al. (12), which included 30 observers from 30 different institutions, and examined Ki-67-stained slides of 20 different breast cancers on whole sections and tissue microarray. Each observer assessed Ki-67 in two different ways: Direct counting and categorical estimation. The study concluded that inter-observer variability of the Ki-67 index for the two methods was significantly high. Tumors with hot spots had higher inter-observer variability, and restricting the measurement area resulted in lower variability.

Table 2. Patient characteristics by pCR status

		pCR (n = 75) No. (%)	Non-pCR (*n* = 112) No. (%)	*p*
Age (years)	51.8±10.7	50±10.5	52±10.8	0.353
Menopausal status	Premenopausal	32 (42.8%)	80 (71.4%)	0.051
	Menopausal	43 (51.1%)	32 (28.6%)	
Tumor size (cm)	<5 cm	45 (60%)	52 (45.7%)	<0.001
	>5 cm	30 (40%)	60 (54.3%)	
Nodal status	Positive	51 (68.6%)	52 (46.7%)	<0.001
	Negative	20 (31.4%)	60 (53.3%)	
Histological type	Invasive ductal carcinoma	62 (82.8%)	109 (97.1%)	<0.001
	Invasive lobular carcinoma	13 (17.1%)	3 (2.85%)	
Nuclear grade	Grade 3	57 (75.7%)	9 (8.6%)	<0.001
	Grade 2	18 (24.3%)	96 (91.4%)	
Vascular invasion	Positive	58 (82.8%)	10 (8.6%)	<0.001
	Negative	12 (17.1%)	102 (91.4%)	
Hormonal receptor status	Positive	63 (84.3%)	48 (42.8%)	<0.001
	Negative	12 (15.7%)	64 (57.1%)	
HER-2 status	Positive	48 (64.3%)	70 (62.8%)	= 0.022
	Negative	27 (35.7%)	42 (37.1%)	
Ki-67	<35%	11 (14.3%)	63 (56.2%)	<0.001
	>35%	64 (85.7%)	49 (43.8%)	

HER-2: Human epidermal growth factor receptor-2; pCR: Pathological complete response

Table 3. Logistic regression analysis of the correlation between pCR and clinicopathological characteristics

Parameter	Odds ratio	Confidence interval	*p*
Ki-67 >35%	5.27	2.44–11.39	<0.001
Tumor size<5cm	3.26	1.675–6.33	<0.001
Nuclear grade 3	89.18	31.48–252.62	<0.001
Hormone-positive status	91.78	12.29–685.38	<0.001
HER-2 positive status	2.23	1.12–4.45	0.023
Lymph node invasion	5.38	2.59–11.21	<0.001
Vascular invasion	341.5	71.41–1633.76	<0.001

HER-2: Human epidermal growth factor receptor-2; pCR: Pathological complete response

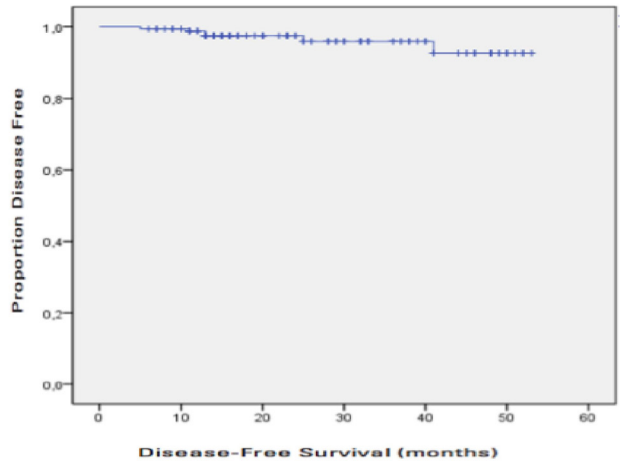

Figure 2. Disease free survival

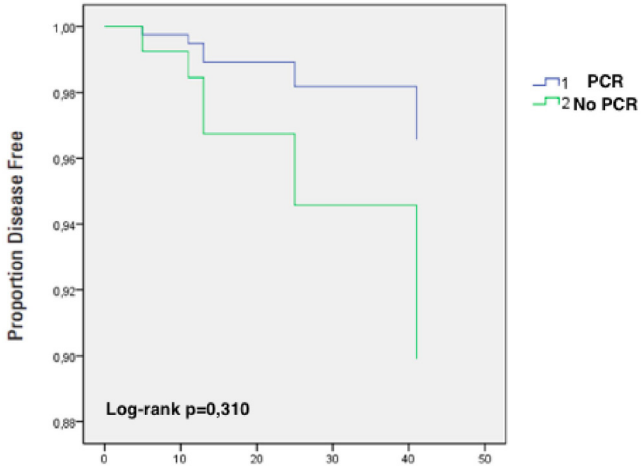

Figure 3. DFS according to PCR

DFS: Disease free survival; PCR: Pathological complete response

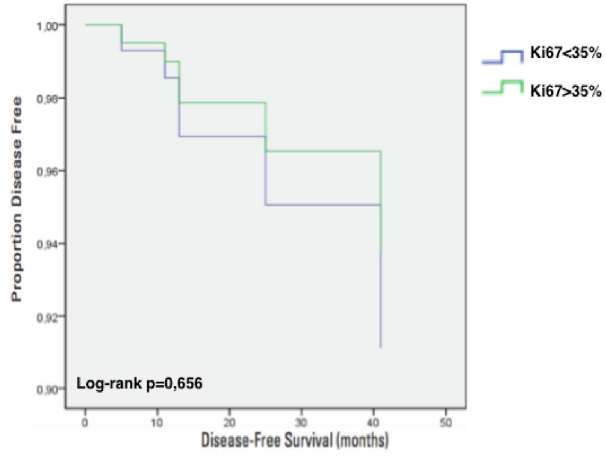

Figure 4. DFS according to Ki-67

DFS: Disease free survival

To solve this problem, automated counting using computer software may be used to objectively measure protein expression in tumor and subcellular compartments (13). It involves the processing of scanned images on whole slides by microscopy and computer analysis. It has recently been developed as a reproducible and more specific method of assessing Ki-67 than visual methods (14). Klauschen et al. (15) described encouraging results from the validation of computer-assisted Ki-67 assessment on over 1,000 breast tumors. The study concluded that it was a valid method for assessing Ki-67 and for predicting overall and progression-free survival (pOS <0.0001, pPFS <0.0002) (15).

There isn't a universally defined cut-off value for categorizing Ki-67 expressions. The Ki-67 cut points are used in various ranges, from 5% to 34% (16). In a study of prognostic factors in breast cancer, the researchers determined a cut-off value for Ki-67 at 12% (17). This value was observed in patients without recurrence, providing a rationale for their categorization. In another related study, a cutoff value of 20% was adopted (18). Kim et al. (18) determined 25% as the optimal cut-off value using ROC curve analysis. However, another study by Alba et al. (19) indicated that the Ki-67 cut-off with the greatest sensitivity and specificity values was 50%. Denkert et al. (20) concluded that Ki-67 cut-off points between 3 and 94% are predictive of pCR. Our study population of 187 patients showed a complete pathological response of 85.7% in patients with a Ki-67 value of more than 35% which was significant (p<0.001).

High Ki-67 level is correlated with an increased probability of achieving pCR in breast cancer patients undergoing NAC (21). This correlation can be explained by the fact that High Ki-67 represents rapidly dividing cells, and that NAC typically targets dividing cells, resulting in enhanced elimination of tumor cells (22). In a meta-analysis conducted by Chen et al. (23), which included 53 studies, and 10,848 patients, it was found that high Ki-67 before NAC was a predictor for pCR in the neoadjuvant setting for breast cancer patients. Indeed, a variety of cut-off points correlated with pCR were used in this meta-analysis, ranging from less than 14% to more than 50% in a total of 41 studies. In a retrospective study by Ingolf et al. (1), which included 77 patients receiving NAC for breast cancer, the study concluded that there was a significant correlation between Ki-67 and pCR as a predictive factor (p = 0.001) (1). The average Ki-67 value was 34.9% but ranged widely between 1–90%. In the group with pCR, the cutoff of Ki67 was 37.4±24%. Similar results were found in a large series by Peter et al. (5), which included 552 patients treated with NAC for invasive breast carcinoma. Ki-67 was identified as a pCR marker by using a cutoff value of more than 13% [odds ratio (OR) 3.5, p = 0.01] (5). It seems that the predictive cutoff values of Ki-67 differ based on the molecular profile according to some authors. Peter et al. (5), showed that higher cutoff values are observed in the hormone receptor-positive tumors (ranging from 36% to 40%) and the triple-negative tumors (30–40%). For the HER-2-positive tumors, Ki-67 varies between 17% and 20% (5). Across all studies, Ki-67 values between 30% and 50% were correlated with better pCR rates in all four breast cancer subtypes. In a study by Wang et al. (24), which included 240 patients, a Ki-67 value of 40% was associated with better pCR. In our series, a Ki-67 value of 35% was predictive of a complete response to NAC in all subgroups (p<0.001).

Tumor size has been considered in numerous studies as a predictor of pCR (25). Consistent with our findings, Chen et al. (25) in their study, which included 1010 BC patients, concluded that tumor sizes less than 4 cm were more likely to attain pCR (p = 0.039). The same

results were observed in the study of Peter et al. (5). The pCR rates were 34.8% in pT1 tumors and 21.5% in pT2 tumors (p<0.0001). This finding aligns with the idea that small tumors might exhibit a more robust response to treatment (5). Our study confirms similar results, with a pCR rate of 60% for tumors <5 cm versus 40% for tumors greater than 5 cm (p<0.001).

Molecular subtype is another factor that has been correlated with pCR (26). In the meta-analysis of Chen et al. (23), Ki-67 was a predictive factor for pCR in all molecular subtypes: in HR+ (n = 7; OR: 2.51), HER2+ (n = 9; OR: 2.76) and triple-negative (n = 10; OR: 2.77) (23). Moreover, the neoadjuvant GeparTrio trial showed that Ki-67 was predictive of response to NAC in nearly all molecular subtypes (21). Kim et al. (18) demonstrated in their study, which included 74 patients, that patients with HER2-positive tumors exhibited a higher rate of pCR (p = 0.040), and a similar trend was observed in ER-negative patients (p = 0.031). In the same way, Peter et al. (5) found that pCR rates were higher in tumors with HER2 over expression (p<0.00001). We also observed a significant association between pCR and HER-2 overexpression (p = 0.0023, OR = 2.227) and HR negative (p<0.001, OR = 91.777). The improvement in pCR rates in these subgroups may be due to the elevated levels of Ki-67 frequently observed in tumors with negative Hormone receptors or exhibiting HER-2 over expression (20). Nonetheless, Petit et al. (27) concluded that the absence of hormone receptor expression (ER and PR) in the high Ki-67 group was a predictor of pCR (p = 0.008 and p = 0.01, respectively). However, HER-2 overexpression was not significantly associated with achieving pCR (p = 0.99).

Tumor grade has shown a clear association with pCR status in many studies (28). In the study of Peter et al. (5) the pCR rate was significantly higher in grade 3 (45.3% compared to 10.6%) than grade 2 (p<0.00001). In another recent study performed by Jarzab et al. (28), including 353 females receiving NAC for breast cancer, increased nuclear grade demonstrated an elevated rate of pCR (31.28% in grade 3 *versus* 8.55% in grade 2, p<0.0001) (28). This finding could be explained by a direct and significant correlation between higher nuclear grades and high Ki-67. In a retrospective study involving 260 breast cancer patients, it was reported that a robust correlation existed between high Ki-67 and elevated nuclear grade (p = 0.010) (29). Furthermore, this study concluded that a higher Ki-67 index was significantly associated with positive lymph nodes and vascular invasion. This discovery may also explain the notable link between lymph node invasion, vascular invasion and pCR in patients exhibiting high Ki-67 levels (30). Our results are consistent with this finding, with pCR rates of 75.7% in nuclear grade 3 (p<0.001, OR = 89,176) and in lymph node involvement (82.8%, p<0.001, OR = 5.388).

Another question is the impact of the chemotherapy regimen on achieving pCR in patients with high Ki-67. The meta-analysis of Chen et al. (23) demonstrated that Ki-67 was also a useful predictor of pCR in patients receiving chemotherapy regimens containing anthracyclines and/or taxanes (n = 13; OR: 2.90), anthracyclines plus taxanes (n = 22; OR: 3.15), and anthracyclines (n = 5; OR: 4.67), compared to taxanes (n = 3; OR: 1.29).

pCR is generally considered a predictor of OS and DFS (31). Von Minckwitz et al. (32) presented a meta-analysis of 6.377 breast cancer patients. The authors concluded that pCR was an effective marker of survival for TNBC, luminal B and HER2-positive patients (p = 0.005). Kong et al. (33) completed a meta-analysis that included 16 studies and 3.776 patients with breast cancer. The authors indicated that pCR was prognostic for relapse-free survival (OR = 3.44), DFS (OR = 3.41), and RFS (OR = 2.45). Similar results were also reported by two large metaanalysis conducted by Cortazar et al. (34) and Spring et al. (35). Patients with TNBC who achieved pCR had significantly better DFS than those who did not (36). Moreover, some authors suggested that a decrease in Ki-67 after NAC contributed to a favorable DFS (37). Yoshioka et al. (38) demonstrated in their study, including 64 patients, that the level of Ki-67 in residual tumors after NAC was strongly associated with increased DFS and OS (p = 0.0004 and p = 0.0003, respectively) (38). Chen et al. (39) showed, in a series of 92 patients with locally advanced breast cancer, that a Ki-67 decrease of over 12.5% was consistent with a better DFS (p = 0.007).

Our study has some limitations. First, our study was conducted retrospectively. Second, the small size of our sample contributed to the variability of certain results compared to other studies. Furthermore, there was no standard cutoff value for Ki-67. It was determined, in our study, using the ROC curve, which combines sensitivity and specificity. Further studies are necessary, involving larger patient groups with analyses of cutoff values, subtypes of BC and outcomes, which will reinforce the present findings.

Our results confirm the predictive role of Ki-67 in patients' response to chemotherapy in the neoadjuvant setting. The findings of the present study suggest that this marker could help select patients who may benefit from chemotherapy. Our analysis showed that a Ki-67 cut-off >35% predicted a better pCR rate. However, this cutoff value remains controversial due to the absence of a standard method of measurement and interpretation with inter- and intra-observer variability. Validation of this cutoff value in a larger population would be desirable.

Ethics Committee Approval: The study adhered to the principles outlined in the Declaration of Helsinki (1964) and received approval from the Local Ethics Committee (CHU Souss Massa, Biomed Laboratory, Faculty of medicine and pharmacy of Agadir, University Ibn Zohr Agadir, approval number: 25_01_2023, date: 25.01.2023).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: G.R., S.B.; Concept: G.R.; Design: G.R., F.B., M.M.; Data Collection and/or Processing: R.M., F.B., S.B., C.S.; Analysis and/or Interpretation: G.R., R.M., F.B., F.R.; Literature Search: V.M.T., R.V., S.S.; Writing: G.R., R.M., F.B., M.M., F.R.

Conflict of Interest: The authors have no conflicts of interest to declare.

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