

A risk score to predict disease-free survival in patients not achieving a pathological complete remission after preoperative chemotherapy for breast cancer

M. Colleoni^{1*}, V. Bagnardi^{2,3,4}, N. Rotmensz², S. Dellapasqua¹, G. Viale⁵, G. Pruneri⁵, P. Veronesi⁶, R. Torrìsi¹, A. Luini⁶, M. Intra⁶, V. Galimberti⁶, E. Montagna¹ & A. Goldhirsch¹

¹Department of Medicine; ²Division of Epidemiology and Biostatistics, European Institute of Oncology; ³Department of Statistics, University of Milan-Bicocca; ⁴Frontier Science and Technology Research Foundation, Southern Europe; ⁵Division of Pathology, European Institute of Oncology and University of Milan School of Medicine and ⁶Division of Senology, European Institute of Oncology, Milan, Italy

Received 29 October 2008; accepted 17 November 2008

Background: We aimed to predict disease-free survival (DFS) in patients who failed to achieve a pathologic complete remission (pCR) after preoperative chemotherapy (PC).

Patients and methods: Data from 577 patients treated with PC and operated at the European Institute of Oncology (EIO) were used to develop a nomogram using Cox proportional hazards regression model based on both categorical (pT, positive nodes, human epidermal growth factor receptor 2 (HER2) status, vascular invasion) and continuous histological variables (estrogen receptors and Ki-67 expression) at surgery. The nomogram was tested on a second patient cohort (343 patients) treated in other institutions and subsequently operated at the EIO.

Results: The nomogram for DFS based on both categorical and continuous variables had good discrimination in the training and the validation sets (concordance indices 0.73, 0.67).

Conclusion: The use of a nomogram based on the degree of selected histopathological variables can predict DFS and might help in the adjuvant therapeutic algorithm design.

Key words: breast cancer, disease-free survival, nomogram, predictive factors, preoperative chemotherapy, primary therapy

introduction

Preoperative treatment is an established therapy for patients with operable breast cancer for whom a reduction of primary tumor size is required in order to allow breast conservation [1–3].

Preoperative chemotherapy (PC) might be beneficial in several ways in addition to allowing breast conserving surgery in some of the patients. In fact, the degree of response to the primary treatment may be used as a prognostic marker. Several large randomized studies have shown that patients achieving a pathological complete remission (pCR) to chemotherapy have better long-term survival than those who respond incompletely to primary chemotherapy. Recent studies, however, indicated that pCR can be achieved in a minority of the patients. In particular, pCR rates range from 30% to 40% in those patients whose tumors do not express estrogen receptors (ERs) or progesterone receptors (PgRs), whereas in patients with endocrine responsive tumors pCR rates range between 2% and 10% [4–6]. Therefore, >75% of the patients currently fail

to achieve a pCR and have an increased risk of relapse and death, even if they received additional systemic therapy.

Limited data are available on factors which are able to predict prognosis of breast cancer after PC in patients at substantial risk of relapse, although several models or nomograms were presented in the past based on both clinical and pathological features [7–9].

We combined all pathological information available at surgery after PC in a nomogram which is able to calculate the overall probability of a specific clinical outcome for an individual patient. We subsequently validated the prognostic model, based on both categorical and continuous histological variables, to be used for the prediction of disease-free survival (DFS) for patients who received primary chemotherapy, resulted as incomplete responders, and had a homogeneous diagnostic and therapeutic environment.

patients and methods

patients

We collected information through the institutional clinical database on all consecutive breast cancer patients operated at the European Institute of Oncology, Milan, Italy, from May 1995 to June 2005. Data on patients'

*Correspondence to: Dr M. Colleoni, Department of Medicine, Division of Medical Oncology, European Institute of Oncology, Via Ripamonti 435, 20141 Milan, Italy. Tel: +39-02-57489439; Fax: +39-02-574829212; E-mail: marco.colleoni@ieo.it

medical history, concurrent diseases, type of surgery, pathological assessment of morphological and biological features and results of staging procedures (blood chemistry, hematological values, bone scan, chest film and upper abdominal ultrasound examination) were combined. We subsequently identified those patients treated with PC. Other eligibility criteria for the study included no previous chemotherapy/hormonotherapy, performance status of zero to two (Eastern Cooperative Oncology Group scale), measurable lesions and age between 18 and 70 years.

These patients were divided into two cohorts according to treatment center.

The first cohort included those patients treated with preoperative therapy at the European Institute of Oncology (EIO) ($N = 577$) and was used as a training set to develop the nomogram. pCRs were evaluated according to the criteria of Kuerer et al. [10]. In particular, the absence of invasive cancer on both the primary breast tumor and axillary lymph nodes qualified for pCR. Consequently, only those patients who presented invasive cancer on either the primary breast tumor and/or axillary lymph nodes were considered for the present study. Patients were treated with PC given in 3-week courses. Patients with partial remission or complete remission were candidates to receive a maximum of six courses. The regimens used during the conduct of the study included anthracycline-containing regimens, taxane-containing regimens and navelbine-containing regimens, as previously reported [4].

The second cohort used as a validation set, included patients treated with PC in other institutions ($N = 343$) and that subsequently underwent surgery at the EIO.

Written informed consent was obtained from all patients. The study was notified to the Institutional Review Board.

pathology and immunohistochemistry

All patients had pathological evaluation carried out at the EIO. The original histological determinations, carried out before the patient was included in this analysis, were used. The histotype evaluation was carried out based on the results at final surgery.

Immunostaining experiments for the localization of ER and PgR, human epidermal growth factor receptor 2 (HER2) protein and Ki-67 antigen were carried out on consecutive tissue sections, as previously reported [4]. The following primary antibodies were used: the mAb to ER (Dako, Glostrup, Denmark; at 1/100 dilution), the mAb to PgR (Dako, 1/800), the MIB-1 mAb to the Ki-67 antigen (Immunotech, Marseille, France, 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 tumor cells was taken as evidence of HER2/neu overexpression (3+).

statistical analysis

The main end point was DFS. DFS was defined as the length of time from the date of surgery to events such as relapse (including ipsilateral breast recurrence), appearance of a second primary cancer (including contralateral breast cancer) or death, whichever occurred first. For survivors, DFS was censored at the last follow-up visit. The DFS distribution was estimated using the Kaplan–Meier method, and log-rank test was used to test differences between survival distributions in the univariable analysis.

The Cox proportional hazard multivariable regression was used to build the nomogram for DFS, considering both categorical (histologic type, pT, lymphonodal status, HER2 status, vascular invasion, type of primary therapy and response to primary therapy) and continuous variables (age, ER, PgR and Ki-67) evaluated at surgery.

Departure from linearity in the relationship between continuous variables and DFS was evaluated by fitting restricted cubic spline models

[11]. Schoenfeld residuals were used to check the adequacy of proportional hazard assumption [12]. Backward variable selection was used to determine variables significantly associated with the outcome. Variables with P value <0.10 were retained in the model.

The nomogram for DFS was created in the training cohort. Predictive accuracy of the nomogram was firstly assessed by internal validation, using bootstrap technique in order to reduce overfit bias [11]. It 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). Discrimination was measured by the Harrell's concordance statistics (c -index), which is the probability that given two randomly selected patients, the survival time predicted by the model is greater for the subject who survived longer. In case of analysis of censored data, the c -index is calculated using all possible pairs of patients at least one of whom has experienced the event. A value of 1 denotes perfect concordance, while a value of 0.5 is no better than chance. Calibration was evaluated by a visual inspection of the plot comparing the 5-year probabilities of event predicted by the model with the Kaplan–Meier 5-year survival proportions observed in groups defined by quantiles of predicted probabilities.

Finally, the nomogram was validated externally: for each patient of the validation cohort, the risk score of event was calculated using the parameter estimates from the model developed in the training cohort. Again, the 5-year predicted probabilities of event were compared with the observed, and the c -index was calculated.

All statistical analyses were carried out using the R software with the Design and Hmisc libraries [13]. All reported P values were two sided.

results

Table 1 displays the characteristics at surgery of the 577 patients in the training cohort and the 343 patients in the validation cohort. The characteristics of the two cohorts did not differ greatly overall, except for type of primary therapy received. Six percent of the patients in the training cohort and 36% in the validation set received anthracyclines combined with taxanes, while additional hormonotherapy was given to 25% of the training cohort and only 4% of the validation cohort. A partial response was observed in the 62% and 57% of the training and validation cohort, respectively. The frequency of patients with more than three positive lymph nodes at surgery was greater in the validation (57%) than in the training (48%) cohort.

Forty-six percent and 43% of patients underwent breast-conserving surgery in training and validation cohort, respectively. Radiotherapy was carried out in 74% of the patients in the training cohort and 77% in the validation sample. The majority of patients (93%) were candidate to adjuvant therapy both in training and validation cohort.

development of the model and internal validation

Among the 577 patients of the training cohort, median length of follow-up was 56 months (range 1–140). DFS at 5 years was 59% [95% confidence interval (CI) 55% to 64%] (Figure 1). Table 2 shows the univariate analysis of the factors measured at surgery. The factors that showed a statistically significant association with survival were type of primary therapy, histotype, residual tumour dimension (pT), number of positive lymph nodes, degree of tumour endocrine responsiveness,

Table 1. Patient characteristics at surgery of the training and validation cohorts

	Cohort			
	Training (n = 577)		Validation (n = 343)	
	n	%	n	%
Primary chemotherapy				
Anthracyclines	289	50.1	201	58.6
Anthracyclines and taxanes	36	6.2	122	35.6
Other	252	43.7	20	5.8
Additional	144	25.0	14	4.1
hormonotherapy				
Response to primary therapy				
Partial response	360	62.4	197	57.4
Stable disease	202	35.0	138	40.2
Progression	15	2.6	8	2.3
Age (years)				
<35	66	11.4	50	14.6
35–49	282	48.9	167	48.7
50–59	153	26.5	90	26.2
60+	76	13.2	36	10.5
Histologic type				
Ductal	494	85.6	288	84.0
Lobular	43	7.5	26	7.6
Other	40	6.9	29	8.4
Residual tumor size at surgery (pT)				
pT0	10	1.7	5	1.5
pT1	245	42.5	145	42.3
pT2	221	38.3	123	35.9
pT3	88	15.3	55	16.0
pT4	13	2.3	15	4.4
Positive lymph nodes				
None	142	24.6	60	17.5
1–3	160	27.7	87	25.4
4+	275	47.7	196	57.1
Degree of ER and PgR expression				
ER and PgR absents	175	30.3	108	31.5
ER or PgR 0%–49%	325	56.3	163	47.5
ER and PgR ≥ 50%	77	13.4	69	20.1
Unknown	0	0.0	3	0.9
HER2 status				
Overexpressed	108	18.7	88	25.7
Not overexpressed	427	74.0	252	73.5
Unknown	42	7.3	3	0.9
Ki-67				
<20%	295	51.1	167	48.7
≥20%	280	48.5	165	48.1
Unknown	2	0.3	11	3.2
PVI				
Absent	313	54.2	170	49.6
Present	81	14.0	44	12.8
Focal	28	4.9	21	6.1
Diffuse	138	23.9	98	28.6
Unknown	17	2.9	10	2.9

ER, estrogen receptor; PgR, progesterone receptor; PVI, peritumoral vascular invasion.

HER2 status, Ki-67 and peritumoral vascular invasion (PVI). When ER and PgR expression, Ki-67 and age were considered as continuous variables, no departure from linearity was observed in their relationship with survival.

In multivariable analysis, statistically significant prognostic/predictive factors of DFS were residual tumour dimension (pT), number of positive lymph nodes, ER expression, HER2/neu status, Ki-67 expression and PVI (Table 3). Figure 2 shows a nomogram to predict 5-year DFS, constructed on the basis of the fitted multivariable proportional hazard model. The nomogram is used by first determining the patient’s score for each predictor. For example, an ER expression of 40% contributes approximately of 31 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 arrive at 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 5-years DFS probability axis will indicate the patient’s probability of remaining free from event for 5 years.

The bootstrap-corrected concordance index for the nomogram was 0.73 (95% CI 0.67–0.80) (discrimination). Figure 3A shows how the predictions from the model at 5 years compared with the actual survival probability 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 DFS observed in the cohort. Dashed line is the ideal relationship where model perfectly predict the actual outcome. The performance of our nomogram is plotted as the solid line that connects the dots. Calibration of our nomogram was good since it did not exhibit systematic over- or underprediction. Only for patients with intermediate prognosis, the model did not perform very well, with almost equal DFS observed probability (59% and 60%) in patients classified in the second and in the third quintile of predicted risk. The Xs indicate bootstrap-corrected estimates of the predicted DFS proportion. Vertical bars indicate 95% CI around the actual probability. Only for the second quintile of predicted risk, the model showed a significant overestimation of the risk of event within 5 years from the time of surgery (56% predicted versus 41% observed).

external validation

Patients in the validation cohort had worse survival compared with patients in the training set (Figure 1). DFS at 5 years was 50% (95% CI 44% to 56%). The median length of follow-up was 50 months (range 1–127).

The discriminative ability and calibration of the nomogram was also evaluated in this cohort. The concordance index was 0.67 (95% CI 0.58–0.75), while predicted and observed 5-year DFS rates were fairly concordant (Figure 3B).

discussion

It has been assumed that pCR is a valid surrogate of long-term survival and cure from breast cancer. A large evidence from retrospective analyses of well-conducted clinical trials supports this notion [2, 3, 14, 15]. However, how to distinguish

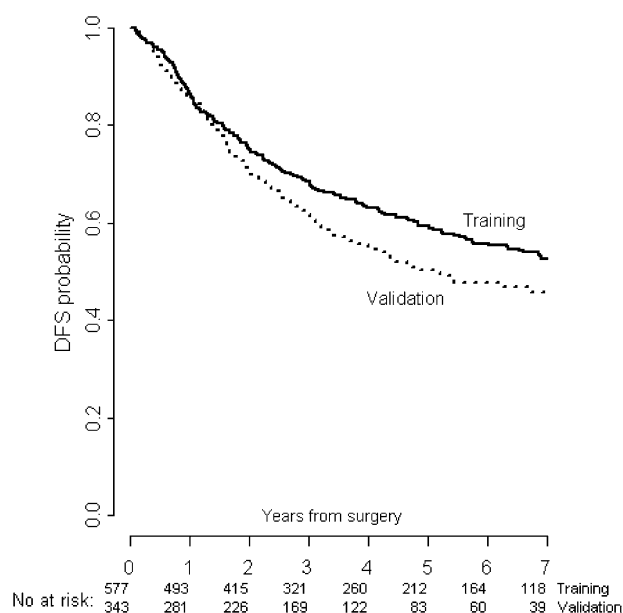


Figure 1. Disease-free survival in the training and validation cohort.

patients at high risk of relapse within those who failed to achieve a pCR remains uncertain. Appropriate identification of prognostic factors and factors predictive of responsiveness to specific adjuvant treatment programs after preoperative therapy continue to represent a major research issue. Intrinsically different subtypes of breast cancer were clearly recognized in the past years based on genetic profile and immunohistochemical demonstration of selected targets [16, 17], but optimal tailoring of adjuvant therapies after PC is still a matter of debate. Treatment strategy should focus mainly on targeted therapies wherever possible, though acknowledging that supplementation with less target-specific chemotherapy is often required.

This analysis provides useful insights into the treatment and prognosis 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. Other groups already developed nomograms based upon large number of subjects which are able to correlate patients' outcome after PC. However, data from past series include information on several aspects of the disease collected in the earlier period, when the various prognostic and predictive factors were not available in the fashion they are today [6]. Moreover, no central pathology review was carried out in some of these studies [7].

In the present study, the surgeons, medical oncologists and most of all pathologists used consistent approaches during the years of reference. Adjuvant systemic therapies, besides an increased attention to precise determination of the biological characteristics of primary tumors, represent another relevant novelty between current and past assessments. Adjuvant treatment proposed was largely based on the degree of nodal involvement as well as on known prognostic features according to the recent St. Gallen Consensus Conference Guidelines [18–20].

Here, we developed a nomogram which is able to predict DFS based upon expression of classical and newer pathologic

Table 2. Five-year DFS according to patient characteristics at surgery

	No. of patients	No. of events	5-year DFS probability	P value
Primary chemotherapy				
Anthracyclines	289	131	0.60	0.0004
Anthracyclines and taxanes	36	21	0.40	
Others	252	94	0.61	
Response to primary therapy				
Partial response	360	146	0.60	0.34
Stable/progressive disease	217	100	0.58	
Age (years)				
<35	66	31	0.57	0.73
35–49	282	120	0.60	
50–59	153	66	0.58	
60+	76	29	0.60	
Histologic type				
Ductal	494	221	0.57	0.018
Lobular	43	12	0.82	
Other	40	12	0.68	
Residual tumor size at surgery (pT)				
pT0	10	6	0.30	<0.0001
pT1	245	86	0.67	
pT2	221	98	0.56	
pT3	88	46	0.56	
pT4	13	10	0.13	
Positive lymph nodes				
None	142	47	0.71	<0.0001
1–3	160	58	0.67	
4+	275	141	0.49	
Degree of ER and PgR expression				
ER and PgR absent	175	111	0.36	<0.0001
ER or PgR 0%–49%	325	110	0.67	
ER and PgR ≥ 50%	25	77	0.76	
HER2 status				
Overexpressed	108	61	0.37	<0.0001
Not overexpressed	427	170	0.62	
Ki-67				
<20%	295	88	0.75	<0.0001
≥20%	280	156	0.44	
Vascular invasion				
Absent	313	105	0.68	<0.0001
Present	81	35	0.59	
Focal	28	13	0.59	
Diffuse	138	86	0.40	

Univariable analysis based on the 577 patients in the training cohort. DFS, disease-free survival; ER, estrogen receptor; PgR, progesterone receptor.

characteristics of the primary tumor. Moreover, the degree of expression of selected features was used considering that the definition of arbitrary thresholds in a biological continuum might be misleading.

Among categorical variables, the nomogram included T stage, number of positive nodes, vascular invasion and HER2. The residual tumor size as well as the diameter of the tumor according to the tumor–node–metastasis staging system have

been demonstrated to significantly correlate with survival [7, 8]. In particular, a poorer outcome was observed for large residual tumors, supporting the development of non-cross-resistant chemotherapy regimens to improve DFS and overall survival rates of patients with incomplete response to neoadjuvant chemotherapy [21].

Table 3. Multivariable proportional hazard regression model predicting DFS

	Parameter estimate	Hazard ratio	95% CI	P value
Residual tumor				
size at surgery (pT)			reference	
pT0–pT1			reference	
pT2	0.327	1.39	1.01–1.90	0.042
pT3–pT4	0.586	1.80	1.24–2.61	0.002
Positive lymph nodes				
None			reference	
1–3	0.164	1.18	0.76–1.82	0.46
4+	0.413	1.51	1.01–2.26	0.045
ER expression				
+10% increase	–0.103	0.90	0.87–0.94	<0.0001
HER2 status				
Not overexpressed			reference	
Overexpressed	0.363	1.44	1.05–1.97	0.024
Ki-67				
+10% increase	0.202	1.22	1.15–1.30	<0.0001
Vascular invasion				
Absent			reference	
Present	0.202	1.22	0.82–1.83	0.33
Focal	0.536	1.71	0.91–3.22	0.097
Diffuse	0.900	2.46	1.79–3.39	<0.0001

Analysis based on the 577 patients in the training cohort.
DFS, disease-free survival; ER, estrogen receptor; CI, confidence interval.

The presence of axillary lymph node metastasis is the best-established independent prognostic factor in women with newly diagnosed breast cancer. As reported in the most recent St. Gallen Consensus Conference, the number of involved axillary lymph nodes is pivotal in the risk evaluation [22]. Similar results, in terms of decreased survival associated with an increasing number of positive axillary nodes, were reported in patients pretreated with primary chemotherapy [23].

Early studies suggested that the presence of PVI may provide additional prognostic information and that PVI is associated with a particularly unfavorable outcome among specific subgroups such as the node-negative population [24, 25]. In particular, the evaluation of the degree of PVI might properly identify patients at higher risk thus sparing unnecessary treatment in a large group of patients after surgery. Although emerging data on the clinical significance of PVI have prompted the introduction of this factor in the therapeutic algorithm of patients candidated to adjuvant treatment, PVI was uncommonly considered as a prognostic factor after PC. We previously showed that the presence of PVI after PC might identify patients with T4 disease at higher risk [26]. The results of the present study, where the degree of PVI was significantly correlated with DFS, further support a prognostic role for this factor.

Limited data are available on the relationship between HER2 expression and outcome after PC and HER was uncommonly taken into consideration in the development of models or nomograms. This might be explained by the small sample sizes, heterogeneity of examinations and methods and especially cutoffs used in the various studies. DFS was reported to be significantly worse for the population that overexpressed HER2 if compared with HER2-negative tumors in two large studies [27, 28]. In particular, in a retrospective analysis including 1731 patients, progression-free survival rates were significantly worse for HER2-positive disease both in the cohort of patients HR positive and HR negative [27]. In the present study, we observed at the

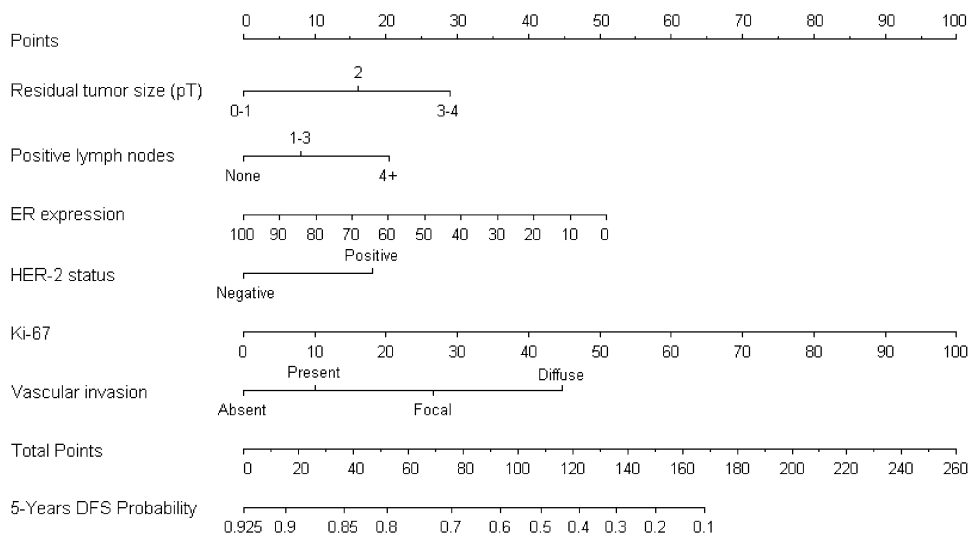


Figure 2. Nomogram predicting 5-year disease-free survival in patients not achieving a pathological complete remission after preoperative chemotherapy for breast cancer.

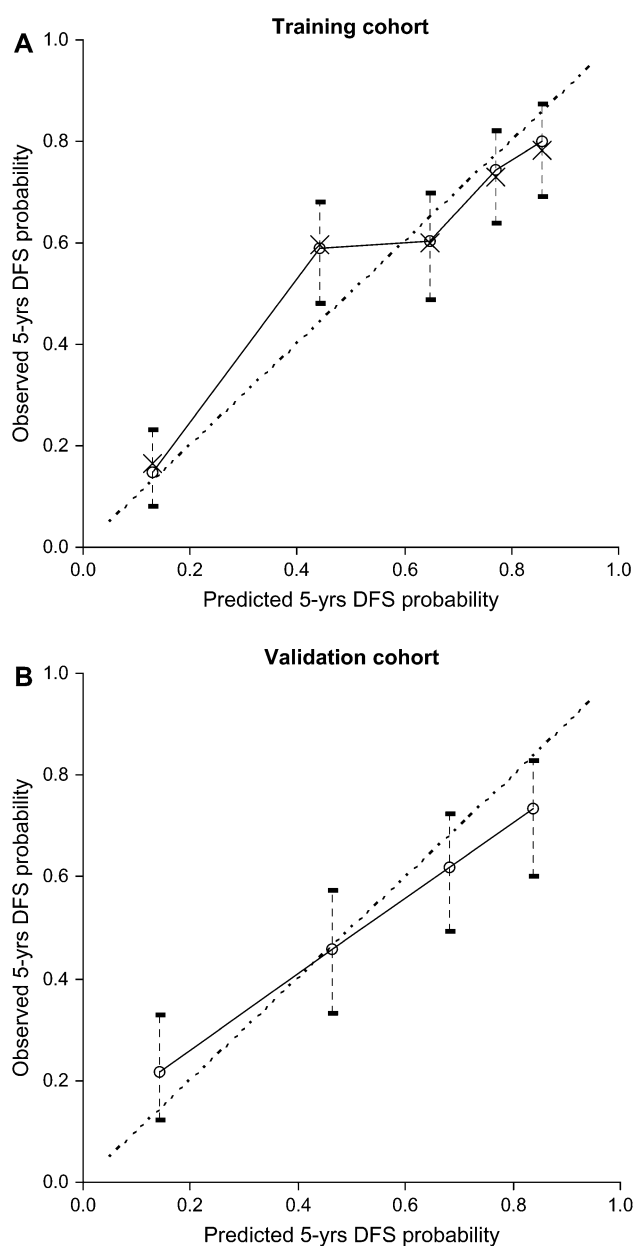


Figure 3. Calibration of the nomogram in training (A) and validation (B) cohorts.

multivariate analysis a worse DFS for patients with HER2-positive tumors supporting the use of tailored therapies for this patient cohort.

Among continuous variables, the nomogram included ER and Ki 67 expression. The evaluation of the number of positive cells has been demonstrated to be relevant in the detection of prognostic and predictive factors in early breast cancer [22]. In particular, the degree of endocrine responsiveness according to the percentage of positive cells is considered as crucial in determining a probability of response to targeted treatments [22]. The value of the degree of endocrine responsiveness was, however, uncommonly taken into consideration in the therapeutic algorithm after preoperative therapy. In previously published studies, analyses were carried out based on a

so-called ‘receptor-negative grouping’, which combines receptor-absent disease with that expressing low receptor levels, and ‘receptor positive grouping’ which combines all patients with tumors expressing ER and/or PgR in $\geq 10\%$ of the cells. As reported in Table 3, in the present study, the level of expression of ER was significantly correlated with the DFS of the patients and, therefore, the degree of expression of ER was introduced in the nomogram. According to these results, the degree of endocrine responsiveness evaluated quantitatively might contribute to a decision about postoperative treatment after PC.

Tumor proliferation fraction is an important predictor of prognosis. Ki-67 labeling index (LI) is a measure of tumor proliferation that has been correlated with outcome in several studies [29, 30] and in a recent meta-analysis conducted in >12 000 patients [31]. It has also been suggested that high Ki-67 LI may be predictive of responsiveness to neo-adjuvant (primary) chemotherapy [32], but it was not included in models or nomograms. More recently, a study focusing on 228 postmenopausal women with endocrine-responsive breast cancers treated within a neo-adjuvant endocrine therapy trial showed at the multivariable analysis of post-treatment tumor characteristics, that Ki-67 expression was independently associated with RFS [33]. The results of the present study indicate that measures of tumor cell proliferation such as Ki-67 expression could potentially identify patients who require further therapy (adjuvant chemotherapy as well as endocrine therapy) after PC in locally advanced breast cancer. The observed correlation between the degree of Ki-67 expression and the patients outcome implies the absolute necessity for reporting quantitative results from KI-67 LI staining with appropriate quality control.

In conclusion, in the present study, we developed a nomogram based upon expression of classical and newer pathologic characteristics of the primary tumor, which might accurately predict the risk of patients who failed to achieve a pCR after preoperative therapy. This nomogram may be useful when a postoperative ‘tailored’ algorithm should be developed in patients already submitted to preoperative treatment.

references

1. Powles TJ, Hickish TF, Makris A et al. Randomized trial of chemoendocrine therapy started before or after surgery for treatment of primary breast cancer. *J Clin Oncol* 1995; 13: 547–552.
2. Fisher B, Bryant J, Wolmark N et al. Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. *J Clin Oncol* 1998; 16: 2672–2685.
3. Bear HD, Anderson S, Brown A et al. The effect on tumor response of adding sequential preoperative docetaxel to preoperative doxorubicin and cyclophosphamide: preliminary results from National Surgical Adjuvant Breast and Bowel Project Protocol B-27. *J Clin Oncol* 2003; 21: 4165–4174.
4. Colleoni M, Minichella I, Mazzarol G et al. Response to primary chemotherapy in breast cancer patients with tumors not expressing estrogen and progesterone receptors. *Ann Oncol* 2000; 11: 1–3.
5. MacGrogan G, Mauriac L, Durand M et al. Primary chemo-therapy in breast invasive carcinoma: predictive value of the immuno-histochemical detection of hormonal receptors, p53, c-erbB-2, MIB1, pS2 and GST pi. *Br J Cancer* 1996; 74: 1458–1465.

6. Kaufmann M, von Minckwitz G, Smith R et al. International expert panel on the use of primary (preoperative) systemic treatment of operable breast cancer: review and recommendations. *J Clin Oncol* 2003; 21: 2600–2608.
7. Rouzier R, Puszta L, Delaloge S et al. Nomograms to predict pathologic complete response and metastasis-free survival after preoperative chemotherapy for breast cancer. *J Clin Oncol* 2004; 22: 2294–2302.
8. Chollet P, Amat S, Belembaogo E et al. Is Nottingham prognostic index useful after induction chemotherapy in operable breast cancer? *Br J Cancer* 2003; 89: 1185–1191.
9. Abrial SC, Penault-Llorca F, Delva R et al. High prognostic significance of residual disease after neoadjuvant chemotherapy: a retrospective study in 710 patients with operable breast cancer. *Breast Cancer Res Treat* 2005; 94: 255–263.
10. Kuerer HM, Newman LA, Smith TM et al. Clinical course of breast cancer patients with complete pathologic primary tumor and axillary lymph node response to doxorubicin-based neoadjuvant chemotherapy. *J Clin Oncol* 1999; 17: 460–469.
11. Harrell FE Jr, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat Med* 1996; 15: 361–387.
12. Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika* 1994; 81: 515–526.
13. Harrell FE Jr. Design and Hmisc libraries. <http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/RS> (last accessed on 1 October 2008).
14. Rouzier R, Extra JM, Klijanienko J et al. Incidence and prognostic significance of complete axillary downstaging after primary chemotherapy in breast cancer patients with T1 to T3 tumors and cytologically proven axillary metastatic lymph nodes. *J Clin Oncol* 2002; 20: 1304–1310.
15. Scholl SM, Pierga JY, Asselain B et al. Breast tumour response to primary chemotherapy predicts local and distant control as well as survival. *Eur J Cancer* 1995; 31: 1969–1975.
16. Regan MM, Viale G, Mastropasqua MG et al. Re-evaluating adjuvant breast cancer trials: assessing hormone receptor status by immunohistochemical versus extraction assays. *JNCI Cancer Spectr* 2006; 98: 1571–1581.
17. Sorlie T, Perou CM, Tibshirani R et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A* 2001; 98: 10869–10874.
18. Goldhirsch A, Wood WC, Senn HJ et al. Meeting highlights: international consensus panel on the treatment of primary breast cancer. *J Natl Cancer Inst* 1995; 87: 1441–1445.
19. Goldhirsch A, Glick JH, Gelber RD et al. Meeting highlights: international consensus panel on the treatment of primary breast cancer. *J Natl Cancer Inst* 1998; 90: 1601–1608.
20. Goldhirsch A, Glick JH, Gelber RD et al. Meeting highlights: international consensus panel on the treatment of primary breast cancer. Seventh International Conference on Adjuvant Therapy of Primary Breast Cancer. *J Clin Oncol* 2001; 19: 3817–3827.
21. Thomas E, Holmes FA, Smith TL et al. The use of alternate, non-cross-resistant adjuvant chemotherapy on the basis of pathologic response to a neoadjuvant doxorubicin-based regimen in women with operable breast cancer: long-term results from a prospective randomized trial. *J Clin Oncol* 2004; 22: 2294–2302.
22. Goldhirsch A, Wood WC, Gelber RD. Progress and promise: highlights of the international expert consensus on the primary therapy of early breast cancer 2007. *Ann Oncol* 2007; 18: 1133–1144.
23. Curé H, Amat S, Penault-Llorca F et al. Prognostic value of residual node involvement in operable breast cancer after induction chemotherapy. *Breast Cancer Res Treat* 2002; 76: 37–45.
24. Lee AK, DeLellis RA, Silverman ML et al. Prognostic significance of peritumoral lymphatic and blood vessel invasion in node-negative carcinoma of the breast. *J Clin Oncol* 1990; 8: 1457–1465.
25. Lauria R, Perrone F, Carlomagno C et al. The prognostic value of lymphatic and blood vessel invasion in operable breast cancer. *Cancer* 1995; 76: 1772–1778.
26. Montagna E, Bagnardi V, Rotmensz N et al. Factors that predict early treatment failure for patients with locally advanced (T4) breast cancer. *Br J Cancer* 2008; 98: 1745–1752.
27. Guarneri V, Broglio K, Kau SW et al. Prognostic value of pathologic complete response after primary chemotherapy in relation to hormone receptor status and other factors. *J Clin Oncol* 2006; 24: 1037–1044.
28. Gregory RK, Powles TJ, Salter J et al. Prognostic relevance of *cerbB2* expression following neoadjuvant chemotherapy in patients in a randomised trial of neoadjuvant versus adjuvant chemoendocrine therapy. *Breast Cancer Res Treat* 2000; 59: 171–175.
29. Domagala W, Markiewski M, Harezga B et al. Prognostic significance of tumor cell proliferation rate as determined by the MIB-1 antibody in breast carcinoma: its relationship with vimentin and p53 protein. *Clin Cancer Res* 1996; 2: 147–154.
30. Mandard AM, Denoux Y, Herlin P et al. Prognostic value of DNA cytometry in 281 premenopausal patients with lymph node negative breast carcinoma randomized in a control trial: multivariate analysis with Ki-67 index, mitotic count, and microvessel density. *Cancer* 2000; 89: 1748–1757.
31. de Azambuja E, Cardoso F, de Castro G Jr et al. Ki-67 as prognostic marker in early breast cancer: a meta-analysis of published studies involving 12 155 patients. *Br J Cancer* 2007; 96: 1504–1513.
32. Archer CD, Parton M, Smith IE et al. Early changes in apoptosis and proliferation following primary chemotherapy for breast cancer. *Br J Cancer* 2003; 89: 1035–1041.
33. Ellis MJ, Tao Y, Luo J et al. Outcome prediction for estrogen receptor-positive breast cancer based on postneoadjuvant endocrine therapy tumor characteristics. *J Natl Cancer Inst* 2008; 100: 1380–1388.