Only grading has independent impact on breast cancer survival after adjustment for pathological response to preoperative chemotherapy

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Our objective was to determine pretreatment factors with an independent impact on survival after adjusting for response to preoperative chemotherapy and to describe parameters predictive for achieving a pathological complete remission (pCR) after preoperative chemotherapy containing an anthracycline. We performed univariate and multivariate analyses to describe the impact of the following pretreatment characteristics of 240 primary breast cancer patients who received preoperative chemotherapy containing an anthracycline at our institution on disease-free survival (DFS), distant disease-free survival (DDFS) and overall survival (OS): age, stage, clinical tumor size, clinical nodal status, grading, and expression of estrogen receptor, progesterone receptor, Her2/neu, Ki67, Bcl-2 and p53. Afterwards, the response to preoperative chemotherapy was added to the multivariate model in order to evaluate which pretreatment parameters retained their prognostic impact. In addition, univariate analysis was performed to describe pretreatment variables predictive for achieving a pCR. With a median follow-up of 6.4 years (range 0–10.4), only grading retained its independent impact on DFS, DDFS and OS [hazard ratio (HR) 1.5, 1.7 and 2.9, respectively; $p<0.05$] after adjusting for the strongest independent prognostic factors pathological T category at surgery (HR 1.6, 1.8 and 1.7, respectively; $p<0.001$) and pathological N category at surgery (HR 2.3, 2.4 and 2.1, respectively; $p<0.001$). Predictive factors for the achievement of pCR ($p<0.05$) were age under 35 years, lower stage or smaller clinical tumor size and higher expression of Bcl-2 at diagnosis. We conclude that only grading retained its independent prognostic impact on DFS, DDFS and OS after adjusting for pathological response of breast tumor and axillary lymph node metastases to preoperative chemotherapy. According to our data, it could be hypothesized that young patients with early tumor stage and small primary tumors might profit most from preoperative chemotherapy. *Anti-Cancer Drugs* 15:127–135 © 2004 Lippincott Williams & Wilkins.

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Introduction

In early breast cancer, preoperative (neoadjuvant) and postoperative (adjuvant) chemotherapy are equally effective with regard to disease-free survival (DFS) and overall survival (OS) [1]. After preoperative chemotherapy, however, breast-conserving surgery is possible more often and tumor response could be a surrogate marker for evaluating the effect of chemotherapy on micrometastases [1]. Patients with pathological complete remission (pCR) after preoperative chemotherapy experience a significantly improved survival as compared to patients with persistent, invasive tumor [2]. A significant increase in the pCR rate might translate into an improved survival [3]. However, although the pathological response and, in particular, the pCR rate appear to be reasonable intermediate markers for survival, this information is not available until the end of preoperative chemotherapy. Therefore, markers that can be evaluated before any therapy with independent prognostic significance, i.e. markers that still predict long-term survival after adjustment for tumor response to preoperative chemotherapy, need to be identified. Furthermore, markers that predict the achievement of a pCR should be evaluated.

In order to determine those pretreatment factors with an independent impact on survival, we collected clinical and tumor biologic parameters that were prospectively measured before and after preoperative chemotherapy in all 240 primary breast cancer patients who received preoperative chemotherapy which contained an anthracycline at our institution between 1992 and 2000. We performed univariate and multivariate analyses to

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evaluate the independent prognostic value of pretreatment factors for DFS, distant disease-free survival (DDFS) and OS after adjusting for response to preoperative chemotherapy, in particular for pathological response of primary breast tumors and axillary lymph node metastases. In addition, we performed univariate analyses to describe parameters predictive for achieving a pCR.

Patients and methods

Patients and systemic treatment

Between 1992 and 2000 240 patients with primary breast cancer International Union Against Cancer (UICC) stage I to III received a median of 4 cycles (range 1–6) of preoperative chemotherapy that included anthracycline at the Department of Gynecology and Obstetrics of the University of Heidelberg. Of these, 143 (60%) patients received additional postoperative chemotherapy. The pretreatment patient and tumor characteristics, clinical response, and pathological T category and pathological N category after preoperative chemotherapy are given in Table 1. The chemotherapy regimens and the number of patients who received those treatments are listed in Table 2. Grading was assessed as described by Elston and Ellis [4]. Expression of Her2/neu, Ki67, Bcl-2, p53, estrogen receptor (ER) and progesterone receptor (PgR) were measured by immunohistochemical staining on primary tumor sections. The following primary antibodies were used (clones in parentheses, all reagents by Dako): Her2/neu (A0485), Ki67 (MIB-1), Bcl-2 (124), p53 (DO7), ER (1D5) and PgR (PR88). The antibody staining was recorded as the percentage of positive tumor nuclei for Ki67 and p53. The Her2/neu and Bcl-2 immunoreaction was scored from 0 to 3. For evaluation of Her2/neu, the Dako criteria were applied and evaluation was strictly limited to specific membrane staining. The staining intensity and number of positive nuclei were evaluated in ER and PgR; receptor positivity was assumed when the semiquantitative score was at least 3 points (out of a maximum of 12 points) [5]. After completion of chemotherapy clinical tumor response (i.e. complete response and partial response) was assessed by palpation according to UICC criteria [6]. Patients underwent surgery within 21–28 days after last chemotherapy. If breast-conserving surgery was not possible due to size of invasive or non-invasive tumor residue, modified radical mastectomy was recommended. If the tumor size allowed breast-conserving surgery, (i) the surgical margins had to be free of invasive or non-invasive breast cancer, or otherwise repeat excision had to be performed; (ii) an adequate cosmetic result had to be anticipated; and (iii) if cosmetically acceptable the whole previously involved area had to be excised. Otherwise (e.g. in case of clinically complete response and an unfavorable ratio of tumor to breast size) a biopsy specimen of adequate size had to be taken from a representative area. To assess the histological tumor response all resection specimens were cut into 0.5-cm slices after overnight fixation. The tumor and surrounding areas were sampled systematically and topographically for histological evaluation of the extent of the invasive and intraductal component, and their relationship to the resection margins. Histological regressive changes were classified into three main categories according to increasing tumor regression: no tumor detectable (pT0), residual non-invasive tumor only (pTis) and residual invasive tumor (pT1–4). All patients without clinical response to preoperative chemotherapy or with pT3–4 tumors received additional postoperative chemotherapy. All patients who underwent breast-conserving surgery received standard radiotherapy in the remaining breast after recovery from the operation or the end of postoperative chemotherapy, whichever was last. In cases of hormone receptor-positive tumor, premenopausal patients received goserelin 3.6 mg s.c. once a month for 2 years or until relapse and postmenopausal patients tamoxifen 20–30 mg/day orally for 2–5 years or until relapse, starting no later than 6 weeks after surgery or the

Table 1 Pretreatment patient and tumor characteristics, clinical response, pathological T category and pathological N category after preoperative chemotherapy

Parameter	*n*	%
All	240	100
Age (years) [median (range), 48 (23–75)], <35/35–50/>50	20/119/101	8/50/42
Stage, UICC I/II/III	6/140/94	3/58/39
Clinical tumor size, T1/T2/T3/T4	9/118/63/50	4/49/26/21
Clinical nodal status, N0/N1/N2	102/115/23	43/48/10
Grading, 1/2/3/NA	11/126/96/7	5/53/40/3
ER[a], pos/neg/NA	122/109/9	51/45/4
PgR[a], pos/neg/NA	97/133/10	40/55/4
ER or PgR pos/ER and PgR neg/NA	122/109/9	51/45/4
Her2/neu[a], 0–2+/3+/NA	119/50/71	50/21/30
Ki67[a], <35% pos cells/ ≥ 35%/NA	41/41/158	17/17/66
Bcl-2[a], 0–2+/3+/NA	67/41/132	28/17/55
p53[a], <50% pos cells/ ≥ 50%/NA	126/47/67	53/20/28
Clinical response, yes/no/NA	126/101/13	53/42/5
Pathological T category after preoperative chemotherapy, pT0/pTis/pT1/pT2/pT3/pT4/NA	7/8/87/88/32/16/2	3/3/36/37/13/7/1
Pathological N category after preoperative chemotherapy, pN0/pN1/pN2/NA	105/113/20/2	44/47/8/1

[a]Measured by immunohistochemistry on primary tumor section.

Table 2 Characteristics of chemotherapy administered and number (percent) of patients who received those treatments

Preoperative chemotherapy	Median (range)	*n* (%)	Postoperative chemotherapy *n* (%)
All		240 (100)	143 (60)
1–6 × (F)EC		206 (86)	126 (53)
1–2 × (T)IE + 1–2 × HD-CT		15 (6)	3 (1)
1–4 × ET		10 (4)	6 (3)
2–3 × IE		5 (2)	4 (2)
3 × E + 3 × T		2 (1)	2 (1)
3 × EC + 3 × IE		1 (<1)	1 (<1)
1 × TIE + 4 × IE		1 (<1)	1 (<1)
No. of cycles	4 (1–6)		
Cumulative dose of epirubicin	360 (90–630)		

E, Epirubicin 150 mg/m^2 d1 q d15; EC, epirubicin + cyclophosphamide either as 90/600 mg/m^2 d1 q d22 or 60/600 mg/m^2 d1 + 8 q d29; ET, epirubicin + paclitaxel either as 90/175 mg/m^2 d1 q d22 or 45/90 mg/m^2 d1 + 2 q d22; FEC, fluorouracil 500 mg/m^2 d1 + epirubicin 90 mg/m^2 d1 + cyclophosphamide 500 mg/m^2 d1 q d22; HD-CT, stem cell supported high-dose chemotherapy; IE, ifosfamide 2500 mg/m^2 d1–3 + epirubicin 40 mg/m^2 d1–3 q d22; T, paclitaxel 250 mg/m^2 d1 q d15; TIE, paclitaxel 45 mg/m^2 d1–3 + ifosfamide 2000 mg/m^2 d1–3 + epirubicin 30 mg/m^2 d1–3 q d22.

last cycle of chemotherapy, whichever was last. Until 1998 tamoxifen was omitted in premenopausal patients. Afterwards all hormone receptor-positive patients received tamoxifen 20–30 mg/day orally for 2–5 years. Menopausal status was determined at diagnosis before start of chemotherapy.

Statistical analysis

DFS, DDFS and OS were taken as clinical outcome variables. DFS was measured from the date of surgery until relapse, death or last contact, DDFS from the date of surgery until distant relapse, death or last contact and OS from the date of surgery until death or last contact, respectively, whichever occurred first. Survival curves were estimated using the Kaplan–Meier product limit method [7]. Univariate analyses (log-rank tests [8]) and multivariate analyses (Cox regression analyses [9]) were performed to identify risk factors associated with DFS, DDFS and OS. The following factors, assessed either before (bf) or after (af) preoperative chemotherapy, were examined in univariate analyses: age (bf; < 35 versus 35–50 versus > 50 years), UICC stage (bf; I versus II versus III), clinical tumor size (bf; T1 versus T2 versus T3 versus T4), clinical nodal status (bf; N0 versus N1 versus N2), grading (bf; 1 versus 2 versus 3), ER status (bf; positive versus negative), PgR status (bf; positive versus negative), expression of Her2/neu (bf; 0–2 + versus 3 +), Ki67 (bf; < 35% positive cells versus ≥ 35%), Bcl-2 (bf; 0–2 + versus 3 +) and p53 (bf; < 50% positive cells versus ≥ 50%), clinical response evaluated by UICC criteria (af; yes versus no), pathological T category (af; pT0 or pTis versus pT1 versus pT2 versus pT3 versus pT4), pCR, defined as no invasive tumor residuals in the breast (af; yes versus no), pathological N category according to the TNM classification (5th edn) (af; pN0 versus pN1 versus pN2), and administration of postoperative chemotherapy (af; yes versus no). Variables that showed statistical significance in univariate analysis ($p < 0.05$) were included in a multivariate analysis. Model selection was performed using forward selection (entry into the model if $p < 0.1$) and backward selection (removal from the model if $p > 0.1$). First, only significant variables measured before preoperative chemotherapy were included in Cox regression analysis (model A). Afterwards, variables assessed after preoperative chemotherapy were added separately to the multivariate analysis process (model B) in order to (i) examine the additional independent value of those factors for DFS, DDFS and OS, respectively, and to (ii) evaluate which variables assessable before preoperative chemotherapy retain their independent prognostic impact on DFS, DDFS and OS, respectively.

In addition, we performed univariate analyses (χ^2 or Fisher tests, if appropriate) to describe factors predictive for achieving pCR. Due to the low number of patients who achieved a pCR ($n = 15$), multivariate analysis did not seem meaningful. All tests were performed using SAS software (version 8.2; Cary, NC).

Results

With a median follow-up period of 6.4 years (range 0–10.4) out of 240 patients, 172 (72%) are alive, 124 (52%) without relapse and 149 (62%) without distant relapse.

Prognostic factors for survival

According to univariate analysis a longer DFS was associated with a lower UICC stage ($p < 0.001$), smaller clinical tumor size ($p < 0.001$), lower clinical nodal status ($p < 0.001$), lower grading ($p = 0.001$), higher expression of Bcl-2 ($p = 0.012$), clinical response to preoperative chemotherapy ($p = 0.012$), lower pathological T category after preoperative chemotherapy ($p < 0.001$) and lower pathological N category after preoperative chemotherapy ($p < 0.001$). A longer DDFS was associated with lower UICC stage ($p < 0.001$), smaller clinical tumor size ($p < 0.001$), lower clinical nodal status ($p < 0.001$), lower grading ($p < 0.001$), higher expression of Bcl-2 ($p = 0.005$), lower expression of p53 ($p = 0.005$), clinical response to preoperative chemotherapy ($p = 0.005$), lower pathological T category after preoperative chemotherapy ($p < 0.001$) and lower pathological N category after preoperative chemotherapy ($p < 0.001$). Prognostic fac-

tors for a longer OS were a lower UICC stage ($p < 0.001$), smaller clinical tumor size ($p < 0.001$), lower clinical nodal status ($p < 0.001$), lower grading ($p < 0.001$), ER ($p = 0.05$) or PgR positivity ($p = 0.006$), lower expression of Her2/neu ($p = 0.015$), higher expression of Bcl-2 ($p = 0.005$), lower expression of p53 ($p < 0.001$), clinical response to preoperative chemotherapy ($p = 0.05$), lower pathological T category after preoperative chemotherapy ($p < 0.001$) and lower pathological N category after preoperative chemotherapy ($p < 0.001$). The administration of additional postoperative chemotherapy had no significant impact on DFS, DDFS and OS, respectively.

Due to a strong correlation between UICC stage and clinical tumor size or nodal status, UICC stage was omitted from the multivariate analyses. In model A (only pretreatment parameters), independent prognostic factors for longer DFS and DDFS were smaller clinical tumor size [for DFS hazard ratio (HR) = 1.5 (95% confidence interval (CI) 1.2–1.9); $p = 0.001$; for DDFS HR = 1.5 (95% CI 1.1–2.0); $p = 0.004$], lower clinical nodal status [for DFS HR = 1.5 (95% CI 1.1–2.1); $p = 0.005$; for DDFS HR = 1.6 (95% CI 1.1–2.2); $p = 0.01$] and lower grading [for DFS HR = 1.5 (95% CI 1.0–2.1); $p = 0.03$; for DDFS HR = 1.7 (95% CI 1.1–2.5); $p = 0.01$]. In model B (separate addition of significant variables assessed after preoperative chemotherapy), pathological T category after preoperative chemotherapy [for DFS HR = 1.6 (95% CI 1.3–2.1); $p < 0.001$; for DDFS HR = 1.8 (95% CI 1.3–2.3); $p < 0.001$] replaced clinical tumor size, and pathological N category after preoperative chemotherapy [for DFS HR = 2.3 (95% CI 1.6–3.2); $p < 0.001$; for DDFS HR = 2.4 (95% CI 1.7–3.6); $p < 0.001$) replaced clinical nodal status. Only grading retained independent prognostic significance (Tables 3 and 4). Clinical response had no independent impact on DFS or DDFS.

In multivariate analysis considering pretreatment variables (model A), independent prognostic factors for longer OS were smaller clinical tumor size [HR = 2.0 (95% CI 1.3–2.9); $p < 0.001$], lower grading [HR = 2.9 (95% CI 1.5–5.6); $p = 0.002$] and lower expression of p53 [HR = 1.9 (95% CI 1.0–3.6); $p = 0.04$]. In model B (separate addition of significant variables assessed after preoperative chemotherapy) pathological T category after preoperative chemotherapy [HR = 1.7 (95% CI 1.3–2.4); $p < 0.001$] replaced clinical tumor size. As a consequence, patients without invasive tumor residue after preoperative chemotherapy (pT0 or pTis) had a Kaplan–Meier estimate of OS at 6 years of 92% in comparison to 83 and 45% for patients with tumor residue between 2 and 5 cm (pT2) or greater than 5 cm (pT3), respectively (Fig. 1). Along the same line, the probability of OS was significantly better for patients without axillary lymph node involvement after preoperative chemotherapy (pN0) in comparison to those with microscopic (pN1) and massive (pN2) involvement (89, 62 and 48%, respectively, at 6 years) (Fig. 2). The pathological N category after preoperative chemotherapy gained independent prognostic significance [HR = 2.1 (95% CI 1.5–3.1); $p < 0.001$], whereas, again, only grading retained its significant impact (Table 5 and Fig. 3). P53 was no longer significant (data not shown) and was omitted from the analyses due to the low number of cases in order to increase the statistical power. Clinical response had no independent impact on OS.

Predictive factors for the achievement of pCR

Predictive factors for achieving pCR in univariate analyses were age under 35 years ($p\chi^2 = 0.007$), low UICC stage ($p\chi^2 < 0.001$), smaller clinical tumor size ($p\chi^2 < 0.001$), and high expression of Bcl-2 ($p\chi^2 = 0.004$, $p_F = 0.008$) (Table 6). Due to the low number of patients with pCR ($n = 15$), a multivariate analysis was not performed.

Table 3 Cox regression analyses: independent prognostic factors for DFS following preoperative chemotherapy including an anthracycline

Parameter	HR (95% CI); *p* value
Model A[a]	
clinical tumor size, T1 versus T2 versus T3 versus T4	1.5 (1.2–1.9); 0.001
clinical nodal status, N0 versus N1 versus N2	1.5 (1.1–2.1); 0.005
grading, 1 versus 2 versus 3	1.5 (1.0–2.1); 0.03
Model B[b] including pathological T category	
clinical tumor size, T1 versus T2 versus T3 versus T4	1.1 (0.8–1.5); 0.44
clinical nodal status, N0 versus N1 versus N2	1.4 (1.0–1.9); 0.03
grading, 1 versus 2 versus 3	1.7 (1.2–2.4); 0.003
pathological T category, pT0/is versus pT1 versus pT2 versus pT3 versus pT4	1.6 (1.3–2.1); <0.001
Model B[b] including pathological N category	
clinical tumor size, T1 versus T2 versus T3 versus T4	1.3 (1.1–1.7); 0.01
clinical nodal status, N0 versus N1 versus N2	1.1 (0.8–1.6); 0.48
grading, 1 versus 2 versus 3	1.5 (1.1–2.2); 0.02
pathological N category, pN0 versus pN1 versus pN2	2.3 (1.5–3.2); <0.001
Model B[b] including clinical response to preoperative chemotherapy	
clinical tumor size, T1 versus T2 versus T3 versus T4	1.5 (1.1–1.9); 0.004
clinical nodal status, N0 versus N1 versus N2	1.5 (1.1–2.1); 0.02
grading, 1 versus 2 versus 3	1.5 (1.1–2.2); 0.02
clinical response to preoperative chemotherapy, yes versus no	0.8 (0.5–1.2); 0.21

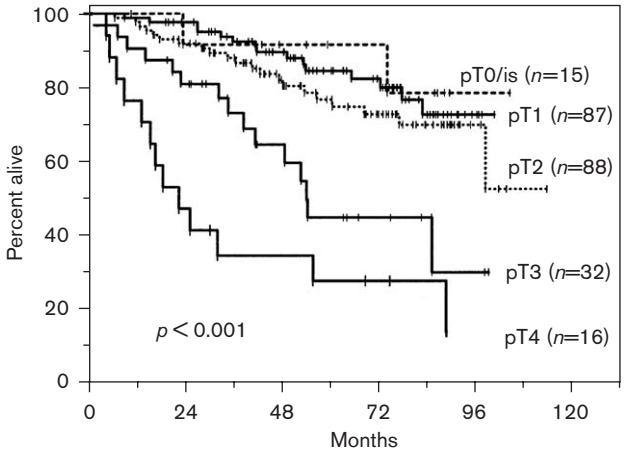
[a]Only significant parameters assessable before preoperative chemotherapy
[b]Separate addition of parameters assessable after preoperative chemotherapy.

Table 4 Cox regression analyses: independent prognostic factors for DDFS following preoperative chemotherapy including an anthracycline

Parameter	HR (95% CI); *p* value
Model A[a]	
clinical tumor size, T1 versus T2 versus T3 versus T4	1.5 (1.1–2.0); 0.004
clinical nodal status, N0 versus N1 versus N2	1.6 (1.1–2.2); 0.01
grading, 1 versus 2 versus 3	1.7 (1.1–2.5); 0.01
Model B[b] including pathological T category	
clinical tumor size, T1 versus T2 versus T3 versus T4	1.1 (0.8–1.5); 0.70
clinical nodal status, N0 versus N1 versus N2	1.4 (0.96–2.0); 0.07
grading, 1 versus 2 versus 3	2.1 (1.4–3.1); <0.001
pathological T category, pT0/is versus pT1 versus pT2 versus pT3 versus pT4	1.8 (1.3–2.3); <0.001
Model B[b] including pathological N category	
clinical tumor size, T1 versus T2 versus T3 versus T4	1.4 (1.1–2.8); 0.02
clinical nodal status, N0 versus N1 versus N2	1.0 (0.7–1.5); 0.99
grading, 1 versus 2 versus 3	1.8 (1.2–2.7); 0.006
pathological N category, pN0 versus pN1 versus pN2	2.4 (1.7–3.6); <0.001
Model B[b] including clinical response to preoperative chemotherapy	
clinical tumor size, T1 versus T2 versus T3 versus T4	1.5 (1.1–2.0); 0.01
clinical nodal status, N0 versus N1 versus N2	1.5 (1.1–2.4); 0.01
grading, 1 versus 2 versus 3	1.7 (1.1–2.6); 0.02
clinical response to preoperative chemotherapy, yes versus no	0.7 (0.5–1.1); 0.1

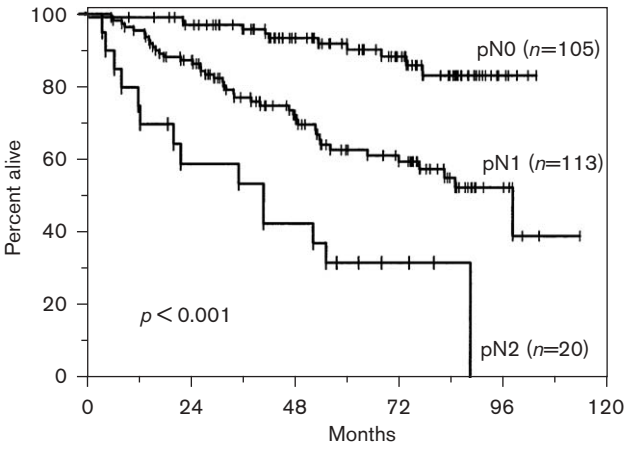
[a]Only significant parameters assessable before preoperative chemotherapy.
[b]Separate addition of parameters assessable after preoperative chemotherapy.

Fig. 1



OS according to pathological T category (pT stage) after preoperative chemotherapy including an anthracycline. *p* value of multivariate analysis.

Fig. 2



OS according to pathological N category (pN stage) after preoperative chemotherapy including an anthracycline. *p* value of multivariate analysis.

Discussion

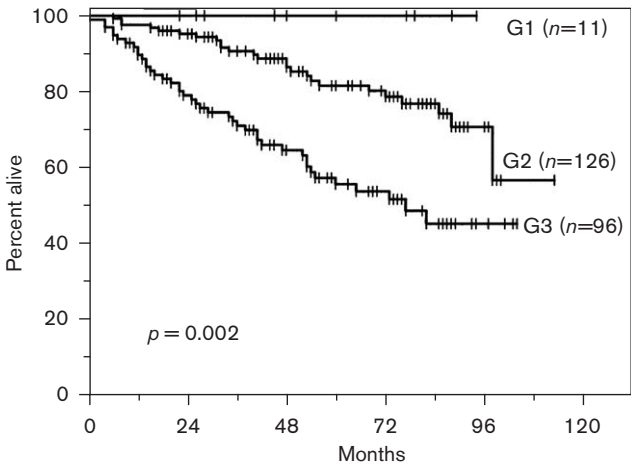
With a median follow-up of 6.4 years (range 0–10.4) the strongest independent prognostic factors for DFS, DDFS and OS of all 240 primary breast cancer patients who received preoperative chemotherapy that contained anthracycline were pathological T category after preoperative chemotherapy (HR 1.6, 1.8 and 1.7, respectively; $p < 0.001$) and pathological N category after preoperative chemotherapy (HR 2.3, 2.4 and 2.1, respectively; $p < 0.001$). Patients with no invasive tumor residue either in breast or axillary lymph nodes survived significantly longer than all other patients. Considering all prognostic factors measured before treatment, only grading (HR 1.5, 1.7 and 2.9, respectively; $p < 0.05$) retained its independent impact on DFS, DDFS and OS after adjusting for pathological response of primary breast tumor and axillary lymph node metastases. In particular, all immunohistochemical markers evaluated before treatment no longer helped to distinguish between patients with good or poor survival. It could be argued that postoperative chemotherapy might have influenced survival. Additional postoperative chemotherapy, however, was only given to non-responders or patients with large tumor residues. Thus, the impact of pathological tumor response to preoperative chemotherapy on survival would have been blurred rather than enhanced. Furthermore, in univariate analysis postoperative chemotherapy had no impact on DFS, DDFS and OS, respectively.

Table 5 Cox regression analyses: independent prognostic factors for OS following preoperative chemotherapy including an anthracycline

Parameter	HR (95% CI); *p* value
Model A[a]	
clinical tumor size, T1 versus T2 versus T3 versus T4	2.0 (1.3–2.9); <0.001
grading, 1 versus 2 versus 3	2.9 (1.5–5.6); 0.002
p53, <50% positive cells versus ≥ 50%	1.9 (1.0–3.6); 0.04
Model B[b] including pathological T category[c]	
clinical tumor size, T1 versus T2 versus T3 versus T4	1.3 (0.9–2.9); 0.18
grading, 1 versus 2 versus 3	1.3 (1.9–5.1); <0.001
pathological T category, pT0/is versus pT1 versus pT2 versus pT3 versus pT4	1.7 (1.3–2.4); <0.001
Model B[b] including pathological N category[c]	
clinical tumor size, T1 versus T2 versus T3 versus T4	1.5 (1.1–2.1); 0.005
grading, 1 versus 2 versus 3	2.5 (1.5–4.1); <0.001
pathological N category, pN0 versus pN1 versus pN2	2.1 (1.5–3.1); <0.001
Model B[b] including clinical response to preoperative chemotherapy[c]	
clinical tumor size, T1 versus T2 versus T3 versus T4	1.9 (1.4–2.6); <0.001
grading, 1 versus 2 versus 3	2.6 (1.6–4.4); <0.001
clinical response to preoperative chemotherapy, yes versus no	0.7 (0.4–1.2); 0.24

[a]Only significant parameters assessable before preoperative chemotherapy.
[b]Separate addition of parameters assessable after preoperative chemotherapy.
[c]Expression of p53 lost significance and was omitted from analysis due to low number of cases.

Fig. 3



OS according to grading (G) as assessed before preoperative chemotherapy including an anthracycline. *p* value of multivariate analysis.

Table 6 χ^2 or Fisher test, if appropriate: predictive value of different parameters for achieving a pathological complete remission following preoperative chemotherapy including an anthracycline

Parameter	*p* value	
	χ^2 test	Fisher test
Age, <35 versus 35–50 versus >50 years	0.007	
Stage, UICC I versus II versus III	<0.001	<0.001
Clinical tumor size, T1 versus T2 versus T3 versus T4	<0.001	<0.001
Clinical nodal status, N0 versus N1 versus N2	0.20	
Grading, 1 versus 2 versus 3	0.29	
ER, pos versus neg	0.45	
PgR, pos versus neg	0.40	
Her2/neu, 0–2+ versus 3+	0.06	0.10
Ki67, <35% pos cells versus ≥ 35%		0.43
Bcl-2, 0–2+ versus 3+	0.004	0.008
P53, <50% pos cells versus ≥ 50%	0.09	0.19

Established pretreatment prognostic factors for primary breast cancer included in consensus recommendations are age, tumor size, nodal status, grading, and hormone receptor status [10,11]. The magnitude of Her2/neu as a prognostic factor varies among studies; however, overall, Her2/neu appears to have only a weak impact on survival [12]. The proliferation marker thymidine-labeling index has actually been found to have independent prognostic value in most of the studies [13]. UPA and PAI-1 have strong prognostic impact in primary breast cancer validated in a prospective randomized trial [14] and in meta-analysis [15]. As of the technical complexity of the analyses, however, the usefulness of these markers has been questioned and, so far, they are not recommended for daily practice [11]. Other molecular markers, e.g. Ki67, Bcl-2 and p53, are either not properly standardized or not validated in multivariate analyses [16]. For example, studies on the predictive or prognostic value of p53 are hampered by a number of methodological issues, including immunologic versus molecular biological analyses. About 20% of the p53 gene mutations do not result in p53 protein accumulation, whereas p53 accumulation may also occur without a gene mutation [17–19].

In contrast, retrospective analyses of multiple trials of preoperative chemotherapy have documented that pathological response to chemotherapy strongly correlates with long-term outcomes. Patients in whom primary tumor and regional lymph node metastases completely disappear experience a significantly longer DFS and OS than patients who only achieve a partial remission or who do not respond [1,20–23]. Therefore, in light of the pathological response of primary breast tumor and axillary lymph node metastases to preoperative chemotherapy, the prognostic impact of established and proposed pretreatment factors might be substantially diminished.

Amat *et al.* [24] evaluated the prognostic significance of clinical and histological characteristics of 451 breast cancer patients treated with preoperative chemotherapy. Similar to our findings, besides number of nodes still involved after preoperative chemotherapy and achievement of a pCR, only the grade of residual tumor appeared to have an independent impact on survival. In addition, clinical tumor response as assessed by palpation, which is a rather crude and subjective measure for tumor regression, had no independent impact on survival after adjustment for pathological tumor response. In conclusion, pathological response to preoperative chemotherapy, i.e. the result of an individual *in vivo* chemosensitivity test, stronger reflects tumor biology and might better predict outcome than all established and proposed prognostic factors. Only grading retained its independent prognostic value when evaluated together with pathological response to preoperative chemotherapy. Although too few data are available to make valid conclusions, we found no appreciable independent impact of Her2/neu, Ki67, Bcl-2, and p53 expression.

In light of the utmost importance of pathological response to preoperative chemotherapy, in general, and achieving pCR, in particular, for long-term survival, there is considerable interest in developing predictive markers for the achievement of a pCR. In univariate analysis, we found age under 35 years ($p\chi^2 = 0.007$), low UICC stage ($p\chi^2 < 0.001$) or smaller clinical tumor size before preoperative chemotherapy ($p\chi^2 < 0.001$) and higher expression of Bcl-2 before preoperative chemotherapy ($p\chi^2 = 0.004$) be associated with a complete disappearance of invasive breast cancer tissue. Due to the low number of patients who achieved a pCR ($n = 15$), multivariate analysis did not seem meaningful. No association was found with clinical nodal status, grading and expression of ER, PgR, Her2/neu, Ki67 or p53.

In several studies, a number of tumor characteristics have been correlated with response to preoperative chemotherapy. However, the different study end points, different numbers of treatment cycles and different definitions of tumor characteristics make it difficult to evaluate their predictive importance. According to recent overview data, a benefit from adjuvant chemotherapy in early breast cancer is age related, with young women profiting most [25]. Therefore, it is not surprising that young breast cancer patients also respond best to preoperative chemotherapy. Most reports suggest no correlation between initial tumor size and overall response to preoperative chemotherapy. In some analyses, however, and in line with our findings, pathological response rates were reported to be inversely proportional to tumor size, i.e. the smaller the tumor, the higher the complete response rate [26,27]. As complete response rates correlate with tumor burden in the metastatic setting, this correlation is not unexpected [28]. The predictive value of Bcl-2 expression for achieving a pathological response with preoperative chemotherapy is uncertain. In two studies there was no correlation of Bcl-2 abnormalities with response [29,30], while in another study the decrease in Bcl-2 expression after chemotherapy but not the pretreatment expression correlated with disease response [31]. Similar to our results, only Makris *et al.* described a non-significant trend for higher response rates for Bcl-2^+ tumors among 90 primary breast cancer patients treated with a neoadjuvant chemoendocrine regimen [32].

Probably due to the low number of patients with pCR we found no correlation of pCR and high grading, hormone receptor negativity or Ki67, which is described in most reports [16]. At least two reports have indicated that for patients with high nuclear grade the probability of response is higher and the probability of a complete response is even much higher as compared to patients with grade I tumors [33,34]. ER/PgR poor or negative tumors showed a better overall and complete response rate as compared to receptor positive disease [35]. High S phase fraction or Ki67 expression was associated with higher overall and complete response rates in several studies [36–39]. Unfortunately, there was no uniformity in the methodology used to perform and analyze the S phase fraction or Ki67, which makes it difficult to assess their predictive value outside of clinical trials.

In contrast, no conclusive data exist on the predictive value of Her2/neu and p53. There is ongoing controversy regarding the correlation of Her2/neu overexpression or amplification with sensitivity or resistance to chemotherapy. In the largest of four clinical trials there was no correlation with response to preoperative chemotherapy [40]. In two trials, overexpression or amplification of Her2/neu was associated with an improved response rate [32,41] and the fourth predicted a decreased response to preoperative chemotherapy [42]. Clinical research with p53 has been plagued by technical problems. Immunohistochemistry fails to identify all molecular abnormalities, and full sequencing is very time-consuming and labor intensive. Although the association of p53 abnormalities and response to preoperative chemotherapy has been analyzed in several reports, the level of evidence for each study is low. Five reports found no correlation between p53 abnormalities and response to preoperative chemotherapy, two studies reported p53 to predict decreased response to preoperative chemotherapy, and in one study response varied depending on p53 status and cytotoxic agents used [19,30,32,40–46].

Conclusion

Considering established and proposed clinical and tumor biologic pretreatment characteristics, only grading re-

tained its independent prognostic impact on DFS, DDFS and OS after adjusting for pathological response of breast tumor and axillary lymph node metastases to preoperative chemotherapy. These findings further underline the importance for developing refined selection criteria for treatment based not only on distinct clinical and molecular markers, but rather on the whole molecular make-up of the cancer, e.g. on gene or protein expression profiles. According to the present analysis, despite its limitations (small sample size, few patients with pCR), young patients (under 35 years) with early tumor stage and small primary tumors might profit most from preoperative chemotherapy.

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