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A nomogram based on the expression of Ki-67, steroid hormone receptors status and number of chemotherapy courses to predict pathological complete remission after preoperative chemotherapy for breast cancer

Marco Colleoni [a,*], *Vincenzo Bagnardi* [b,c,d], *Nicole Rotmensz* [b], *Giuseppe Viale* [e], *Mauro Mastropasqua* [e], *Paolo Veronesi* [f], *Anna Cardillo* [a], *Rosalba Torrisi* [a], *Alberto Luini* [f], *Aron Goldhirsch* [g]

[a] *Department of Medicine, Research Unit in Medical Senology, European Institute of Oncology, Milan, Italy*
[b] *Division of Epidemiology and Biostatistics, European Institute of Oncology, Milan, Italy*
[c] *Department of Statistics, University of Milan-Bicocca, Milan, Italy*
[d] *Frontier Science and Technology Research Foundation, Southern Europe, Chiasso, Switzerland*
[e] *Division of Pathology, European Institute of Oncology and University of Milan, School of Medicine, Milan, Italy*
[f] *Division of Senology, European Institute of Oncology, Milan, Italy*
[g] *Department of Medicine, European Institute of Oncology, Milan, Italy*

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ABSTRACT

Background: Tools able to predict pathological complete response (pCR) to preoperative chemotherapy might improve treatment outcome.

Patients and methods: Data from 783 patients with invasive ductal carcinoma treated with preoperative chemotherapy and operated at the European Institute of Oncology were used to develop a nomogram using logistic regression model based on both categorical (clinical T and N, HER2/neu, grade and primary therapy) and continuous variables (age, oestrogen receptor (ER), progesterone receptor (PgR), Ki-67 expression and number of chemotherapy courses). The performance of the resulting nomogram was internally evaluated through bootstrapping methods. Finally the model was externally validated on a patient set treated in other institutions and subsequently operated at the EIO.

Results: At multivariable analysis the probability of pCR was directly associated with Ki-67 expression (OR for 10% increase in the percentage of positive cells, 1.15, 95% confidence interval (CI), 1.03, 1.29) and number of chemotherapy courses (OR for one cycle increase, 1.31, 95% CI, 1.12, 1.53) and inversely associated with ER and PgR expression (ORs for 10% increase in the percentage of positive cells, 0.86, 95% CI 0.79, 0.93 and 0.82, 95% CI 0.69, 0.99, respectively). The nomogram for pCR based on these variables had good discrimination in training as well in validation set (AUC, 0.78 and 0.77).

Conclusion: The use of a nomogram based on the number of preoperative courses, degree of Ki-67 and steroid hormone receptors expression may be useful for predicting the probability of pCR and for the design of the proper therapeutic algorithm in locally advanced breast cancer.

* *Corresponding author:* Address: Department of Medicine, Research Unit in Medical Senology, European Institute of Oncology, Via Ripamonti 435, 20141 Milan, Italy. Tel.: +39 02 57489439; fax: +39 02 574829212.
E-mail address: marco.colleoni@ieo.it (M. Colleoni).

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1. Introduction

Preoperative therapy is indicated for patients with operable breast cancer to improve surgical options. Preoperative therapy might in fact achieve a sufficient tumour shrinkage to allow breast conserving surgery in some patients (depending also on breast size, tumour size and conditions allowing radiation therapy to the conserved breast).[1–3]

Furthermore, in some patients preoperative chemotherapy might provide early information on responsiveness of the disease. In fact, the response to the primary treatment may be used as a prognostic marker, because it was demonstrated to be associated with a longer disease-free survival (DFS) compared with no-response.[4–7] In particular the best response pathological complete remission (pCR) predicts overall outcome in terms of DFS. Several large randomised studies have shown that patients achieving a pCR to primary chemotherapy have far better long-term survival than those who fail to respond (incomplete responders).[4,8]

Recently published studies indicated that the likelihood of pCR might be related to the biological features of the tumour. Indeed, the rate of pCR following preoperative chemotherapy was significantly higher for patients whose tumours did not express both oestrogen receptor (ER) and progesterone receptor (PgR). In particular, pCR rates of 30–40% have been obtained in patients whose tumours did not express both ER and PgR, but only in 2–10% of patients with endocrine responsive tumours.[5,9,10]

Other biological features might also be related to pCR after preoperative chemotherapy. A high proliferative fraction (elevated Ki-67 labelling index)[11] and high tumour grade[12] identified patients with tumours responsive to chemotherapy in the preoperative setting. HER2 overexpression or amplification represents a target for preoperative treatment with the humanised monoclonal antibody against its extracellular domain, but is also a predictive factor of response to preoperative systemic therapies. A significant positive correlation between HER2 positivity and pCR rate in patients treated with neoadjuvant chemotherapy was recently shown.[13] These factors might therefore assist in the identification of patients who might benefit from chemotherapy, in particular within the subset of patients with endocrine responsive disease.

In earlier studies, however, analyses were performed based on biological variables dichotomised as positive or negative (e.g. steroid hormone receptors) through classical cut-offs and no data are currently available on the predictive role of the extent of expression of the biological features in the preoperative setting. We therefore combined all pathological and clinical information available at biopsy before therapy in a nomogram to assess the overall likelihood of a response for an individual patient. We subsequently validated the prognostic model, based on both categorical and continuous variables, to be used for the prediction of pCR for patients who received primary chemotherapy.

2. Patients and methods

2.1. Patients

We collected information through the institutional clinical database on all consecutive breast cancer patients operated at the European Institute of Oncology (IEO), Milan, Italy, between May 1995 and December 2008. Data on patient's medical history, concurrent diseases, type of surgery, pathological assessment of morphological and biological features and results of staging procedures (blood chemistry, haematological values, bone scan, chest film and upper abdominal ultrasound examination) were combined. We subsequently identified those patients treated with preoperative chemotherapy. Other eligibility criteria for the study included no previous chemotherapy/hormonotherapy, performance status 0–2 (ECOG scale) and measurable lesions.

Patients treated with preoperative chemotherapy at the IEO ($N = 1089$) were selected as the training set to develop the predictive model. No pCR was observed within the subgroup of patients with invasive lobular carcinoma (ILC) and consequently we excluded from the study those patients with ILC or mixed ILC and invasive ductal carcinoma (IDC) (85 patients). For this reason, the model is applicable only for patients with IDC. Furthermore, the exclusion of patients without complete information about ER, PgR, HER2/neu and Ki-67 expression (221 patients) led to a total of 783 patients in the training set. A second cohort of patients with IDC treated with preoperative chemotherapy in other Institutions and that subsequently underwent surgery at the IEO were used to validate the final model ($N = 101$).

pCR was evaluated according to Kuerer et al.'s criteria.[8] In particular the absence of invasive cancer on both the primary breast tumour and axillary lymph nodes qualified for pCR. Patients were treated with preoperative chemotherapy given usually in 3-week courses. In the absence of progressive disease a maximum of 8 courses was prescribed. The regimens used during the conduct of the study included anthracycline-containing regimens, taxane-containing regimens as well as other drug combinations as previously reported.[11] From February 2002, patients with tumours expressing ER and/or PgR $\geqslant$ 10% of the cells were candidated to receive concurrent preoperative endocrine therapy (letrozole plus/minus LH–RH analogue according to menopausal status). Moreover, starting from April 2004 concurrent preoperative trastuzumab was proposed to patients with HER2 overexpressed and/or amplified disease. The study was notified to the Ethical Committee.

2.2. Pathology and immunohistochemistry

All patients had pathological evaluation performed at the IEO. The original histological determinations obtained through core-needle biopsies, performed before the patient was included in the study, were used.

Immunostaining experiments for the localisation of ER and PgR, HER2 protein and Ki-67 antigen were performed on consecutive tissue sections, as previously reported.[9] The following primary antibodies were used: the monoclonal antibody (MAb) to ER (clone 1D5 at 1/100 dilution, Dako, Glostrup, Denmark), the Mab to PgR (clone 1A6, 1/800, Dako), the MIB-1 Mab to the Ki-67 antigen (Dako, 1/1200) and the polyclonal antiserum (Dako, 1/3200) to the HER2 protein.

The immunostained slides were evaluated independently by two of the authors. Only nuclear reactivity was taken into account for ER, PgR and Ki-67 antigen, whereas only an

intense and complete membrane staining >10% of the tumour cells was taken as evidence of HER2/neu overexpression (3+).

2.3. Statistical analysis

The main end-point was the achievement of pCR. The nomogram for pCR was created in the training cohort. Multivariable logistic regression was used to build the nomogram, considering both categorical (clinical T, clinical lymph node status, HER2/neu status, grade, type of primary therapy) and continuous variables (age, ER, PgR, and Ki-67 expression, number of preoperative chemotherapy courses) evaluated at diagnosis.

Departure from linearity in the relationship between continuous variables and pCR was evaluated fitting restricted cubic splines models.[14] Backward variable selection was used to determine variables significantly associated with the outcome. The statistical significance of each factor was assessed using Likelihood Ratio Test (LRT). Variables with p-value less than 0.05 were retained in the model.

As a sensitivity analysis, the nomogram building process was repeated only in patients not receiving concomitant chemotherapy and trastuzumab.

Predictive accuracy of the nomogram was evaluated with respect to discrimination (i.e. the ability of the model to classify a patient with the outcome from a patient without the outcome) and calibration (i.e. the agreement between the outcome frequencies observed in the data and the predicted probabilities of the model).[15]

Discrimination was measured by the area under the ROC curve (AUC) that is the probability that a randomly selected patient with pCR had a predicted probability of pCR higher than a randomly selected patient without pCR. A value of 1 denotes perfect discrimination, while a value of 0.5 is no better than chance. Calibration was firstly evaluated by a visual inspection of the plot comparing the observed proportions versus the mean predicted probabilities of event in groups defined by quantiles of predicted probabilities. In the training set, the discriminative ability of the model was assessed using bootstrap technique, in order to reduce overfit bias[16]. Hosmer and Lemeshow goodness-of-fit test[17] was also used to assess calibration internally.

Finally, the nomogram was validated externally. The coefficients from the model developed on patients treated at IEO were used to calculate the estimated probabilities of pCR among the 101 patients with baseline histology evaluated at the IEO, treated with preoperative chemotherapy in other Institutions, and that subsequently underwent surgery at the IEO.

Again, the predicted probabilities of pCR were compared with the observed, and the AUC was calculated. To evaluate the calibration in the validation cohort, unreliability (U)-statistic was used.[14] All statistical analyses were performed using the R software with the Design and Hmisc libraries.[18] All reported p-values were two sided.

3. Results

Among the 783 patients of the training cohort, the observed pCR rate was 12.6%. Table 1 shows the characteristics of the cohort evaluated at diagnosis and their association with pCR probability. Those showing a statistically significant association at univariable analysis with pCR were grade, extent of ER and PgR immunoreactivity, HER2 status, Ki-67 labelling index, type of primary chemotherapy and number of courses.

Age, ER, PgR, Ki-67 and number of courses were considered as continuous variables in the development of the nomogram. No departure from linearity was observed in their relationship with response.

Table 1 – Patient characteristics at baseline and observed pCR rates (training set).

Characteristic	Number of patients	Number of pCR (%)	p-value *
All patients	783	99 (12.6)	–
Age, years			
<35	88	13 (14.8)	0.47
35–49	394	55 (14.0)	
50–59	189	19 (10.1)	
60+	112	12 (10.7)	
Menopausal status			
Premenopausal	336	42 (12.5)	0.92
Postmenopausal	447	57 (12.8)	
Grade			
1–2	394	32 (8.1)	<.0001
3	347	63 (18.2)	
Unknown	42	4 (9.5)	
Clinical T classification			
T2	394	52 (13.2)	0.89
T3	159	19 (11.9)	
T4	230	28 (12.2)	
Clinical nodal status			
Positive	591	67 (11.3)	0.11
Negative	169	27 (16)	
Unknown	23	5 (21.7)	
ER/PgR expression			
ER and PgR absents	295	74 (25.1)	<.0001
ER or PgR 1–49%	326	23 (7.1)	
ER and PgR ⩾50%	162	2 (1.2)	
HER2/neu status			
Positive	215	36 (16.7)	0.034
Negative	568	63 (11.1)	
Ki-67			
<20%	134	5 (3.7)	0.0007
⩾20%	649	94 (14.5)	
Primary chemotherapy			
Anthracyclines	339	44 (13)	0.0003
Anthracyclines and taxanes	145	27 (18.6)	
Trastuzumab	96	18 (18.8)	
Others	203	10 (4.9)	
Number of courses			
3–4	215	14 (6.5)	0.002
5–6	310	48 (13.4)	
7–8	210	37 (17.6)	
Concomitant hormonotherapy †			
Yes	318	6 (3.5)	0.24
No	170	19 (6.0)	

Abbreviations: pCR, pathological complete response; ER, oestrogen receptor; PgR, progesterone receptor.

* Chi-square test comparing pCR rates among levels of the considered variables. Unknowns were excluded in the calculation.

† Patients with ER and PgR absents were not considered.

The parameter estimates of the full multivariable model are shown in Table 2.

After backward selection, predictive factors for pCR that remained statistically significant were ER expression (Odds Ratio, OR, for 10% increase in the percentage of positive cells, 0.86, 95% CI 0.79, 0.93), PgR expression (OR for 10% increase in the percentage of positive cells 0.82, 95% CI 0.69, 0.99), Ki-67 (OR for 10% increase in the percentage of positive cells 1.15, 95% CI 1.03, 1.29) and number of preoperative chemotherapy courses (OR for one course increase 1.31, 95% CI, 1.12, 1.53) (Table 2). The *p*-value from the LRT comparing the full model with the reduced model was 0.24, indicating that the goodness-of-fit of the two models was similar.

After excluding 96 patients treated with concomitant chemotherapy and trastuzumab, the same factors were selected and the parameter estimates did not substantially change (OR 0.85, 0.77, 1.16 and 1.24 for ER, PgR, Ki-67 and number of preoperative courses, respectively).

Fig. 1 shows the nomogram constructed on the basis of the final multivariable logistic regression model to predict pCR. The nomogram is used by first determining the patient's score for each predictor. For example, an ER expression of 40% contributes approximately 48 points; this is determined by comparing the location of the 40 value on the 'ER' axis to the 'Points' scale above and drawing a vertical line between the two axes. The scores for all considered predictors are determined in a similar manner and are summed to account for a Total Points value. This value is plotted on the Total Points axis. A vertical line drawn from the Total Points axis straight down to the pCR probability axis will indicate the patient's probability of pCR.

Two examples of actual individuals in the IEO cohort illustrate the range of pCR probability predicted by the model. A patient with ER expression of 95%, PgR expression of 90%, Ki-67 expression of 6% and receiving 4 courses of chemotherapy obtained a total score of about 34 points, corresponding to a predicted pCR probability less than 0.5%. At the opposite, a patient with ER and PgR expression absents, Ki-67 expression of 80% and receiving 7 courses of chemotherapy scored about 290 points, corresponding to a predicted pCR probability of about 40%. The model AUC (discrimination) was 0.779 in the training set (Fig. 2A). The corrected AUC after bootstrap sampling was similar (0.773).

Fig. 2B shows how the pCR probabilities predicted from the model compared with the observed pCR probabilities for the patients in the training cohort, grouped into quintiles of the predicted risk score (calibration). The *x*-axis is the prediction calculated from the nomogram and the *y*-axis is the actual pCR rate observed in the cohort. Dashed line is the ideal relationship where model perfectly predicts the actual outcome.

Table 2 – Multivariable logistic regression models predicting pathological complete response after primary chemotherapy. Full and final models.

Characteristics	Full model			Final model		
	Parameter estimate	OR (95% CI)	*p*-value LRT	Parameter estimate	OR (95% CI)	*p*-value LRT
Intercept	−2.4073	–	–	−3.448	–	–
Age						
+10 years increase	−0.009	0.99 (0.97, 1.02)	0.47			
Grade						
G3 versus G1–G2	0.2767	1.32 (0.79, 2.21)	0.29			
Clinical T						
T3 versus T2	−0.2911	0.75 (0.41, 1.38)	0.63			
T4 versus T2	−0.145	0.87 (0.49, 1.54)				
Clinical N[*]						
Positive versus negative	−0.5377	0.58 (0.34, 1.01)	0.055			
ER expression						
+10% increase	−0.0148	0.86 (0.78, 1.09)	0.003	−0.153	0.86 (0.79, 0.93)	0.0002
PgR expression						
+10% increase	−0.0212	0.81 (0.67, 1.17)	0.022	−0.195	0.82 (0.69, 0.99)	0.034
HER2/neu status						
Positive versus negative	0.03	1.03 (0.56, 1.91)	0.92			
Ki-67						
+10% increase	0.00982	1.10 (0.97, 1.15)	0.13	0.141	1.15 (1.03, 1.29)	0.013
Primary chemotherapy						
AC+TAX versus AC	−0.1109	0.90 (0.50, 1.62)	0.69			
Trastuzumab versus AC	0.0506	1.05 (0.46, 2.39)				
Others versus AC	−0.4709	0.62 (0.28, 1.38)				
Number of courses						
+1 cycle increase	0.2578	1.29 (1.09, 1.54)	0.004	0.267	1.31 (1.12, 1.53)	0.001
Concomitant HT						
Yes versus No	0.1521	1.16 (0.58, 2.32)	0.67			

Abbreviations: OR, odds ratio; CI, confidence interval; AC, anthracyclines; TAX, taxanes; HT, hormotherapy; LRT, likelihood ratio test.

[*] The 23 missing values were treated as a separate category in the multivariable logistic model.

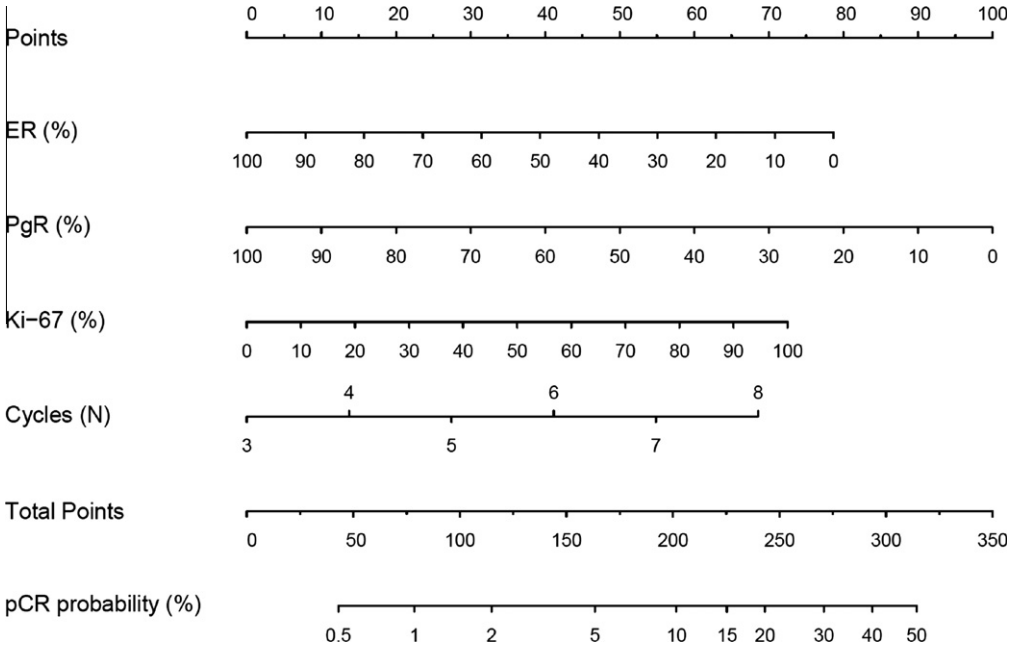

Fig. 1 – Nomogram predicting the probability of pathological complete response (pCR) after primary chemotherapy.

In the training cohort, calibration of our nomogram was good, since it did not exhibit systematic over- or under-prediction (p-value for the Hosmer and Lemeshow statistic = 0.89).

Table 3 shows the characteristics of the 101 patients included in the validation set. These patients had a lower probability of pCR if compared with patients in the training set (5.9% versus 12.6%, $p = 0.05$). Moreover patients in the training set had tumours with higher Ki-67 expression (Ki-67 $\geqslant$ 20%, 83% versus 73%; $p = 0.01$) and lower ER/PgR expression (ER and/or PgR positive, 62% versus 72%, $p = 0.05$) if compared with the validation set. Regimens containing anthracyclines and taxanes were used less frequently (19% versus 40%, $p < 0.01$) and 3–4 courses were given less frequently (29% versus 59%, $p < 0.01$) in the training set than in the validation set. The discriminative ability and calibration of the nomogram were also evaluated in this cohort. The AUC was 0.770 (Fig. 2A), while predicted and observed pCR rates were fairly concordant (Fig 2B), with no significant difference (p-value for the U statistics = 0.34).

Using the ROC curve in the training set, the optimal cut-off level of the pCR probability predicted by the nomogram (i.e. the value maximising the sum of sensitivity and specificity) was 13.6%.

Among the 38 patients in the validation set whose predicted probability was greater than 13.6%, five patients had a pathological complete response. Among the remaining 63 patients in the validation set whose predicted probability was lower than 13.6%, one pCR was observed.

Notably, HER2 status was not significantly associated with the likelihood of pCR at the multivariable analysis. However, a borderline significant interaction between HER2 status and PgR expression was detected ($p = 0.068$). A significant trend towards lower probability of pCR with increasing PgR expression in HER2 negative, ER positive (>0% of the cells) tumours was observed (p-trend: 0.009, Fig. 3A), whereas in HER2 positive, ER positive, the expression of PgR was not significantly associated with the probability of pCR (p-trend: 0.95, Fig. 3B). Nevertheless, adding the interaction term to the final model did not appreciably change its predictive accuracy (AUC 0.782). Still, the nomogram including the interaction is shown in Supplemental Fig. S1.

The number of courses of therapy might be of less importance for treatment decisions since current guidelines on preoperative chemotherapy supports a longer treatment duration. We therefore developed a further model based only upon biological variables (ER, PgR and Ki-67 expression). The exclusion of the number of courses to the model did not appreciably change its predictive accuracy and a good discrimination was maintained in the training as well in the validation set (AUC, 0.757 and 0.768). The nomogram based on biological variables alone is shown in Supplemental Fig. S2.

4. Discussion

It has been assumed that pCR is a valid surrogate marker of long-term survival and cure in patients with locally advanced breast cancer treated with preoperative chemotherapy. A large evidence from retrospective analysis of well-conducted clinical trials supports this assumption,[4–6] although the definition of pCR has varied across clinical trials throughout the literature. More recently it has been agreed that the ideal definition of pCR is the absence of residual invasive cancer within both the breast and lymph nodes. In fact the presence of nodal disease after preoperative therapy predicts for a poorer prognosis and should be probably considered as residual disease.[8,19]

How to identify patients most likely to achieve pCR remains unclear. In fact, despite the recognition in the past years of different subtypes of breast cancer based on expres-

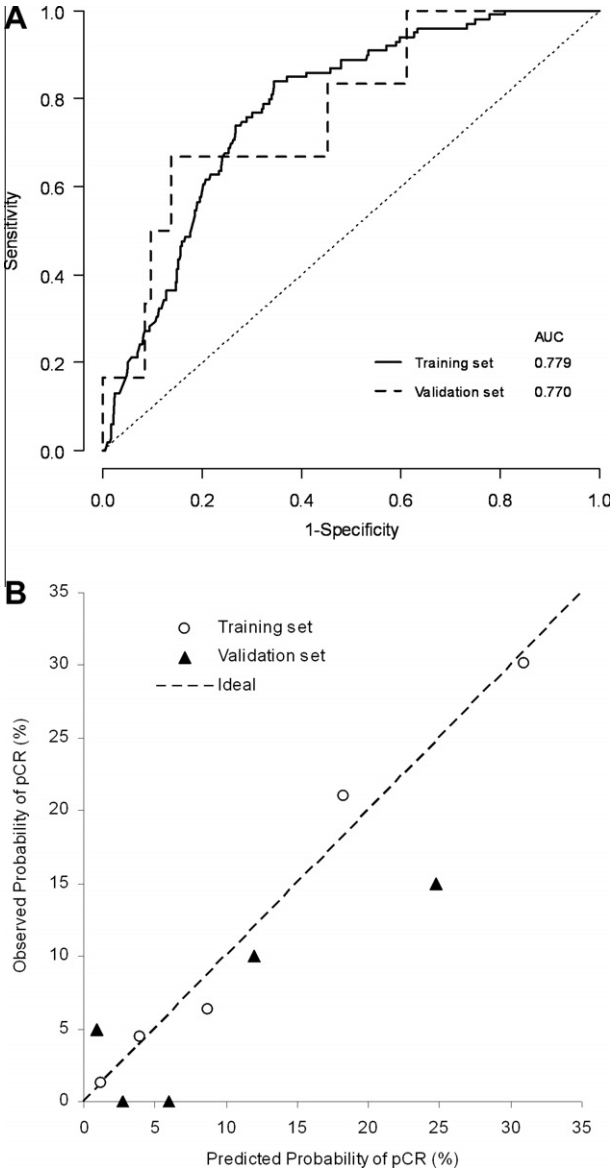

Fig. 2 – Predictive accuracy of the nomogram to predict pathological complete response (pCR) after primary chemotherapy. (A) AUC in the training and the validation set (B) predicted and observed pCR rates in the training and the validation set.

Table 3 – Patient characteristics at baseline and observed pCR rates (validation set).

Characteristic	Number of patients	Number of pCR (%)
All patients	101	6 (5.9)
Age, years		
<35	13	1 (7.7)
35–49	49	2 (4.1)
50–59	31	3 (9.7)
60+	8	0 (0)
Menopausal status		
Premenopausal	52	2 (3.8)
Postmenopausal	49	4 (8.2)
Grade		
1–2	25	1 (4)
3	24	2 (8.3)
Unknown	52	3 (5.8)
Clinical T classification		
T2	51	5 (9.8)
T3	24	1 (4.2)
T4	26	0 (0)
Clinical nodal status		
Positive	73	5 (6.8)
Negative	27	1 (3.7)
Unknown	1	0 (0)
ER/PgR expression		
ER and PgR absents	28	4 (14.3)
ER or PgR 1–49%	49	1 (2)
ER and PgR ⩾50%	24	1 (4.2)
HER2/neu status		
Positive	10	1 (10)
Negative	62	4 (6.5)
Unknown	29	1 (3.4)
Ki-67		
<20%	27	1 (3.7)
⩾20%	74	5 (6.8)
Primary chemotherapy		
Anthracyclines	41	4 (9.8)
Anthracyclines and taxanes	41	1 (2.4)
Herceptin	7	1 (14.3)
Others	12	0 (0)
Number of courses		
3–4	60	4 (6.7)
5–6	30	1 (3.3)
7–8	11	1 (9.1)
Concomitant hormonotherapy[*]		
Yes	18	0 (0)
No	55	2 (3.6)

Abbreviations: pCR, pathological Complete Response; ER, Oestrogen Receptor; PgR, Progesterone Receptor.
[*] Patients with ER and PgR absents were not considered.

sion profiles and immunohistochemical identification of selected markers,[20,21] the accurate identification of factors predictive of response to preoperative treatment programs continues to represent a major research issue.

This study provides useful insights into the treatment of breast cancer because it is based on a large number of patients, collected in a relatively short time, thus allowing adoption of modern procedures. The pathologists, surgeons and medical oncologists used consistent approaches during the years of reference. Other authors already developed nomograms based on large number of patients to predict pCR after preoperative chemotherapy.[12,22] However, data from past series include information on several characteristics of the disease collected in the earlier period, when the various prognostic and predictive factors were not available as they are today.

In the present study we developed a nomogram to predict pCR based upon the extent of expression of biological characteristics of the primary tumour as well as duration of preoperative therapy. The latter point however might be less relevant for clinical purposes. In fact a longer treatment duration (e.g. 6–8 courses) has been shown to compare favourably with shorter duration (3–4 courses) in large randomised trials in terms of response.[2,8] Therefore, it is commonly accepted that for patients candidate to preoperative therapy, at least 6

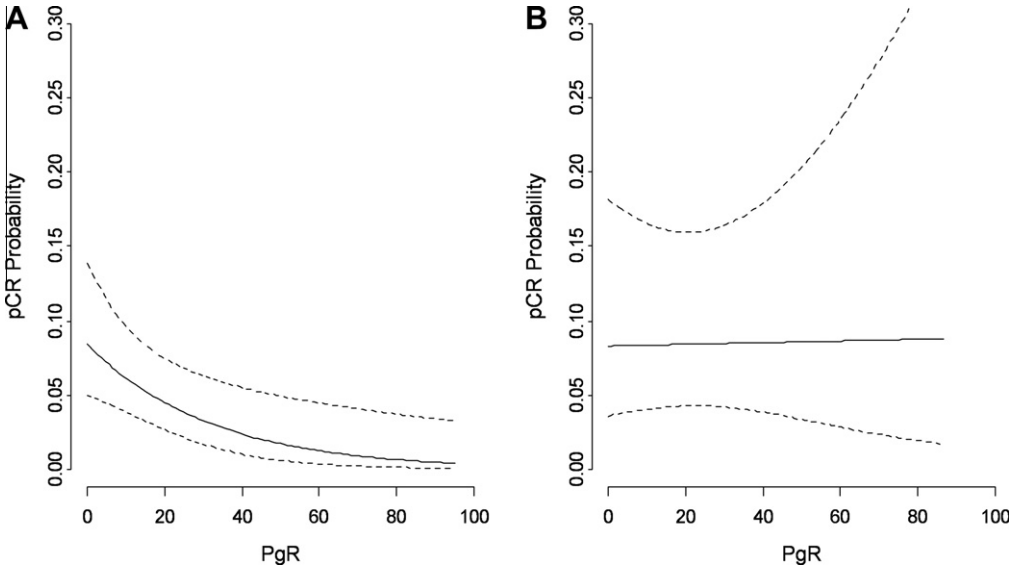

Fig. 3 – Relationship between PgR expression and probability of pCR in ER positive tumours (>0% of the cells), stratified by HER2 status. (A) HER2 negative, (B) HER2 positive.

courses of chemotherapy should be planned and given preoperatively over 4–6 months.[8] The nomogram based only on biological variables maintained however a good discrimination in the training as well in the validation set (AUC, 0.757, and 0.768 (Supplemental Fig. 2).

We found a significant correlation between the degree of expression of Ki-67, ER and PgR and the response (pCR) to preoperative chemotherapy. In fact, although helpful for the purpose of a pragmatic decision on who may benefit from targeted treatments and therefore for the therapeutic algorithm, the definition of arbitrary thresholds in a biological continuum might be inappropriate. In particular, the quantitative assessment of biological features (e.g. ER and PgR expression) may be important to better define treatment response, rather than simply relying on the positive and negative designations, that encompass a mixture of patients with variable degree of responsiveness such as of endocrine responsiveness. Previous studies showed a different pattern of response to chemotherapy according to the extent of steroid hormone receptors. In particular, no or limited benefit from the addition of chemotherapy to endocrine therapy was observed in pre- and postmenopausal women with tumours showing higher expression of ER.[23,24] Also the higher expression of PgR was correlated in previous studies with a lower degree of response to adjuvant chemotherapy and higher response to endocrine treatment.[25] It is possible that the high expression of ER and PgR identifies a Luminal A phenotype characterised by a less aggressive course of the disease as well as poor response to preoperative chemotherapy, as shown in the present study. The Luminal A phenotype has been in fact associated to an improved outcome, albeit the lack of pCR after preoperative chemotherapy.[26]

The results of the present study indicate that measures of tumour cell proliferation such as Ki-67 labelling index could potentially identify responsive patients to preoperative chemotherapy in locally advanced breast cancer. Proliferation- and genomic grade-related gene signatures have already been shown to be associated with chemotherapy sensitivity in both ER– and ER+ breast tumours.[27] Moreover, the use of proliferation indexes might result in a highly effective separation of subgroups within the ER-positive patients into different populations (luminal A and luminal B), with a different outcome when compared across classical prognostic features.[28] The result of the present study confirms the importance of proliferation markers in the therapeutic algorithm and supports its incorporation into therapy decisions.

Limited data are available on the relationship between HER2 status and response to preoperative chemotherapy,[13] and HER2 was uncommonly taken into account in the development of previous models or nomograms. This might be explained by the small sample sizes, heterogeneity of examinations and methods, and especially cut-offs used in the various studies. In the present study we observed a borderline significant interaction between HER2 overexpression and PgR expression. In particular a predictive role of PgR was observed only in the subgroup of patients with HER2 negative disease whereas no effect was observed in the HER2 positive population. These results might be explained with the identification of a specific HER2 negative subpopulation with high expression of ER but low expression of PgR, already defined and characterised by a higher probability of response to chemotherapy if compared with the HER2 negative subgroup expressing high levels of ER and PgR.[26] In the former subgroup of patients, hyperactive cross-talk between ER and growth factor signalling pathways was recently reported, leading to a more aggressive course of the disease.[29]

A small subgroup of patients in the present study received also concomitant trastuzumab. The introduction of preoperative trastuzumab resulted in significantly improved pCR rates in recently published randomised trials[30,31] therefore possibly influencing the herein reported results. However, in a further analysis conducted with the exclusion of those patients who received preoperative trastuzumab results remain unchanged therefore confirming the validity of the proposed model.

In conclusion, in the present study, conducted in a single Institution, we developed a nomogram that can be used to predict the global chemosensitivity to primary chemotherapy in patients with breast cancer. The magnitude of the effect of preoperative chemotherapy in early breast cancer significantly correlates with the degree of expression of biological features identified through immunohistochemistry as well as the number of courses. Further studies are warranted to further define the predictive value of the available nomograms in other series of patients. Meanwhile, the application of this nomogram may be useful when an algorithm should be developed in candidate patients to preoperative treatment.

Conflict of interest statement

Dr. M. Colleoni has Research Funding and Honoraria to disclose (Novartis); there are no further financial disclosures from other authors.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2010.04.008.

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