Predicting Early Failure after Adjuvant Chemotherapy in High-Risk Breast Cancer Patients with Extensive Lymph Node Involvement

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ABSTRACT

Purpose: **There is limited knowledge of risk factors for breast cancer recurrence within 2 years. This study aimed to predict early failure and identify high-risk patients for prognostic and therapeutic purposes.**

Experimental Design: **We studied 739 patients from a randomized trial who were <56 years of age and had ≥4 or more positive lymph nodes, no distant metastases, and no previous other malignancies. After complete surgical treatment, patients received conventional-dose anthracycline-based chemotherapy or a high-dose scheme of anthracycline-based plus alkylating chemotherapy. We assessed clinical and (immuno)histological parameters to predict recurrence within 2 years.**

Results: **Early failure occurred in 19% ($n = 137$). Median survival after early failure was limited to 0.7 year. Estrogen and progesterone receptor negativity and visceral relapse predicted poor prognosis. Early failure was associated with young age, large tumors, high histological grade, angio-invasion, apical node metastasis, and ≥10 involved nodes. Estrogen receptor, progesterone receptor, and p27 negativity; HER2 overexpression; and p53 positivity also predicted early failure. The surgical or chemotherapy regimen and histological type did not. The same parameters except tumor size were associated with early death. Grade III, ≥10 involved nodes, and estrogen receptor negativity were independently associated with early failure and together identified a subset of patients (7%) with 3-fold increased early failure and 5-fold increased early death.**

Conclusions: **Early failure is associated with poor survival. The combination of three commonly determined parameters constitutes a strong predictive model for early failure and death.**

INTRODUCTION

Axillary lymph node status is the strongest prognostic factor in patients with primary operable breast cancer. Compared with patients without axillary lymph node involvement, 10-year survival of lymph node-positive breast cancer patients is decreased from ~65–80% to ~25–48%. The prognosis worsens with increasing numbers of involved lymph nodes. Patients with four or more positive nodes are regarded as a particularly unfavorable subgroup. Further subdivision into categories (*e.g.*, 4–10 *versus* ≥10 positive lymph nodes) can also be useful for prognostic purposes (1, 2).

Meta-analyses of randomized clinical trials have shown that adjuvant chemotherapy improves the prognosis of all breast cancer patients, irrespective of the risk of relapse. In absolute numbers, however, patients with lymph node metastases benefit most from adjuvant treatment (3).

Conventional-dose adjuvant chemotherapy improves patient outcome mainly by reducing the incidence of first local or regional and distant soft-tissue relapses, whereas first recurrences in bone or viscera are less influenced (4). Clinical trials in metastatic breast cancer, in which dose intensity of chemotherapy was the most important variable, have shown that the response rate to chemotherapy rises with increasing dose intensity (5–8). More intensive treatments might also reduce the incidence of bone and visceral relapses and have a greater influence on survival. We recently published a multicenter randomized clinical trial investigating whether increasing the dosage of adjuvant chemotherapy improves the outcome of operable breast cancer patients with four or more positive lymph nodes (9). Within the framework of this trial, we evaluated the association between several tumor characteristics and short-term disease progression. Recurrence of breast cancer within 2 years of initial treatment predicted a dismal outcome (10, 11). Identifying the patient subgroup that will have such short-term progression can be clinically relevant. The main aim of this study was therefore to define factors that predict early failure in lymph node-positive breast cancer.

PATIENTS AND METHODS

High-Dose *versus* Conventional-Dose Chemotherapy Trial. We conducted a multicenter prospective randomized Phase III trial investigating the effectiveness of high-dose adjuvant chemotherapy in patients with operable breast cancer (9). Patients had undergone modified radical mastectomy or breast-conserving surgery with complete axillary clearance. Major eligibility criteria were histologically confirmed stage IIA, IIB, or IIIA carcinoma of the breast with involvement of four or more axillary lymph-nodes, age under 56 years, no evidence of distant metastases, and no previous other malignancies. The trial

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design consisted of two treatment arms to which patients were randomly assigned:

(*a*) The "conventional-dose" chemotherapy arm, comprising five courses of 5-fluorouracil (500 mg/m^2), epirubicin (90 mg/m^2), and cyclophosphamide (500 mg/m^2); and

(*b*) The "high-dose" chemotherapy arm, comprising four courses of 5-fluorouracil–epirubicin–cyclophosphamide and a high-dose trialkylator regimen [cyclophosphamide (6 g/m^2), thiotepa (480 mg/m^2), and carboplatin (1.6 g/m^2)]. During the third 5-fluorouracil–epirubicin–cyclophosphamide course, peripheral-blood stem cells were mobilized with granulocyte-colony-stimulating factor and harvested. During high-dose chemotherapy patients received granulocyte-colony-stimulating factor and peripheral blood stem cell infusion.

Within 6 weeks after chemotherapy, all patients underwent conventional external beam radiotherapy to the breast or chest wall and to the regional lymph node areas (axilla and internal mammary chain), according to local protocols. Patients with hormone receptor-positive tumors received 5 years of adjuvant tamoxifen.

Informed consent was obtained from all patients, and the study was approved by the institutional review committees at each of the participating centers.

Patients. Between 1993 and 2000, 885 patients were entered in nine institutes. For 745 patients, H&E-stained and paraffin-embedded unstained tumor slides were available for this study. Six patients died of toxic complications and without evidence of a breast cancer recurrence. We analyzed the remaining 739 patients. Histological features were centrally revised by one of the authors (J. L. P.). The following tumor characteristics were recorded: largest diameter, WHO type, histological grade according to Elston and Ellis (12), presence of angio-invasion, total number of retrieved lymph nodes, number of lymph nodes with metastatic tumor cells, and status of apical node.

Immunohistochemistry. Slides from formalin-fixed, paraffin-embedded tumors were stained with antibodies against HER2 [3B5 (13); 1:10,000 dilution], P53 (DO7; 1:8,000 dilution; mouse IgG2b; DAKO, Glostrup, Denmark), estrogen receptor (1D5; 1:150; mouse IgG1; DAKO), progesterone receptor (PR1; 1:3000 dilution; mouse IgG1; ImmunoVision, Brisbane, CA), p27 (Clone DCS-72.F6; 1:200 dilution; mouse IgG1; NeoMarkers, Fremont, CA). Staining was performed according to an earlier published protocol (14). Replacement of the primary antibody with 1% bovine albumin solution in PBS served as negative control. Archival breast cancer samples with known strong expression were used as positive controls.

Staining patterns recognized as positive were circumferential membrane-bound staining (HER2) and nuclear staining [estrogen receptor (ER), progesterone receptor (PR), p27, and p53]. HER2 was scored with the system that has recently come into use for clinical testing (0 = negative; 1+ $\geq$10% cells weakly positive and not circumferential; 2+ $\geq$10% of cells with moderate circumferential staining; 3+ $\geq$10% of cells with strong circumferential staining). Other immunohistochemical results were scored semiquantitatively on a three-point scale for percentage of positively staining tumor cells (negative = 0%; focal staining = up to 50%; homogeneous staining = 50–100%).

Definition of Early Failure and Statistical Analyses. Early failure was defined as any recurrence of breast cancer (local, regional, or distant) $<$2 years from the time from randomization. Early death was defined as death within the same period. Randomization took place after surgery and definitive pathological diagnosis and before the start of chemotherapy.

The association of all parameters with early death and early failure was tested for statistical significance with the two-sided Pearson's χ^2 test in cross-tabulations. Multivariate analyses were made in a binary logistic regression model. Survival analysis was performed according to the Kaplan–Meier method and with the log-rank test for comparing groups. $P < 0.05$ was considered statistically significant. Additionally, we applied Bonferroni α-level adjustment for multiple testing in the single-variable tests. All data were analyzed with the Statistical Product and Service Solutions software (SPSS 10.0.7 for Windows; SPSS, Chicago, IL).

Pooled Data from Both Treatment Arms. This trial was originally designed to assess the possible effects of high-dose chemotherapy on relapse and survival. As has recently been published, no statistically significant survival advantage was found for dose intensification *per se* (9). We therefore analyzed the data from both treatment arms pooled in one study population.

RESULTS

Patients. A total of 739 patients were analyzed for this study. Mean patient age at randomization was 44.5 years (range, 25.6–55.6; median, 45.5 years). Mastectomy was performed in 572 patients (77%). All other patients underwent breast-conserving surgery with complete axillary dissection. Randomization assigned 369 patients to the conventional-dose chemotherapy treatment group and 370 patients to the experimental high-dose treatment arm.

Tumor Characteristics. The mean tumor diameter was 3.6 cm (range 0.5–14.5 cm; median, 3.0 cm). Most tumors were invasive ductal carcinomas (61%), including seven cases of extensive *in situ* carcinoma with microinvasive foci. Forty-five percent of tumors were histological grade III, reflecting the unfavorable nature of the disease in the selected population.

Angio-invasion was present in 70% of cases, consistent with the lymph node-positive status of the patients. We investigated an average of 15.5 axillary lymph nodes/patient (range, 4–41; median, 15). The mean number of tumor-positive lymph nodes was 8.8 (range, 4–33; median, 8). The apical node was tumor positive in 67% of cases. This corresponds with stage IIIC disease according to the 6th edition of the American Joint Committee on Cancer staging system for breast cancer (15).

Follow-Up. At the time of analysis, the mean duration of follow-up was 4.3 years (range, 0.6–9.1 years; median, 4.1 years). Early recurrence of breast cancer occurred in 137 patients (19%; Table 1). Early death occurred in 69 patients (9%). Early death did not occur as a first event.

Patients with early locoregional failure (n = 40) had recurrences in the remaining breast(s) and scar, chest wall skin (including lymphangitis), and contralateral and ipsilateral regional lymph nodes. Patients with early distant failure (n = 119) had metastases in various—often multiple—sites, mainly bone

Table 1 Early failure in 739 patients

Type of early event	*n*	Percentage of total ($n = 739$)
Any breast cancer relapse	137	19
Locoregional recurrence	18	2
Distant metastasis	97	13
Locoregional and distant recurrence (simultaneous)	22	3
Early death (<2 years)	69	9

($n = 54$), liver ($n = 46$), lung ($n = 22$), and brain ($n = 16$) as well as in cervical, retroperitoneal, mediastinal, and hilar lymph nodes; they also developed pleuritis, meningitis, and peritonitis.

Survival after Early Failure. Median survival after early failure was 0.7 years (Table 2; Fig. 1, *A* and *B*), and 124 patients (91%) died during follow-up. Parallel to the study by Goldhirsch *et al.* (10), we compared three groups on the basis of first relapse site:

(*i*) Locoregional relapse: patients with only locoregional involvement;

(*ii*) Nonvisceral metastasis: patients with skeletal or extraregional skin or lymph node metastases but no organ involvement; and

(*iii*) Visceral metastasis: patients with any involved organ (*e.g.*, brain, liver, or lung), bone marrow involvement, or pleural, peritoneal, or meningeal effusion.

The site of the recurrences was predictive of post-early failure survival ($P = 0.04$). The patients with only nonvisceral relapse (combination of groups *i* and *ii*) had significant survival advantage over those with visceral involvement (group *iii*; $P = 0.02$).

Immunohistochemical Markers. ER staining was positive in 71% of tumors, and 58% had homogeneous staining. PR staining was positive in 57% and homogeneous in 27% of tumors. P53 staining was positive in 47% of tumors, and 20% had homogeneous staining. Seventy percent of tumors had P27 staining, and 13% had homogeneous staining. HER2 was positive (3+) in 23% of assessed tumors.

Associations with Early Failure and Early Death. Both early failure and early death occurred significantly more often with young age, larger tumors, high histological grade, angio-invasion, with ≥10 axillary lymph node metastases, and with metastasis to the apical node (Table 3). Negative ER, PR, and p27 status; HER2 overexpression; and positive p53 status were also associated with early failure and with early death.

Of the parameters associated with early failure, only ER ($P = 0.001$) and PR ($P = 0.02$) were predictive of survival after early failure; negative hormone receptor status was associated with worse outcome (Table 2; Fig. 1, *C* and *D*).

Early failure and death were not associated with the randomly assigned chemotherapy regimen (high- *versus* conventional-dose), with the type of surgical treatment used (mastectomy *versus* breast conserving surgery), or with the primary tumor type (WHO classification).

Multivariate Analysis and Predictive Model. The parameters that were predictive of early treatment failure and early death were entered in a binary logistic multivariate analysis, with early failure and early death as the dependent variables. An independent association with early failure was thus established for age <40 years ($P = 0.03$), histological grade III ($P = 0.009$), ≥10 positive lymph nodes ($P = 0.002$), and negative ER status ($P = 0.004$). Significant independent association with early death was established for histological grade III ($P = 0.03$) and ER status ($P < 0.001$).

Three factors—grade III, ER-negative tumors, and ≥10 positive lymph nodes—were used as a model for the identification of high-risk patients, which was tested for its predictive value (Table 4). Among 726 assessable cases with early failure and early death prevalences of 134 (18%) and 68 (9%), respectively, 52 patients qualified as high-risk (7%). The sensitivity of the model was 21% for early failure and 27% for early death. The positive predictive values were 54% for early failure and 35% for early death. The relative risks for high-risk *versus* low-risk patients were 6.3 for early failure and 6.6 for early death.

Table 2 Survival after early failure in 137 patients

	Patients with EF[a] (*n*)	Patients with EF who died during follow-up (*n*)	Patients alive 1 year after EF (%)	Median survival in years (95% confidence interval)	*P* (log-rank)
All	137	124	36	0.7 (0.6–0.8)	
Site of first relapse					0.04
Nonvisceral only	31	27	53	1.0 (0.3–1.7)	
Locoregional only	18	17	33	0.9 (0.6–1.1)	
Visceral	88	80	30	0.6 (0.4–0.8)	
ER ($n = 135$)					0.001
Homogeneous	42	35	64	1.5 (1.1–1.8)	
Focal	14	13	29	0.5 (0.2–0.8)	
Negative	79	75	22	0.6 (0.5–0.8)	
PR ($n = 135$)					0.02
Homogeneous	13	10	54	1.0 (0.0–2.2)	
Focal	24	22	54	1.0 (0.5–1.6)	
Negative	98	91	28	0.6 (0.5–0.8)	

[a] EF, early failure; ER, estrogen receptor; PR, progesterone receptor.

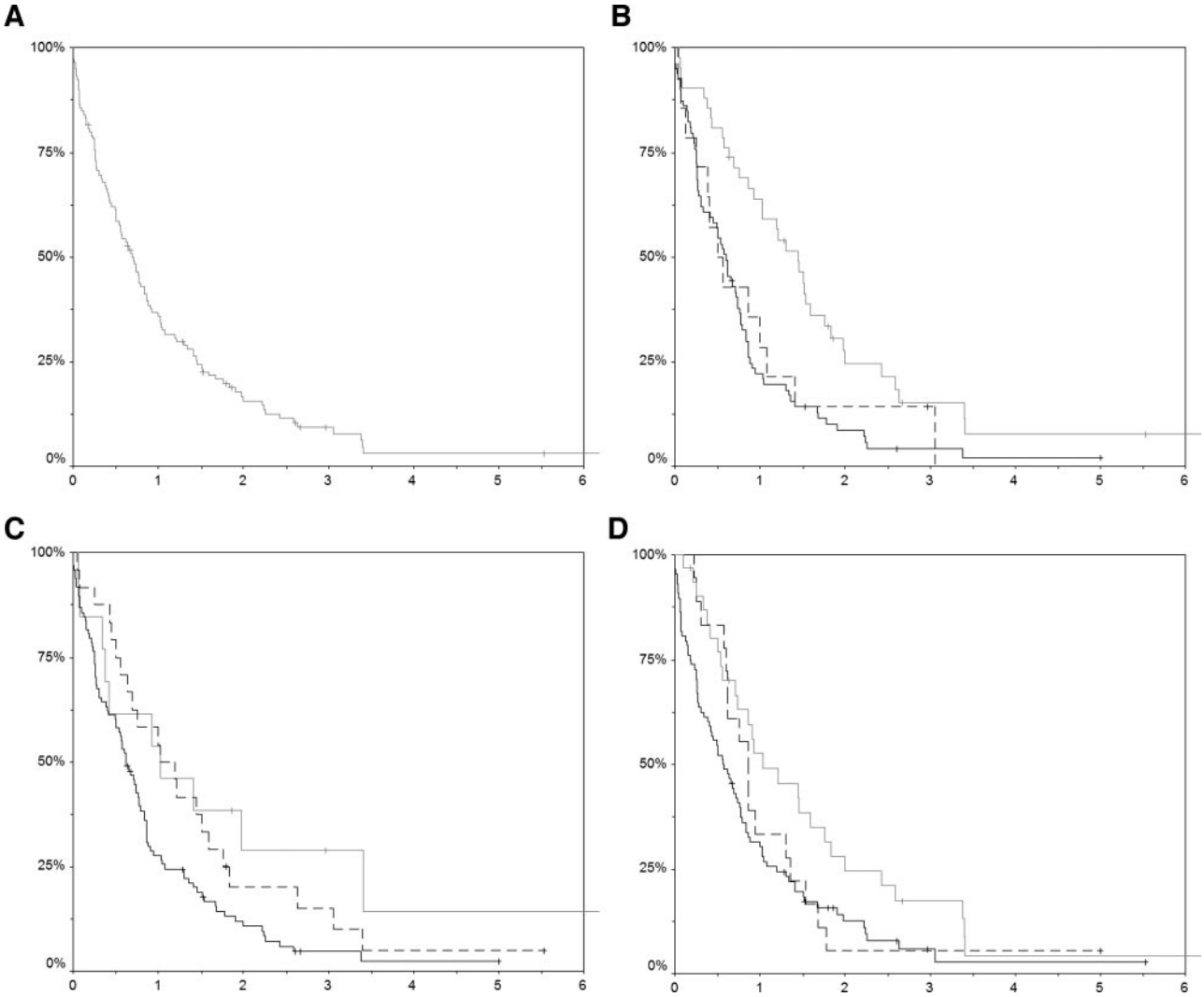

Fig. 1 Kaplan–Meier survival curves for 137 patients after early failure (percentage of surviving patients against time in years). *A*, all 137 patients; *B*, post-early failure survival by site of recurrence ($P = 0.04$). *Solid gray line*, patients with only nonvisceral metastases ($n = 31$); *dashed black line*, patients with only locoregional recurrence ($n = 18$); *solid black line*, patients with visceral metastases ($n = 88$). *C*, post-early failure survival by estrogen receptor (ER) status ($P = 0.007$). *Solid gray line*, patients with homogeneous ER staining ($n = 42$); *dashed black line*, patients with focal ER staining ($n = 14$); *solid black line*, patients with negative ER staining ($n = 79$). *D*, post-early failure survival by progesterone receptor (PR) status ($P = 0.02$). *Solid gray line*, patients with homogeneous PR staining ($n = 13$); *dashed black line*, patients with focal PR staining ($n = 24$); *solid black line*, patients with negative PR staining ($n = 98$).

DISCUSSION

Breast cancer survival statistics show that a subset of patients develop distant metastases and die early. For example, the slope of the survival curve for premenopausal node-positive breast cancer patients distinctly decreases after 5 years of follow-up: mortality decreases from 30–40% in the first 5 years to 15–20% in the second 5 years (3). A disease-free interval <2 years is one of the most accurate predictors of a dismal outcome (10): the risk of breast cancer death is highest among women who have a recurrence within 2 years (11). A reliable identification of the women who are at high-risk of such early failure is of prognostic significance. It could be useful for selectively offering intensified follow-up controls or additional (experimental) forms of treatment.

We have analyzed 739 breast cancer patients under 56 years of age, with extensive lymph node involvement. They were entered in a randomized clinical trial of adjuvant chemotherapy with an anthracycline-based regimen or with an additional high-dose alkylating regimen. We compared patients who had disease progression within 2 years (early failure) *versus* those who did not.

On the basis of the post-early failure survival data we found that the prognosis for patients with early failure is poor, particularly for patients with early visceral metastases: not many more than one-third of the early failure patients survived longer than 3 years after initial treatment. This validates the use of parameters associated with early failure as prognostic factors.

Single-variable analyses of the relationships between the

Table 3 Statistical associations of patient and tumor parameters with early failure and early death

Parameter		Total (*n*)	EF[a]+ *n*	EF+ %	EF−	*P* (χ^2)	ED+ *n*	ED+ %	ED− (*n*)	*P* (χ^2)
All		739	137	19	602		69	9	670	
Chemotherapy dose	Conventional dose	369	78	21	291	0.07	39	11	330	0.25
	High dose	370	59	16	311		30	8	340	
Surgery	Mastectomy	572	109	19	463	0.50	55	10	517	0.63
	Breast conserving	167	28	17	139		14	8	153	
Age	<40 years	193	51	26	142	0.001[b]	25	13	168	0.045
	≥40 years	546	86	16	460		44	8	502	
Tumor classification (n = 690)	pT1 (<2 cm)	113	17	15	96	0.014	8	7	105	0.11
	pT2 (2–5 cm)	406	68	17	338		34	8	372	
	pT3 (≥5 cm)	171	45	26	126		23	14	148	
WHO tumor type (n = 739)	IDC	452	86	19	366	0.64	41	9	411	0.98
	ILC	126	21	17	105		12	10	114	
	Mixed invasive type	85	13	15	72		9	11	76	
	Other	76	17	22	59		7	9	69	
Histological grade (n = 724)	Grade I	125	6	5	119	<0.001[b]	2	2	123	<0.001[b]
	Grade II	273	37	14	236		16	6	257	
	Grade III	326	93	29	233		51	16	275	
Angio-invasion (n = 732)	None	223	28	13	195	<0.001[b]	14	6	209	<0.001[b]
	Focal	316	54	17	262		23	7	293	
	Extensive	193	55	29	138		332	17	161	
No. of lymph nodes with tumor (n = 711)	4–10	462	65	14	397	<0.001[b]	35	8	427	0.022
	≥10	249	66	27	183		32	13	217	
Apical node status (n = 688)	No tumor	225	30	13	195	0.013	14	6	211	0.044
	With metastasis	463	98	21	365		51	11	412	
ER (n = 718)	Negative (no staining)	209	79	38	130	<0.001[b]	53	25	156	<0.001[b]
	Focal (<50%)	94	14	15	80		7	7	87	
	Homogeneous (≥50%)	415	42	10	373		8	2	407	
PR (n = 719)	Negative (no staining)	306	98	32	208	<0.001[b]	59	19	247	<0.001[b]
	Focal (<50%)	221	24	11	197		5	2	216	
	Homogeneous (≥50%)	192	13	7	179		5	3	187	
HER2 (n = 721)	Negative (0, 1+, 2+)	558	93	17	465	0.009	45	8	513	0.020
	Positive (3+)	163	42	26	121		23	14	140	
p53 (n = 715)	Negative (no staining)	382	62	16	320	0.002[b]	28	7	354	0.003[b]
	Focal (<50%)	193	31	16	162		16	8	177	
	Homogeneous (≥50%)	140	41	29	99		24	17	116	
p27 (n = 624)	Negative (no staining)	184	53	29	131	<0.001[b]	28	15	156	0.008
	Focal (<50%)	358	62	17	296		29	8	329	
	Homogeneous (≥50%)	82	6	7	76		4	5	78	

[a] EF, early failure; ED, early death; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; PR, progesterone receptor.

[b] $P < 0.004$ (0.05/14), indicating that the parameter is statistically significant after Bonferroni α-level adjustment for multiple testing.

tested factors and early failure and early death were mostly consistent with previously reported data.

Large tumor size, poor histological grade, extensive axillary node involvement, apical node involvement, and ER and PR negativity are all commonly used in surgical treatment planning or in the choice of an adjuvant treatment strategy, because they are established prognostic factors associated with poor outcome. Many parameters are also interrelated: negative ER status is associated with negative PR status and with high histological grade, and angio-invasion is associated with lymph node metastases.

Young age—variably defined from study to study as younger than 25 to younger than 40 years of age—has previously been established as a negative prognostic factor, and histological differences are described from breast carcinoma in older patients, *e.g.,* increased histological grade and angio-invasion (16–20). These associations were also found in our population. In accordance with earlier published studies, we further found HER2 overexpression in 23% of tumors, mostly of high histological grade; HER2 overexpression has been associated with increased risk of early failure and death (21–25). Increased p53 expression is generally associated with poor survival and high histological grade (26–29). This was essentially confirmed in our series: p53 positivity was associated with high histological grade and with early failure. Also in line with the published literature [reviewed by Cariou *et al.* (30) and by Chiarle *et al.* (31)] is the inverse correlation between p27 expression and histological tumor grade and significantly more

Table 4 Predictive model for early failure with three unfavorable tumor characteristics

	Total	EF[a]+	%	EF−	$P(\chi^2)$	ED+	%	ED−	$P(\chi^2)$
Total	726	134	19	592	<0.001	68	9	658	<0.001
Unfavorable combination (ER−, grade III, and ≥10 positive lymph nodes)	52	28	54	24		18	35	34	
Other	674	106	16	568		50	7	624	

Note. With versus without unfavorable characteristics: Odds ratio for EF = 6.3 (95% confidence interval: 3.5–11.2). Odds ratio for ED = 6.6 (95% confidence interval: 3.5–12.6).

[a] EF, early failure; ED, early death.

frequent early failures in focally staining and negative tumors (32). We have no explanation for the much lower proportion of tumors that stained homogeneously for p27 in our study population: 13% compared with ≥30% in other studies (33, 34).

In the multivariate analysis, most of the tested parameters were not independent prognostic factors. Probably this is the result of the strong association of these factors with histological tumor grade and ER status, which may have masked other independent effects.

The combined independent predictors histological grade III, negative ER status, and involvement of ≥10 axillary lymph nodes identified women with a 3-fold increased risk of early failure and 5-fold increased risk of early death. Such patients likely are an interesting group for prognostic reasons and are possible candidates for primary treatment with alternative regimens. With any single one of these factors, a more significant subgroup of patients with an increased risk of poor prognosis is identified, but none is by itself associated with even a 2-fold increased risk of early failure. These parameters are already routinely determined in most, if not all, breast cancer patients and are therefore applicable without further additional effort or cost, making the model practical for clinical purposes. Certainly the limited number of patients thus identified as high-risk does limit the clinical relevance of this predictive model. In this group 7% of the patients met the criteria for being considered "high risk." Our analyses were done in premenopausal, lymph node-positive breast cancer patients. The model is likely to be applicable for lymph node-positive postmenopausal patients as well. Whether it is applicable to lymph node-negative patients is not known.

In conclusion, our findings in this large, well-defined set of lymph node-positive breast cancer patients confirm that early failure is a strong negative prognosticator. The presented data underline the strength of several known prognosticators and add their clinical use in a combination model that is easily applicable.

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