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Predictive value of tumour cell proliferation in locally advanced breast cancer treated with neoadjuvant chemotherapy

T. Aas[a], S. Geisler[b], G.E. Eide[c], D.F. Haugen[b], J.E. Varhaug[a], A.M. Bassøe[d], T. Thorsen[e], H. Berntsen[b], A.-L. Børresen-Dale[f], L.A. Akslen[g], P.E. Lønning[b,*]

[a]*Department of Surgery, Haukeland University Hospital, N-5021 Bergen, Norway*
[b]*Section of Oncology, Department of Medicine, Haukeland University Hospital, N-5021 Bergen, Norway*
[c]*Centre for Clinical Research and Section for Medical Statistics, Haukeland University Hospital, N-5021 Bergen, Norway*
[d]*Department of Radiology, Haukeland University Hospital, N-5021 Bergen, Norway*
[e]*Department of Biochemical Endocrinology, Haukeland University Hospital, N-5021 Bergen, Norway*
[f]*Department of Genetics, The Norwegian Radiumhospital, N-0310 Oslo, Norway*
[g]*Department of Pathology, The Gade Institute, Haukeland University Hospital, N-5021 Bergen, Norway*

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Abstract

We previously reported that defects in apoptotic pathways (mutations in the *TP53* gene) predicted resistance to doxorubicin monotherapy. The aim of this study was to evaluate whether cell proliferation, as assessed by mitotic frequency and Ki-67 levels, may provide additional predictive information in the same tumours and to assess any potential correlations between these markers and mutations in the *TP53* gene and erbB-2 overexpression. Surgical specimens were obtained from ninety locally advanced breast cancers before commencing primary chemotherapy consisting of weekly doxorubicin (14 mg/m^2) for 16 weeks. 38% of the patients had a partial response (PR) to therapy, 52% had stable disease (SD) while 10% had progressive disease (PD). Univariate analysis showed a significant association between a high cell proliferation rate (expressed as a high mitotic frequency) and resistance to doxorubicin ($P=0.001$). Further analyses revealed this association to be limited to the subgroup of tumour expressing wild-type *TP53* ($P=0.016$), and *TP53* mutation status was the only factor predicting drug resistance in the multivariate analyses. The finding that a high mitotic frequency, as well as a high Ki-67 staining, correlated to *TP53* mutations ($P=0.001$ for both), suggests *TP53* mutations are the key predictor of drug resistance, although cell proliferation may play an additional role in tumours harbouring wild-type *TP53*. Regarding overall (OS) and relapse-free survival (RFS), multivariate analyses (Cox' proportional hazards regression) revealed a high histological grade and negative oestrogen receptor (ER) status to be the variables that were most strongly related to breast cancer death ($P=0.001$ and $P=0.001$, respectively). A key reason for this difference with respect to the factors predicting chemotherapy resistance could be due to the adjuvant use of tamoxifen in all patients harbouring ER-positive tumours.

Keywords: Locally advanced breast cancer; Cell proliferation; Apoptotic factors; Chemotherapy resistance

1. Introduction

Resistance to chemotherapy remains the key obstacle to cancer cure. The knowledge that many drugs are effective only in dividing cells [1,2], precipitated much work on the relationship between cell kinetics and drug sensitivity. More recently, studies on drug resistance have focused on oncogene overexpression, like erbB-2 [3,4] and mutations in genes involved in apoptosis, like *TP53* [5–9].

We previously found mutations in the *TP53* gene predicted resistance to doxorubicin monotherapy in patients with primary locally advanced breast cancers [10,11]. When measuring erbB-2 expression, erbB-2 overexpression was correlated to drug resistance, but was strongly associated with mutations in the *TP53* gene [11]. In the same material, we also found a correlation between histological high grade and drug resistance [10].

It remains open to debate whether cell proliferation is an independent predictor of chemoresistance, may add

* Corresponding author. Tel.: +47-55-975000; fax: +47-55-972046.
E-mail address: plon@haukeland.no (P.E. Lønning).

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additional information to subgroups stratified on the basis of *TP53* mutation status, or if the different parameters are correlated. To address these important questions, we measured proliferation parameters (Ki-67 expression and mitotic frequency) in tumour samples collected from the same patients from whom we previously assessed the predictive role of *TP53* mutations and overexpression of erbB-2 in relation to primary resistance to therapy with anthracyclines [11].

2. Patients and methods

2.1. Patient material

During 1991–1996, 94 women with locally advanced breast cancer, T_3/T_4 and/or N_2, were enrolled in the protocol. 3 patients were excluded from the study for the following reasons: 2 patients had tumours consisting of mainly ductal carcinoma *in situ* with minor invasive components, and 1 patient received treatment with cyclophosphamide in addition to doxorubicin treatment. A fourth patient had a cystic tumour, which made the assessment of clinical response impossible. This last patient was excluded from the statistical analyses of predictive factors with respect to response to doxorubicin, but was included in the survival analyses; the other three were excluded from the evaluations of response and survival.

In the group of 90 patients assessable for response to chemotherapy, 54 patients had T_3 and 33 had T_4 tumours. 3 patients had T_2 tumours, but were classified as locally advanced based on an N_2 axillary status. 12 patients, in addition to their locally advanced tumour, also had minor distant metastasis at the time of diagnosis (which was allowed according to the protocol). These patients were excluded from analyses of survival, leaving 90 patients for the response evaluation and 79 for the survival analyses. The median age at diagnosis was 64 years (range 32–88 years).

2.2. Treatment

The treatment and evaluation procedures have been described in detail elsewhere in Refs. [10–12]. Briefly, all patients underwent a pretreatment staging procedure and had an open incision biopsy prior to chemotherapy treatment. Both snap-frozen and formalin-fixed, paraffin-embedded tumour tissue samples were obtained. The primary treatment consisted of weekly doxorubicin (14 mg/m^2) scheduled for 16 weeks with a 4-weekly assessment of clinical response according to the International Union Against Cancer (UICC) criteria [13]. This was performed mainly by one observer. Response to treatment was evaluated by measuring the tumour using callipers. Thus, responses were classified either as a complete response (CR; complete disappearance of all tumour lesions), partial response (PR; reduction $\geqslant 50\%$ in the sum of all lesions calculated for each as the product of the largest diameter and the one perpendicular to it), progressive disease (PD; increase in the diameter product of any individual tumour lesion by $\geqslant 25\%$), and stable disease (SD; anything in between a PR and a PD).

The terms SD and PR are pragmatic terms, each describing a status of 'growth arrest' with or without any certain degree of tumour size reduction and apoptosis. For studies with the primary aim of exploring the cause of chemoresistance, it may be useful to group these patients as 'responders' because they all are likely to express some kind of effects to the therapeutic agent [14]. In contrast, tumours with a PD may represent a distinct group, easily discriminated clinically from the others as macroscopic growth clearly signals that the bulk of the tumour mass is not sensitive to the therapeutic agent. In our study, if the patients expressed a PD according to the UICC criteria, doxorubicin treatment was terminated immediately, and alternative treatment procedures implemented. Patients considered technically operable had surgery followed by radiotherapy immediately after termination of the neo-adjuvant chemotherapy, while the remaining patients were treated on an individual basis. Women with receptor-positive tumours (oestrogen receptor (ER) and/or progesterone receptor (PgR) $\geqslant 10$ fmol/mg, $n = 69$ in total, Table 1) had treatment with tamoxifen (30 mg daily for 5 years) implemented after surgery. Follow-up time was defined from inclusion into the study up to 31 December 1998 (median 62.5 months, range 2–93).

2.3. Histopathology

Histological grading was performed using the criteria of Elston and Ellis [15] based on the assessment of tubular formation, nuclear pleomorphism and mitotic counts. The mitotic frequency was recorded separately as the number of mitotic figures per 10 High Power Fields (HPF, $\times 400$). Both histological grade and mitotic frequency were determined by one observer, and were available for all 90 patients. In this series, 23 patients (26%) had tumours with histological grade 1, 42 with grade 2 (47%), and 25 tumours were classified as grade 3 (28%).

2.4. Steroid receptor analyses

ER and PR receptors were measured by using ligand-binding assays [16].

2.5. Immunohistochemistry

Paraffin-embedded tissue sections were subjected to microwave epitope retrieval (750 W for 7.5 min and

Table 1
Association between mitotic frequency or Ki-67 and other biological factors

Variables	Mitotic frequency (median)	*P* value	Ki-67 (median)	*P* value
Tumour diameter		0.3		0.03
<68 mm	4		13.2	
⩾68 mm	6		16.9	
Histological grade		0.001		0.001
Grade 1	1		6.5	
Grade 2	4		13.5	
Grade 3	27		26.0	
Oestrogen receptors		0.05		0.03
<10 fmol/mg	8		23.8	
⩾10 fmol/mg	4		13.2	
Progesterone receptors		0.6		0.6
<10 fmol/mg	4.5		15.7	
⩾10 fmol/mg	5		15.3	
TP53 mutations		0.001[a]		0.001*
Wild-type	4		10.5	
Mutations not affecting L2/L3	14		23.5	
Mutations affecting L2/L3	15		20.2	
Bcl-2 expression		0.01		0.003
Negative <6	10		19.0	
Positive ⩾6	3		9.0	
c-erbB-2 expression		0.11		0.04
Negative (0-1)	4		12.5	
Positive (2-3)	13		19.0	

[a] *P* value is related to 'all *TP53* mutations' versus wild-type p53. In previous reports, we found *TP53* mutations affecting the L2/L3 domain to be associated with drug resistance, while mutations outside this domain did not [9,10]. As seen here, the mitotic frequency and Ki-67 values were similiar in the subgroups of *TP53* mutated tumours.

500 W for 5 min) in citrate buffer (pH 6.0). Then the sections were left at room temperature for 20 min and thereafter rinsed in distilled water for 5 min before incubation at room temperature for 1 h with the rabbit anti-human Ki-67 antibody Code No. A 047 (DAKO, Copenhagen, Denmark). The A047 antibody recognises a nuclear antigen that is present throughout the cycle of proliferating cells, but absent in quiescent cells, and which is widely accepted as a marker of proliferation [17]. The sections were examined at low magnifications (×40 and ×100) to identify 'hot spot' areas with the most intense and frequent nuclear staining. Ki-67 expression was defined as the percentage of positive tumour cell nuclei calculated by counting at least 1000 tumour cells within the selected 'hot spot' areas at a magnification of ×1000 [18]. Ki-67 expression could be estimated in 89 tumours. Immunohistochemical stainings for c-erbB-2 and bcl-2 were previously described in Ref. [11].

2.6. TP53 *mutations*

The methods and results concerning mutations of the TP53 gene are previously described elsewhere in Refs. [11,19,20].

2.7. *Statistics*

In evaluating response to therapy, we compared patients with response defined as tumour regression or tumour growth arrest (PR and SD) on the one hand and patients with PD (non-responders) on the other hand, similar to our previous reports [10–12]. The rationale for doing so is given in Section 2.2.

The 'cut-off' values for Ki-67 and mitotic frequency were in accordance with previous publications from the same laboratory [12,18]. Correlations between mitotic frequency and expression of Ki-67 were evaluated using the Spearman's rank correlation coefficient [21]. The associations between proliferation factors and different variables (age, tumour diameter, histological grade, *TP53* mutations, steroid receptors), and between proliferation factors and treatment response were (whenever possible) (2×2 groups) performed by the Fisher's Exact test [22] due to the small numbers in the subgroups. For the other parameters (three or more categories), the Chi-square test including the Yates correction was adopted. In addition, the Mann–Whitney U-test [23] was performed to compare expression values for the different variables (Ki-67, mitotic frequency, age, tumour diameter) in groups experiencing a different response to doxorubicin treatment.

A multiple logistic regression analysis [24] was used to determine which variables had independent effects on the likelihood of response. Due to the strong correlation between them, each of the proliferation markers (mitotic frequency and Ki-67 staining) was evaluated in separate analyses. *TP53* mutations were classified in accordance to mutations involving L2/L3 domain as this subgroup was found to predict for chemoresistance in univariate analyses and in our earlier publications [10,11].

Survival analyses were performed on the subgroup of 79 patients without known distant metastases at the time of diagnosis, and survival curves were estimated by the Kaplan–Meier method [25] using the log-rank test [26] for disclosing differences between categories of each variable. Variables found to have a prognostic inpact in univariate analysis ($P<0.05$) were further analysed by use of the Cox proportional hazards model [27]. Deaths due to causes other than breast cancer were treated as censored observations. All statistical calculations were conducted using the Statistical Package for the Social Sciences (SPSS) software.

3. Results

3.1. *Response to therapy*

34 patients (38%) had a PR, 47 patients (52%) had a SD, while 9 patients (10%) had a PD during

chemotherapy. 79 patients underwent surgical treatment, mastectomy and limited axillary surgery with removal of clinically suspected lymph node metastases, according to the study protocol. 4 of these had their surgery following radiotherapy. 11 patients had no surgical treatment; in 5 cases due to PD, 5 had distant metastases, but a good locoregional disease control, and one patient with a good locoregional control was not operated upon due to concomitant medical disorders.

3.2. Proliferation markers

Median mitotic frequency was 5 per 10 HPF, (range 0–120), while the mean frequency was 14.9 per 10 HPF. The median and mean values for Ki-67 expression were 15.3 and 16.4%, respectively (range 0.75–58.0). Fig. 1 shows a plot of Ki-67 staining and the mitotic frequency for individual tumours subdivided according to chemotherapy response.

There was a highly significant association between the level of Ki-67 expression and mitotic frequency ($P = 0.001$). The median values of mitotic frequency and Ki-67 in subgroups according to other variables are shown in Table 1. Increased mitotic frequency was significantly associated with a high histological grade ($P = 0.001$), *TP53* mutations (without any difference between the subgroups of *TP53* mutations, see Ref. [11]) ($P = 0.001$), low bcl-2 expression ($P = 0.01$) and negative ER status ($P = 0.05$).

Increased Ki-67 expression was significantly associated with a high histological grade ($P = 0.001$), *TP53* mutations ($P = 0.001$), low bcl-2 expression ($P = 0.003$), large tumour diameter ($P = 0.03$), negative ER status ($P = 0.03$) and c-erbB-2 positivity ($P = 0.04$).

In addition to the results demonstrated in Table 1, we found a negative ER status to be significantly associated

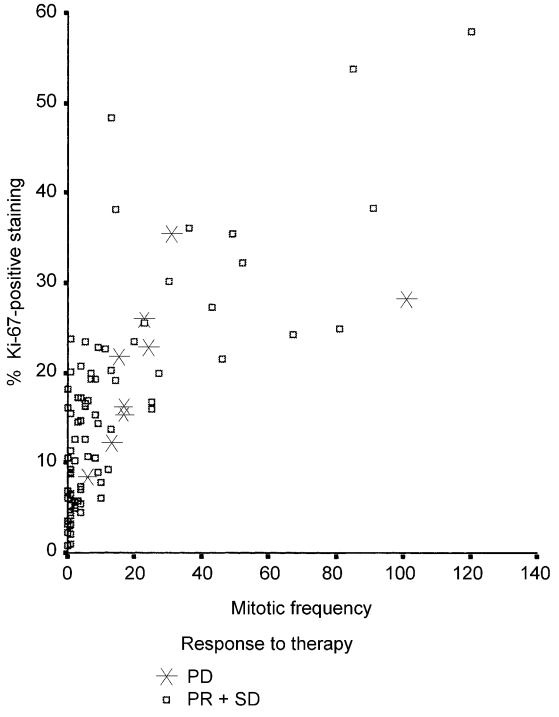

Fig. 1. Plot for Ki-67 and mitotic frequency for individual tumours subdivided according to response (PR + SD versus PD). PR, Partial Response; SD, Stable Disease; PD, Progressive Disease.

Table 2
Variables evaluated to identify parameters predicting response to chemotherapy

Variables	Number of patients		
	PR + SD	PD	*P* value
Age (years) ($n = 90$)			0.4[b]
< 64	44	4	
⩾ 64	37	5	
Tumour category ($n = 90$)			0.4[a]
T2	3		
T3	50	4	
T4	28	5	
N-category ($n = 90$)			0.5[a]
N0	28	2	
N1	31	3	
N2	22	4	
Tumour diameter ($n = 90$)			0.06[b]
< 68 mm	45	2	
⩾ 68 mm	36	7	
Histological grade ($n = 90$)			0.023[a]
Grade 1	22	1	
Grade 2	40	2	
Grade 3	19	6	
Mitotic frequency ($n = 90$)			0.001[b]
< 5	46	0	
⩾ 5	35	9	
Oestrogen receptor ($n = 88$)			0.1[b]
< 10 (negative)	15	4	
⩾ 10 (positive)	64	5	
Progesterone receptor ($n = 88$)			0.7[b]
< 10 (negative)	18	2	
⩾ 10 (positive)	61	7	
Ki-67 staining ($n = 89$)			0.23[b]
< 15.3	42	3	
⩾ 15.3	38	6	
TP53 mutations ($n = 90$)			0.008[a]
Wild-type	60	4	
Mutations not affecting L2/L3	7		
Mutations affecting L2/L3	14	5	
Bcl-2 expression ($n = 89$)			0.018[a]
Negative < 6	38	8	
Positive ⩾ 6	42	1	
c-erbB-2 expression ($n = 89$)			0.04[a]
Negative (0-1)	67	5	
Positive (2-3)	13	4	

Median values: age: 64 years; tumour size: 68 mm; Ki-67: 15.3; mitotic frequency: 5. Oestrogen and progesterone receptors are defined as positive if ⩾ 10. Evaluated by the Chi-square[a] or Fisher's Exact test[b] (see text). c: mutations affecting L2/L3 [11]. See text for Mann–Whitney U-test for individual values and for multivariate analysis.

with a low bcl-2-expression ($P=0.035$). Furthermore, there was a trend towards statistical significance between ER negativity and c-erB-2 positivity ($P=0.07$), and between ER negativity and high histological grade ($P=0.06$). A large tumour diameter was significantly associated with *TP53* mutations ($P=0.003$), high tumour grade ($P=0.03$) and low bcl-2 expression ($P=0.03$).

3.3. Predictive markers for doxorubicin resistance

Table 2 shows the associations between possible predictive variables for response to doxorubicin therapy for the 90 patients with evaluable disease and representative tumour samples. We found that a high mitotic frequency significantly predicted resistance to chemotherapy ($P=0.001$). All 9 patients with PD had a mitotic frequency higher than the median level. The median mitotic frequency for all non-responders (PD) was 17 (range 6–101), compared with 4 (range 0–120) for the rest of the patients (PR + SD).

When analysing tumours expressing mutated or wild-type *TP53* mutations separately, we found a significant correlation between mitotic frequency and resistance to doxorubicin only for tumours expressing wild-type *TP53* (tumours with a high mitotic frequency had an inferior response to therapy, $P=0.016$), while for tumours harbouring *TP53* mutations, no such correlation was found ($P=0.544$).

We previously [11,12] reported a significant association between high histological grade and doxorubicin resistance for the total group of patients ($P=0.023$); 6 out of 9 patients with PD had tumours with histological grade 3, compared with 11 out of 45 with SD and 8 out of 36 with PR. Assessing the subgroup of tumours with wild-type *TP53* separately, we found a significant correlation between treatment resistance and a high histological grade ($P=0.005$), while tumours with *TP53* mutations did not show such a correlation ($P=0.875$).

Using the Mann–Whitney U-test, there was a non-significant trend for an association between high values of Ki-67 expression and a lack of response to chemotherapy ($P=0.079$). Median Ki-67 expression for non-responders was 22% (range 8–36%), compared with 14% (range 0.75–58%) for responders. Subgroup analyses of tumour expressing wild-type or *TP53* mutations did not give any more information; none of the groups demonstrated any correlation between Ki-67 and resistance to doxorubicin.

We also observed an association between a large tumour diameter and resistance to doxorubicin (Mann–Whitney test, $P=0.005$). The median tumour diameter in non-responders was 90 mm, compared with 67 mm in responders. There was no association between age, T- or N-categories, steroid receptor expression and resistance to chemotherapy. However, when considering the subgroup of patients obtaining a partial response as a separate group, a positive ER status significantly predicted for a PR to doxorubicin ($P=0.008$); only 1 patient with a PR had an ER-negative tumour.

The predictive value of specific *TP53* mutations, erbB-2 positivity, and bcl-2 negativity to doxorubicin resistance in our patient material was recently reported in Ref. [11].

In the multivariate analyses (logistic regression entering all the 90 patients) mutations of *TP53* affecting the L2/L3 domain proved to be the only factor with a significant impact on the resistance to treatment ($P=0.02$). However, the analyses demonstrated a strong correlation between the various factors.

3.4. Correlation between biological markers and relapse-free and overall survival

Fig. 2 shows overall survival related to the response to chemotherapy. As expected, the group of patients with PD was significantly different from the other groups (SD and PR), and had a worse prognosis (see Fig. 2 legend for details). No significant difference between the PR and SD groups was observed.

Both relapse-free (RFS) and overall survival (OS) were significantly better for patients with a low tumour cell proliferation (Fig. 3a and b, see legend for details). In addition, patients with large primary tumours (diameter above median size of 68 mm), patients with ER-negative tumours and patients with PR-negative tumours had a worse prognosis (RFS: $P=0.008$, $P=0.003$ and $P=0.004$; OS: $P=0.003$, $P=0.0002$ and $P=0.04$, respectively). The correlations between RFS or

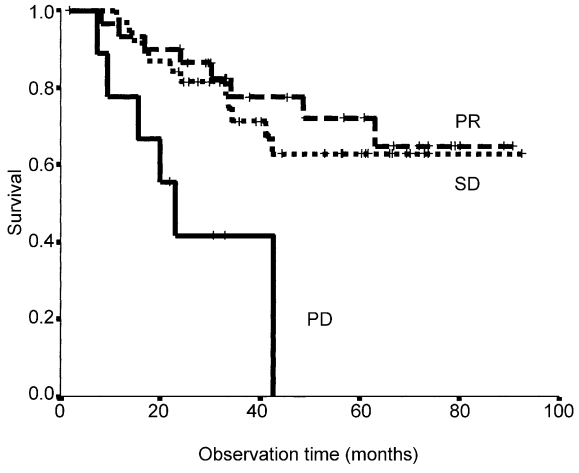

Fig. 2. Overall survival (OS) for patients with a PR, SD and PD during treatment with neoadjuvant chemotherapy. OS of the PD group compared with all other patients ($P=0.0004$), with the SD group ($P=0.003$) and with the PR group ($P=0.0009$). There was no significant difference in OS between the PR and SD group ($P=0.6$).

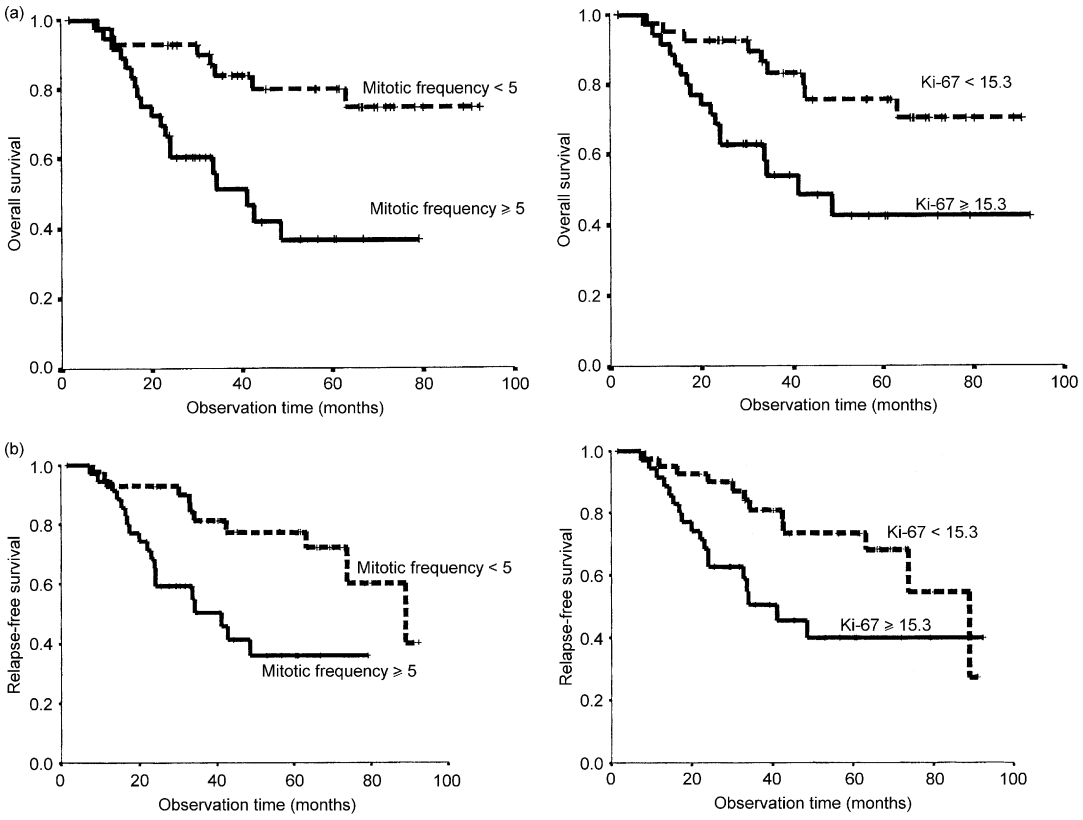

Fig. 3. Overall survival (a) and relapse-free survival (RFS) (b) for patients with a Ki-67-negative staining level $<15.3\%$ (median value) and patient with a Ki-67-positive staining level $\geqslant 15.3\%$ (RFS: $P=0.02$, and OS: $P=0.004$). Overall survival (a) and RFS (b) for patients with mitotic frequency <5 mitotic figures and $\geqslant 5$ mitotic figures per 10 high-powered fields (HPF)×400 (RFS: $P=0.002$, and OS: $P=0.004$).

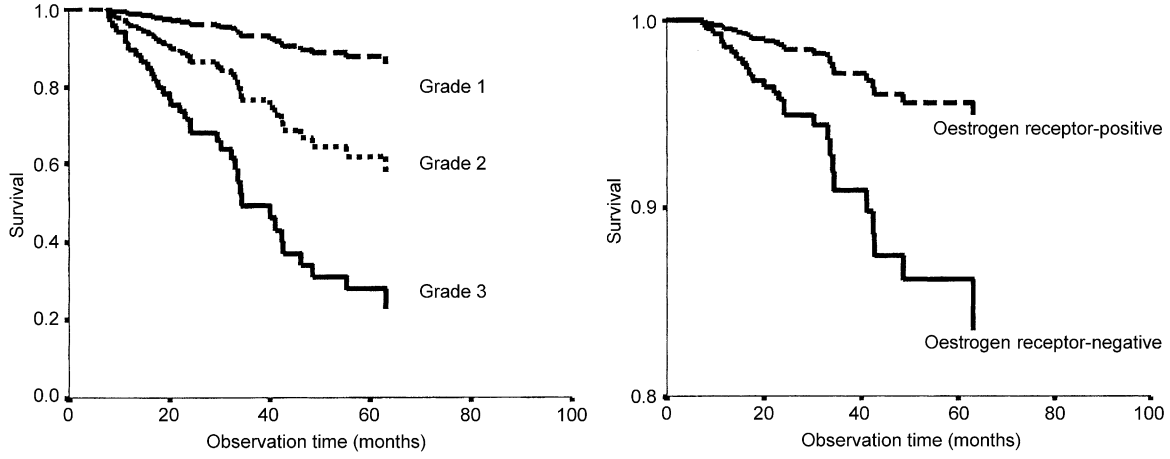

Fig. 4. Survival curves from Cox multivariate analyses with histological grade and oestrogen receptor status.

OS and *TP53* mutations, high histological tumour grade, c-erbB-2 positivity, and low bcl-2 in univariate analyses are given elsewhere in Ref. [11]. Neither tumour status (T_2, T_3, T_4) nor clinical node status (N_0, N_1, N_2) were associated with RFS or OS.

In multivariate survival analysis (Cox' proportional hazards regression method), high histological grade ($P=0.001$) and ER-negativity ($P=0.001$) were found to be independent prognostic factors (Table 3, Fig. 4).

Table 3
Cox proportional hazards regression of prognostic covariates in the survival model

Variables	β	Standard error	Hazard ratio	95% CI	P value
Histological grade	1.55	0.36	4.70	2.30–9.61	0.001
Oestrogen receptor positivity	−1.46	0.44	0.23	0.10–0.56	0.001

95% CI, 95% Confidence Interval.

4. Discussion

We previously focused on defects in the apoptotic pathways (*TP53* mutations) and proto-oncogene expression (c-erbB-2 overexpression) as potential causes of chemoresistance [10,11]. While both parameters correlated with a lack of response, they did not provide a full explanation for treatment failure as some patients with unfavourable alterations in these parameters responded to therapy, while some non-responders expressed wild-type *TP53* and lacked erbB-2 overexpression. This suggested that other mechanisms of modulating drug resistance are operating these tumours.

The cellular growth rate is thought to be important when considering the sensitivity to cytotoxic agents [2]. Moreover, genes involved in apoptosis, such as *TP53*, may interact with the cell cycle. Thus, we evaluated the predictive role of tumour cell proliferation by examining the mitotic frequency and Ki-67 positive staining, to look for any independent and potentially additive role for cell proliferation to drug sensitivity. We used a tumour population that we had previously evaluated for mutations in the *TP53* gene as well as for erbB-2 overexpression [11].

As previously reported by others [28–31], we found a significant association between increased tumour cell proliferation (high mitotic frequency, high expression of Ki-67) and mutations in *TP53*. Mutations in *TP53* might eliminate G1 as well as G2 arrest, possibly through its effect on downstream genes like *Gadd45* and p21 [33,34]. Interestingly the association between *TP53* mutations and high tumour cell proliferation was independent of the 'mutation subgroup'. This observation contrasts with our findings with respect to chemoresistance where mutations affecting the L2/L3 domain were found to be those associated with a lack of response to therapy [10,11]. It remains poorly understood whether *TP53* mutations are the primary cause of the increased proliferation or whether a rapid mitotic activity may facilitate mutations, thus further increasing the tumour growth rate.

Taking together the finding that *TP53* mutations were the factor with the strongest correlation to predict chemoresistance in the multivarate analysis, and the strong correlation between the high mitotic frequency and *TP53* mutations, this suggests that *TP53* mutations may be the primary cause of resistance and mitotic frequency is a co-variate. On the other hand, the finding of a significant association between mitotic frequency and doxorubicin resistance in the group of tumours harbouring wild-type *TP53*, but not in those harbouring *TP53* mutations, suggests other factors may be associated with cell proliferation and play a role in the drug resistant phenotype in a subgroup of tumours. Notably, the number of tumours with PD and wild-type *TP53* is small and it should be noted that a similar finding was not observed for Ki-67. Therefore, this observation has to be interpreted with care.

The role of tumour cell proliferation as a predictive factor for clinical response to chemotherapy is controversial. While some studies indicated that cytotoxic therapy was most effective against breast cancers with a high proliferation rate [35–39], most of the others were not able to confirm this [40–45]. Our results are surprising, in as much as they indicate a high proliferation rate to be associated with an inferior response. To our knowledge, only one previous study reports findings similar to ours, showing an association between low proliferation (grade 1 tumours) and response to chemotherapy [46]. Interestingly, in that study, patients with metastatic breast cancer were treated with combination chemotherapy (5-fluorouracil, epirubicin, cyclophosphamide (FEC)) in relatively low doses given once a week or every fourth week. The authors found the highest numbers of responders among patients with lower grade tumours, regardless of the treatment schedule. Whether this finding relates to 'low-dose' anthracycline regimens in general is uncertain. There is no general consensus regarding the impact of dose density or intensity on outcome in breast cancer, except for erbB-2-positive tumours where a dose–response relationship to anthracycline-containing chemotherapy in the normal dose range has been reported [47].

Our present findings indicate a highly significant association between reduced bcl-2 expression and increased mitotic counts and Ki-67 expression, as reported by others [48]. Furthermore, reduced bcl-2 expression was associated with other factors predicting a lack of clinical response, like a large tumour diameter, negative ER status and a high histological grade, *TP53* mutations, and overexpression of erbB-2 [11]. This is in general agreement with previous studies [30–32,49–51]. The association between a low expression of bcl-2 and lack of response to therapy might merely reflect co-expression of bcl-2 with other parameters influencing drug resistance.

The fact that the most important predictive factors for resistance to chemotherapy, *TP53* mutations, was not a significant prognostic factor with respect to RFS and OS, is interesting. While a lack of response to doxorubicin therapy (PD), was associated with *TP53* mutations as well as a poor outcome, only minor differences with regard to RFS and OS between those having a PR and SD were seen, and the differences were independent of the *TP53* status. An important contribution to this observation could be that all patients with ER-positive tumours were treated with adjuvant tamoxifen for 5 years following local therapy. However, this observation also underlines potential pitfalls of using survival and RFS as potential parameters of chemotherapy sensitivity, as discussed in detail elsewhere [14]. Our findings are also in accordance with the

results of others [52,53] who found a pathological complete response to be the only factor correlated to survival among patients treated with neoadjuvant chemotherapy.

In conclusion, we found an association between tumour cell proliferation markers and resistance to doxorubicin monotherapy in patients with locally advanced breast cancer. The finding that in a subgroup of tumours expressing wild-type *TP53,* drug resistance was highest in tumours expressing a 'high proliferation rate', was unexpected, and should promote further studies. However, assessing the influence of each parameter in a multivariate analysis confirmed *TP53* mutations as the strongest predictor for drug resistance and suggested that the other factors associated with resistance in the univariate analyses may be co-variates.

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