

## ORIGINAL ARTICLE

# Predictive factors of pathological response to neoadjuvant chemotherapy in patients with breast cancer

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## Summary

**Purpose:** To identify predictive factors connected with pathologic response in patients with breast cancer (BC) having received neoadjuvant chemotherapy (NACT).

**Methods:** 49 patients with BC were investigated before and after treatment in this prospective research. Different chemotherapy regimes were administered. The Miller-Payne scoring system was used to assess the tumour response. The nuclear proliferation markers Ki67 and the expression of topoisomerase IIa (Topo IIa) were evaluated.

**Results:** Six patients (12.2 %) achieved pathological complete response (pCR). Noticeable decrease of tumor cellularity was detected in all BC subtypes and pCR in the triple-negative BC (TNBC) group ( $p=0.007$ ) was observed. Poorly dif-

ferentiated tumors could be considered as predictive factors of pCR ( $p=0.07$ ). Ki67 appeared to be a predictive marker of achieving pCR ( $p<0.001$ ) with a threshold of 28% (AUC=0.89, 95% CI 0.75-0.96). The additional factor of reaching pCR was operable BC ( $p=0.04$ ). The expression level of Topo IIa ( $p=0.50$ ) and using different regimens of NACT ( $p=0.97$ ) did not influence pCR achievement.

**Conclusion:** To sum it up, poorly differentiated carcinomas with high cellularity in the primary tumor, TNBC, Ki 67 with a threshold above 28% and operable BC can be considered as early predictors of reaching pCR.

**Key words:** breast cancer, Ki 67, neoadjuvant chemotherapy, pathologic response

## Introduction

The use of preoperative or neoadjuvant chemotherapy (NACT) in the complex treatment of breast cancer (BC) leads to clinical and radiological tumor response correlating with the degree of pathological response in most of the cases [1].

Criterion for determining the response to NACT is the tumor pathological complete response (pCR). This is observed in 10-40 % of patients [2]. pCR is defined as no evidence of invasive carcinoma in the primary breast tumor and in the lymph nodes. Achievement of pCR following NACT is associated with good long-term outcomes [3,4].

Several studies have demonstrated the effect of intrinsic subtypes on tumor regression [5,6]. Luminal A carcinomas do not show pCR, while significant overexpression of HER2 and triple-negative BC (TNBC) show a considerable pathological response [7]. Luminal B carcinomas are distinguished by a heterogeneous response. Despite lower incidence of pCR, luminal B (HER2-positive) patients showed a good prognosis [8]. In comparison, in HER2-positive patients with negative hormonal status achievement of pCR correlated with favourable outcomes [9].

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There is a considerable variability in the methods of pathological evaluation for NACT and in the interpretation for subsequent clinical decisions [10]. A standard definition and approach to assessing the pCR to demonstrate the therapy efficacy of pCR should be used.

Pathologic changes in tumors are quite typical, despite a large number of cytotoxic drugs in NACT, including resizing, cellularity, histological type, tumor grade and lymph node response [11,12]. There are several systems for assessment of pathologic response: NSABP B-18, Miller-Payne, Chevallier, Sataloff, RCB defining a category of pCR, partial response (pPR) and no response (pNR) [11,12]. Some algorithms require the absence not only invasive carcinoma in breast but in lymph nodes as well [13,14].

The disadvantages of the NSABP B-18 system are the lack of evaluation of lymph nodes, lymphovascular invasion and only one category of pPR. Chevallier's system combines cases of ductal carcinoma *in situ* with pCR, making it difficult to interpret the data. Sataloff's system shares the response to treatment in the primary tumor and lymph nodes. RCB system is based on the calculation of residual tumor burden (RCB) [13], which could be determined from bidimensional diameters of the primary tumor bed, the proportion of invasive carcinoma, the number of axillary lymph nodes with metastasis and the diameter of the largest metastasis in lymph node. All the listed data can be transferred into a mathematical index with the definition of four RCB categories by a special formula. To determine all the values of response in clinical practice considering the advantages of the system is rather difficult.

In our opinion Miller-Payne histological grading system was more useful. One of the advantages of this system includes multistep scale based on the comparison tumor cellularity before and after NACT [15].

A number of authors think tumor cellularity one of the most important factors in assessing the response to NACT and the lack of it can predict favorable prognosis [1,15,16].

The purpose of this prospective study was to identify the predictive clinical and pathological factors associated with the achievement of pCR in BC patients having received NACT.

## Methods

### Patients

49 patients who received NACT between 2015 and 2017 at the National Cancer Institute (Ukraine) were analyzed in this prospective study.

The study was based on patients' data with a primary tumor with T1, N1-2, M0 or T2, N0-2, M0 or T3, N0-1, M0 or T4, N1-3, M0. The average age of patients was  $53.4 \pm 10.9$  (range 23-75). The majority of the patients (73.5%) had hormone receptor (HR) positive intrinsic BC subtypes: luminal A-like tumors - 22.5% (11), luminal B-like (HER2/neu-negative) - 38.8% (19), luminal B-like (HER2/neu-positive) - 12.2% (6). Aggressive carcinomas were 16.3% (8) of TNBC and 10.2% (5) of overexpressing HER2/neu.

A core biopsy was performed to obtain tumor tissue for study, after which patients received at least 2 cycles of NACT. Anthracyclin-based chemotherapy (61.2%; 30 patients), combination of anthracyclines with taxanes (30.6%; 15 patients) and platinum drugs (8.2%; 4 patients) were administered in the treatment. Platinum drugs were used for patients with edematous-infiltrative form of BC. Patients did not receive trastuzumab because of different reasons.

NACT was given every 21 days. We used FAC regimen in most of the cases - doxorubicin  $50 \text{ mg/m}^2$  plus cyclophosphamide  $500 \text{ mg/m}^2$  and 5-fluorouracil -  $500 \text{ mg/m}^2$ ; less frequently, the AT regimen - doxorubicin  $50 \text{ mg/m}^2$  plus docetaxel  $75 \text{ mg/m}^2$  or paclitaxel  $175 \text{ mg/m}^2$ , AC regimen - doxorubicin  $60 \text{ mg/m}^2$  plus cyclophosphamide  $600 \text{ mg/m}^2$  and TC regimen - docetaxel  $75 \text{ mg/m}^2$  plus cyclophosphamide  $600 \text{ mg/m}^2$ . Platinum drugs (cisplatin  $75 \text{ mg/m}^2$  or carboplatin AUC 5) were given sequentially for AC or AT regimens.

After NACT, patients were subjected to definitive surgery, including axillary lymph nodes dissection. All the patients signed informed consent. The local ethics committee approved the study protocol.

### Histopathologic review

The histological analysis of the core biopsy with histologic tumor type, the tumor grade (Nottingham grading), estrogen receptor (ER), progesterone receptor (PR), and HER2 were determined. The size of the tumor, the presence of lymphovascular invasion and the status of lymph nodes were assessed in the evaluation of the surgical specimen.

Miller-Payne scoring system was used to assess pathologic response with the evaluation of residual tumor cells [15]. Assessment of tumor cellularity was conducted by a visual analogue scale [13]. The definition of pCR was noninvasive residual disease in breast and axillary lymph nodes.

Based on the data obtained, all the tumors were classified into 5 subgroups (MPG): pCR (MPG5), three pPR groups (MPG2, MPG3, MPG4) and pNR group (MPG1). MPG3, MPG4 and MPG5 were positive response for treatment.

ER, PR and HER2/neu statuses were evaluated in tumor tissue after biopsy and after surgery in the local laboratory. According to Allred score, tumors were considered HR-positive if estrogen and progesterone receptors were present in  $\geq 1\%$  of tumor cells. Expression of the HER2 protein was determined using the immunohistochemical (IHC) method or *in situ* fluorescent hybridization (FISH). Assessment was made using semi-quantitative method for IHC and quantitative for FISH,

along with ASCO-CAP Guidelines for HER2/neu testing [17]. Tumors were classified as HR+/HER2-, HR+/HER2+, HR-/HER2+ and triple-negative (ER-/PR-/HER2-).

The staining of nuclear proliferation marker Ki-67 was also performed using IHC.

Only nuclear staining was considered to assess the expression of the protein topoisomerase II $\alpha$  (Topo II $\alpha$ ). The number of positive tumor cells was evaluated on a scale from 1 to 4 (1 - from 0 to 5% of stained tumor cells; 2 - from 6 to 25%; 3 - from 26 to 75%; 4 - more than 75%) proposed by Sandri et al [18]. According to this scale, values 1 and 2 were evaluated as absence of expression, and 3 and 4 - as presence of expression of Topo II $\alpha$  protein.

### Statistics

The results are presented as mean  $\pm$  standard deviation (SD), minimum and maximum values for continuous variables. Nominal variables were described by absolute and relative frequencies (%). The  $\chi^2$  or Fisher's

test were used to compare the distribution of qualitative variables. Univariate analysis to determine predictive factors of pCR was performed using logistic regression model. 95% confidence interval (CI) for odds-ratios (OR) was calculated to assess the relationship between the factor and the resultant trait. We used the receiver operating characteristic (ROC) curve to compare the sensitivity and specificity for all possible cut-offs. The area under the curve (AUC) was also calculated. All the analyzes were performed using the statistical package MedCalc v.18.11.3 (MedCalc Software Inc., Broekstraat, Belgium, 1993-2019). Differences were considered statistically significant at  $p < 0.05$ .

### Results

The pathological response was evaluated in tumor specimens before and after NACT in 49 patients (pPR in 87.8%, and pCR in 12.2%). Miller-Payne score for responses is listed in Table 1. Patients with a positive response made up the largest group (65.3%). MPG3 response was noted with a cellularity of  $44.4 \pm 21.8\%$  in half of the cases. MPG4 tumor with a cellularity of only  $3 \pm 2.8\%$  was observed in 4.1% of cases, and pCR in 12.2% (6 patients).

Analysis of pathological response depending on tumor grade was conducted further (Table 2). G1 and G2 tumors showed a low level of pathological response (mainly MPG2 and MPG3). There was no

**Table 1.** Distribution of the tumor response on Miller-Payne scale

Miller-Payne score	n=49 n (%)	Cellularity $\bar{x} \pm SD$ (%)	Range (Min-Max)
MPG2	17 (34.7)	80.6 $\pm$ 14.2	50-95
MPG3	24 (49.0)	44.4 $\pm$ 21.8	10-70
MPG4	2 (4.1)	3 $\pm$ 2.8	1-5
MPG5	6 (12.2)	0 $\pm$ 0	0-0

**Table 2.** Pathologic response depending on the primary tumor grade

Histologic tumor grade	Miller-Payne score n (%)				
	MPG2	MPG3	MPG4	MPG5	Total
G1	3 (50.0)	3 (50.0)	0 (0.0)	0 (0.0)	6 (12.2)
G2	9 (37.5)	14 (58.3)	1 (4.2)	0 (0.0)	24 (49.0)
G3	5 (26.3)	7 (36.8)	1 (5.3)	6 (31.6)	19 (38.8)

P value=0.07

**Table 3.** The impact of various regimens of NACT on the pathological response

Regimen of NACT	Miller-Payne score n (%)				
	MPG2	MPG3	MPG4	MPG5	Total
A	10 (32.3)	15 (48.4)	1 (3.2)	5 (16.1)	31 (63.3)
T	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)	2 (4.1)
AT	5 (41.7)	5 (41.7)	1 (8.3)	1 (8.3)	12 (24.5)
A+P	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	2 (4.1)
AT+P	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)	2 (4.1)

P value=0.97

A: anthracyclines; T: taxanes; AT: anthracyclines and taxanes; AP: anthracyclines and platinum drugs; AT+P: anthracyclines, taxanes and sequentially platinum drugs

significant pathological response with the loss of a large amount of tumor cellularity. The pCR (MPG5) was observed only in the subgroup of poorly differentiated tumors (G3) in 31.6% of the cases.

The impact of NACT on the level of pathological response is shown in Table 3. The categories of positive response dominated. Anthracycline-containing chemotherapy (63.3%) and the combination of anthracyclines with taxanes (24.5%) showed better results. Among 6 patients having achieved pCR, 16.1% were treated by anthracycline-containing NACT and 8.3% had combinations of anthracyclines plus taxanes.

Chemotherapy with taxanes only or platinum supplement showed moderate responses (MPG2 and MPG3). Despite of different levels of pathological response, there were no significant correlations between cytotoxic drugs and tumor response (p=0.97).

Luminal carcinomas demonstrated pPR (mainly) with continuing high tumor cellularity (MPG2,

MPG3). As an exception, pCR was detected in one patient with a luminal B-like (HER2-negative) tumor with high Ki-67 (Table 4). Since there was a small number of patients in the total sample, the HER2/neu-positive tumors were shown as a combination of overexpression HER2/neu and luminal B-like (HER2/neu-positive). This group demonstrated various response results from MPG2 (36.4%) to MPG5 (9.1%). HER2 overexpression was detected in 5 of 11 patients (45.5%). Only 1 (20%) of them had pCR. TNBC group had the highest level of pCR (50%). The tumor response interacted differently due to tumor subtype (p=0.002).

ROC analysis was used to analyze the relationships between Ki-67 and the degree of pathological response. Figure 1 shows the ROC curve, with the AUC=0.89 (95% CI 0.75-0.96). The presence of a strong connection showed statistically significant difference in AUC more than 0.5 (p<0.001).

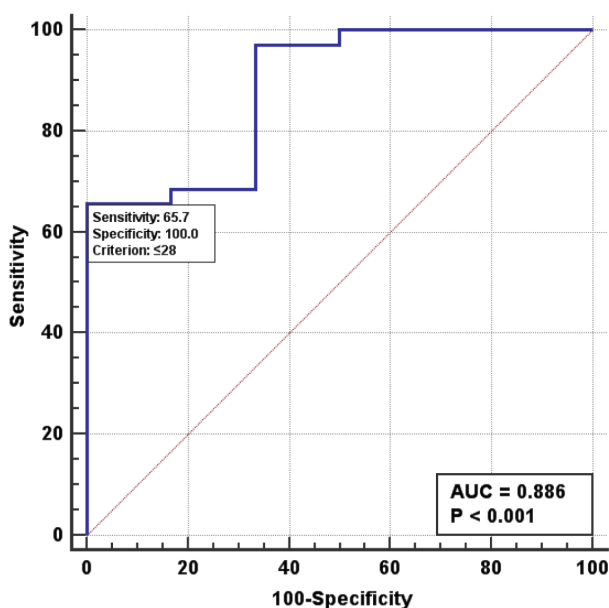
The initial level of Ki-67 in some way predetermined the degree of pathological response that at

**Table 4.** Pathological response rates by intrinsic BC subtypes

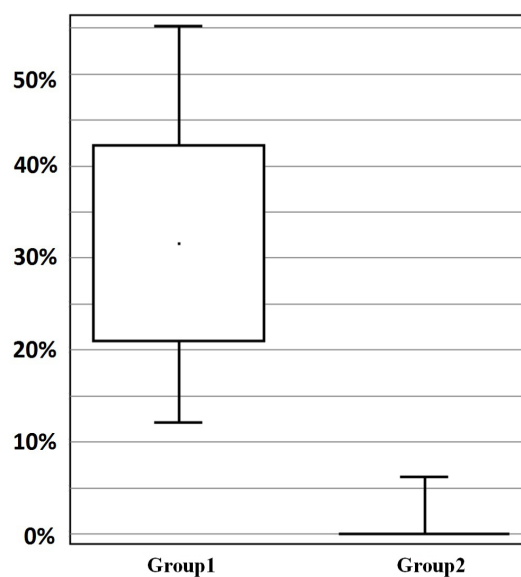
Intrinsic BC subtypes	Miller-Payne score n (%)				Total
	MPG2	MPG3	MPG 4	MPG 5	
Luminal A and Luminal B	12 (40.0)	17 (56.7)	0 (0.0)	1 (3.3)	30 (61.2)
Triple Negative	1 (12.5)	3 (37.5)	0 (0.0)	4 (50.0)	8 (16.3)
HER2/neu positive	4 (36.4)	4 (36.4)	2 (18.1)	1 (9.1)	11 (22.5)

P value=0.002

HER2/neu-positive tumors includes overexpression HER2/neu and luminal B (HER2+)



**Figure 1.** ROC curve of univariate model for predicting the probability of achieving pCR built on Ki-67 marker.



**Figure 2.** Comparison of the BC stages and the probability of achieving pCR (p=0.04). Group 1: early operable BC (stages I and II), Group 2: locally advanced BC (stage III).

**Table 5.** Coefficients of univariate logistic regression model to evaluate the association between pCR and other variables

Independent variables	Regression coefficients, $b \pm m$	Significance level, $p$	Odds ratio (95% CI)
Luminal vs TN	3.37 $\pm$ 1.24	0.007	29 (2.6-329)
HER2/neu positive vs TN	2.30 $\pm$ 1.26	0.07	10.0 (0.8 - 119)
N+ vs N0	1.53 $\pm$ 0.93	0.099	4.6 (0.8-28.4)
AT vs anthracyclines	0.75 $\pm$ 1.15	0.52	-
Taxanes vs anthracyclines	18.9 $\pm$ 13000	-	-
Other regimens (AT+P, A+P) vs anthracyclines	18.9 $\pm$ 13000	-	-
Stage 3 vs stage 2	1.66 $\pm$ 1.14	0.15	-

Luminal subtypes include Luminal A and Luminal B (HER2-negative)

**Table 6.** Distribution of expression Topo IIa depending on the level of pathological response

Expression of Topo IIa	Miller-Payne score n (%)			
	MPG2	MPG3	MPG4	MPG5
Negative	5 (55.6)	3 (33.3)	0 (0.0)	1 (11.1)
Positive	12 (30.0)	21 (52.5)	2 (5.0)	5 (12.5)

P value=0.50

the threshold of Ki-67>28% a significant probability of tumor regression after NACT was expected.

At the selected threshold of Ki-67, the sensitivity was 65.7% (95% CI 47.8-80.9%), and specificity 100% (95% CI 54.1-100%). Thus, the pPR such as MPG2, MPG3 was noted at Ki-67 27.6  $\pm$  22.4% and 28.6 $\pm$ 18.2%, respectively. Pathological response with low cellularity (MPG4) and pCR were detected at Ki-67 61.2  $\pm$  22.7%.

An analysis of univariable logistic regression model was conducted for the estimation the association between pCR and other variables (Table 5). According to the model, the chances of achieving pCR were noted in the TNBC group in comparison with luminal carcinoma ( $p=0.007$ ; OR=29; 95% CI 2.6-329) and overexpressed HER2 BC ( $p=0.07$ ; OR=10; 95% CI 0.8-119). There was no association between the different chemotherapy regimens, involvement of lymph nodes and achieving pCR.

The expression level of Topo IIa protein was not correlated to the response to various cytotoxic drugs ( $\chi^2=1.48$ ,  $p=0.69$ ). The expression of Topo IIa was in no apparent correlation ( $p=0.50$ ) with pathological response (Table 6).

Comparing the stages of BC and the probability of achieving pCR (Figure 2), significant difference between the groups was obtained (Fisher's exact test,  $p=0.04$ ). The frequency of reaching pCR at the early operable stages (I and II) was 22.2% (95% CI 8.0-40.4), while at stage III it was 0% (95% CI 0.0-8.7).

## Discussion

Numerous studies noted that the prognosis of patients after NACT is closely related to the degree of tumor pathological response which can be used as a surrogate marker for better outcomes [1,4,19]. In our research pCR was observed only in 6 patients (12.2%) from 49. This rate was comparable to the results of other authors [6]. Increasing the pathologic response of some BC subtypes could possibly be achieved by administering more intense neoadjuvant treatment [2]. Patients with highly aggressive BC were much more responsive to chemotherapy and achieving pCR than patients with luminal subtypes. It was shown that molecular subtypes proved to be significant predictors of response to the treatment and the achievement of pCR ( $p=0.002$ ). The highest number of pCR was obtained in patients with TNBC, and these results coincide with other investigations [6,7,20]. Some authors [21,22] considered that the pCR frequency increased with aggressive HER2+ BC when trastuzumab was added to NACT. In our study pCR was achieved only in one patient because of the potential confounding of the results by non-using of trastuzumab as neoadjuvant therapy.

In accordance with earlier observations [23,24], the histological type and grade of tumor differentiation were predictors to treatment response. A trend to a significant impact of tumor grade on

the severity of pathological response was demonstrated ( $p=0.07$ ).

Well-differentiated tumors showed a low level of pathological response. It is typical for luminal carcinomas with low proliferative activity. Moderately differentiated tumors were the most heterogeneous group due to intrinsic BC subtypes with high variability in proliferative activity. The highest fluctuations in the response to treatment - from pPR to pCR were found. The only case of pCR in a patient with a luminal B-like (HER2 negative) is likely to be the exception rather than the rule. On the other hand, it could indicate tumor heterogeneity and sufficient diagnostic variability of the core biopsy.

In no case we observed a complete absence of a pathological response (MPG1). On the contrary, we observed a noticeable decrease in the number of total cellularity of the tumor associated with the influence of relevant cytotoxic drugs. The residual tumor cellularity is an important factor in assessing the response, and it correlates with the prognosis [6,25].

The proliferation marker Ki-67 is used to assess baseline risk to adapt adjuvant therapy and it is a component of several multidimensional models of prediction after setting NACT [11]. Adding Ki-67 may improve the prediction of pCR [5].

In addition to the grade of tumor differentiation and aggressive intrinsic BC subtypes, the predictive factor appeared to be Ki-67 with a threshold of 28% and more, with the probability of achieving pathological response. Ki-67 is known to be one of the most reliable biomarkers, and it may predict outcome with greater likelihood of a tumor regression [26].

One of the potential benefits of NACT is the ability to decrease the size of primary tumor, thus permitting breast-conserving surgery. NACT can be used in operable and early-stage disease patients as well. We identified additional factors predicting the possibility of pCR such as early operable BC, with frequency of 22.2% versus 0% for a locally advanced process (Fisher's exact test,  $p=0.04$ ). At the same time, lymph node involvement cannot predict any pathological response ( $p=0.099$ ).

Despite the attainment of best pathological response when using anthracycline-containing NACT, no impact to achieving pCR was detected ( $p=0.97$ ).

There are diverse biological markers, some of them are consistent with the expected associated patient outcomes. Several studies have suggested that Topo II- $\alpha$  expression is related to response to anthracycline chemotherapy for BC [27-29].

Overexpression or amplification of Topo II $\alpha$  in HER2-enriched tumor was positively correlated with higher pCR rates [30,31].

This study did not reveal a predictive value of Topo II $\alpha$  expression associated with achieving a pathological response ( $p=0.50$ ).

Different authors have shown that the predictive value of Topo II $\alpha$  expression may be partly due to the techniques for measuring and an unestablished threshold value for Topo II $\alpha$  expression. In addition, Topo II $\alpha$  is differentially expressed among BC molecular subtypes and its amplification does not correlate to the protein expression, and these two events might be characteristic of different kinds of tumors [32].

The assessment pCR is being incorporated actively in clinical practice but it is a relatively "late" endpoint [33]. That's why we attempted to identify early predictors of the effectiveness of NACT and the achievement of pathological response.

Due to the data obtained, poorly differentiated carcinomas with high cellularity, TNBC, Ki-67 with a threshold above 28% and early operable stages can be considered as early predictors of pathologic response.

To sum up, the use of additional predictive factors allows real-time evaluation of the response to treatment and can potentially be used for personalized therapy of BC patients.

## Authors' contributions

Drs. D. Ryspayeva, A. Lyashenko I. Dosenko and O. Koshyk had full access to all the data in the research and took responsibility for the integrity of the data and the accuracy of the data analysis.

*Study concept and design:* D. Ryspayeva and I.Smolanka;

*Collection, analysis and interpretation of data:* D. Ryspayeva, A. Lyashenko, I. Dosenko, O. Kostryba, M. Krotevych and O. Koshyk;

*Statistical analysis:* D. Ryspayeva, A. Lyashenko and O. Koshyk;

*Writing of the manuscript:* D. Ryspayeva and O. Koshyk;

*Critical revision of the manuscript:* all authors;

*Administrative, technical or material support:* D. Ryspayeva, I.Smolanka, O. Kostryba, M. Krotevych and O. Koshyk;

*Study supervision:* D. Ryspayeva

## Conflict of interests

The authors declare no conflict of interests.

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