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Prognostic role of clinical, pathological and biological characteristics in patients with locally advanced breast cancer

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Summary Forty-two patients with clinical stage IIIA or IIIB breast cancer were treated with neoadjuvant chemotherapy followed by mastectomy and radiotherapy. The median follow-up was 32 months (range 10–72 months) and the median time to progression was 17 months (range 10–30 months). A multivariate analysis showed that a longer disease-free survival (DFS) was related to more chemotherapy cycles given ($P = 0.003$), a better pathological response to chemotherapy ($P = 0.04$) and fewer positive axillary lymph nodes ($P = 0.05$). A better overall survival (OS) was related to more chemotherapy cycles given ($P = 0.03$) and better pathological response to chemotherapy ($P = 0.04$). In patients with residual tumour after neoadjuvant chemotherapy, high levels of staining for Ki-67 was correlated with a worse DFS ($P = 0.008$). Other biological characteristics, including oestrogen receptor status, microvessel density (CD31 staining), P-glycoprotein (P-gp) staining and nuclear accumulation of p53, were not independent prognostic factors for either DFS or OS. If both P-gp and p53 were expressed, DFS and OS were worse in the uni- and multivariate analysis. The preliminary results of this phase II study suggest that coexpression of P-gp/p53 and a high level of staining for Ki-67 after chemotherapy are associated with a worse prognosis, and that prolonged neoadjuvant chemotherapy and the attainment of a pathological complete remission are important factors in determining outcome for patients with this disease.

Keywords: prognostic factor; locally advanced breast cancer; neoadjuvant chemotherapy

Neoadjuvant chemotherapy followed by either radiotherapy, surgery or both has improved the prognosis in patients with locally advanced breast cancer (LABC) (Hortobagyi et al, 1994). The many studies performed to date are, however, different in patient population studied, local therapy applied and chemotherapy scheme used. This makes it difficult to compare results and the optimal treatment scheme still has to be established. In stage I and II breast cancer, clinical and pathological variables have prognostic significance and are used as a guide for adjuvant therapies (Carter et al, 1989). Biological characteristics such as nuclear accumulation of mutant p53, microvessel density (MVD) and tumour cell proliferation are being used increasingly to further refine our ability to predict the prognosis of patients with early breast cancer (Weidner et al, 1991; Isola et al, 1992; Allred et al, 1993; Railo et al, 1993; Gasparini et al, 1994*a*). Recently, we reported that the expression of P-glycoprotein (P-gp), may be indicative of a worse prognosis in primary stage I–II breast cancer (Linn et al, 1995). Much less is known regarding these prognostic factors in LABC. This paper reports the preliminary results of a prognostic factor analysis on a group of women treated with neoadjuvant chemotherapy for LABC, incorporating both traditional and more recently developed factors.

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PATIENTS AND METHODS

Patients

Patients with stage IIIA and stage IIIB breast cancer according to the AJCC criteria (Beahrs et al, 1993) were enrolled into a study with neoadjuvant doxorubicin 90 mg m^{-2} and cyclophosphamide 1000 mg m^{-2} (Pinedo et al, 1996) and GM-CSF 250 μg m^{-2} (Honkoop et al, 1996). As established in a previous dose-finding study, a dose reduction of 10% relative to the previous dose level was applied in cycles 2 and 4 in every patient (Hoekman et al, 1991). Initially, it was the intention to give four to six cycles, dependent upon the rapidity of achieving a clinical complete or nearly complete remission, and the toxicity for the individual patient. When the study progressed and toxicity appeared tolerable, we aimed at the administration of six cycles whenever possible. This decision was based on the fact that gross residual tumour was often observed at pathological examination of the mastectomy specimens when fewer cycles were given. In 24 patients an incisional biopsy was performed for diagnosis. In 18 patients, referred from other hospitals, other diagnostic procedures were performed: a subclavicular biopsy in nine patients and a fine-needle aspiration in another nine patients (Honkoop et al, 1997). All patients underwent mastectomy according to Madden with axillary dissection, followed by radiotherapy (4005 cGy in 15 fractions to the thoracic wall, the internal mammary nodes, the axilla and the supraclavicular fossa). No adjuvant chemotherapy or hormones were applied.

Processing of the histological material

The fresh mastectomy specimens or biopsies were processed using standard pathological techniques with fixation in 4% buffered

Table 1 Antibodies used for immunohistochemical staining on paraffin slides

Antibody	Directed against	Source	Mono/polyclonal	Host species	Dilution
JSB-1	P-glycoprotein (P-gp)	Gift from Professor Dr RJ Scheper Amsterdam, the Netherlands	Monoclonal	Mouse	1:50
DO-7	Wild type p53	Dako, Glostrup, Denmark	Monoclonal	Mouse	1:500
Ki-67	Cells not in G_0	Dako, Glostrup, Denmark	Polyclonal	Rabbit	1:100
JC70	CD31	Dako, Glostrup, Denmark	Monoclonal	Mouse	1:40
ER	Oestrogen receptor	Abbott Diagnostics, Chicago, USA	Monoclonal	Mouse	1:1

Table 2 Survival of LABC ($n = 42$) by univariate analysis for prechemotherapy variables

Variable	n	DFS 2 years (%)	P (UV)	P (MV)	OS 2 years (%)	P (UV)	P (MV)
Age (years)							
≤ 46	19	77	0.12	*	90	0.09	0.1
> 46	23	58			68		
Tumour size (cm)							
≤ 9	22	70	0.15	*	88	0.3	*
> 9	20	58			80		
Stage							
IIIA	21	72	0.13	*	88	0.3	*
IIIB	21	60			80		
Oestrogen receptor							
(+)	14	60	0.5	*	96	0.08	0.3
(–)	25	55			72		
Cd31							
Low	12	68	0.2	*	90	0.8	*
High	9	78			100		
p53							
Low	10	68	0.8	*	92	0.7	*
High	17	64			82		
P-gp							
Low	9	60	0.8	*	100	0.9	*
High	18	64			88		
Ki-67							
Low	13	66	0.9	*	92	0.9	*
High	14	64			82		
P-gp/p53							
Positive	11	38	0.006	0.04	52	0.003	0.04
Negative	17	82			100		

*Not included in multivariate analysis. UV, univariate analysis; MV, multivariate analysis.

formaldehyde. Sections (4 μm thick) were cut and stained with haematoxylin and eosin (H&E). Antibodies used for immunohistochemical staining are listed in Table 1. Staining for Ki-67, p53, CD31 and P-gp was carried out on formalin-fixed, paraffin-embedded pre- and post-chemotherapy material. Staining for CD31 was only performed on prechemotherapy breast biopsies but not on infraclavicular biopsies. The avidin–biotin immunoperoxidase method (van der Valk et al, 1990; Linn et al, 1995) was used, and a microwave antigen retrieval technique was applied (Shi et al, 1991). Samples were considered positive for P-gp if ≥ 20% of tumour cells were stained (Schneider et al, 1989) and positive for p53 if at least 1% of tumour cell nuclei were stained with DO-7 (Thor et al, 1992). Oestrogen receptor staining was performed on frozen sections according to the manufacturer's protocol. The histoscore was applied and the receptor was considered positive when the score was >100 (Bosman et al, 1992). Microvessels were counted at 400 magnification using a 40× objective in one area (consisting of four fields, diameter 0.445 μm) with the highest MVD at low magnification ('hot spot') (Weidner et al, 1991).

Definition of pathological response

Pathological response was graded as complete (PCR) if no residual tumour was found in the mastectomy specimen or axillary lymph nodes; microscopic when macroscopic examination was normal but scattered foci of tumour were visible on microscopy (MPR); macroscopic when tumour was seen macroscopically; and diffuse when no tumour was seen microscopically but there was extensive infiltration on microscopic examination. Patients with PCR and MPR were regarded as one group having minimal residual disease (MRD), the other patients were regarded as having gross residual disease (GRD) (Honkoop et al, 1997).

Table 3 Survival of LABC ($n = 42$) by univariate analysis for post-chemotherapy factors

Variable	*n*	DFS 2 years (%)	P (UV)	P (MV)	OS 2 years (%)	P (UV)	P (MV)
Number of cycles							
≤ 4	5	0	0.0007[a]	0.003	32	0.009	0.03
5	13	55			73		
6	24	78			95		
Clinical response							
CR	21	64	0.8	*	90	0.7	*
PR	20	58			72		
SD	1	NR			NR		
Pathological response							
Minimal/no tumour	23	80	0.03	0.04	94	0.05	0.04
Gross residual tumour	19	45			68		
Axillary nodes							
(+)	24	54	0.05	0.05	70	0.14	*
(–)	18	80			94		
Axillary nodes							
0	18	80	0.02[a]	0.04	94	0.04	0.05
1–3	8	68			80		
4–9	13	48			62		
≥ 10	1	NR			NR		
Oestrogen receptor							
(+)	10	75	0.7	*	90	0.1	*
(–)	23	66			78		
CD31							
Low	17	60	0.7	*	82	0.8	*
High	14	68			79		
p53							
Low	15	52	0.5	*	84	0.17	*
High	16	48			64		
P-gp							
Low	13	54	0.8	*	84	0.5	*
High	17	56			74		
Ki-67							
Low	25	64	0.03	0.008	82	0.08	NS
High	5	20			58		
P-gp/p53							
Positive	12	23	0.03	0.05	40	0.008	0.04
Negative	16	70			100		

CR, complete remission; PR, partial remission; SD, stable disease; NR, not reliable due to only one patient in this group. [a]Trend test across the groups.

Prognostic factor analysis and statistics

Clinical and pathological variables included in prognostic significance analysis for disease-free survival (DFS) and overall survival (OS) are listed in Tables 2 and 3. For statistical analysis, grouping was performed using logical categories for the discrete variables. For continuous variables the cut-off was the median value (except for P-gp and p53 as mentioned above). Kaplan–Meier curves were plotted and differences analysed using the Mantel–Cox test. *P*-values below 0.05 were considered significant. Multivariate analysis of prognostic variables was performed using the Cox regression model with the limit to enter a variable in the analysis being set at $P \leq 0.1$. All tests were carried out with the Biomedical Package (BMDP, Statistical Solutions, Cork, Ireland).

RESULTS

The pretreatment characteristics of the patients are shown in Table 4. As per the initial protocol 18 patients received less than six cycles; five patients received four cycles and 13 patients received five cycles. Although the sample sizes were small for the different groups, there seemed to be no differences in the pretreatment characteristics of patients who received more than four cycles, five cycles or six cycles as in all groups stage IIIA and stage IIIB were equally represented (data not shown). The clinical response rate was 98% with 21 patients having a clinical complete response, 20 patients having a partial response and one patient with stable disease. Pathological and clinical response is depicted in Table 5. Fifteen patients have relapsed and eight patients have died with a median follow-up from start of therapy of 32 months (range 10–72 months). The median time to progression from start of therapy was 17 months (range 10–30 months). Figure 1 shows OS and DFS for the whole group of patients.

Univariate analysis

Table 2 and Table 3 show OS and DFS at 2 years for pre-chemotherapy and postchemotherapy variables respectively. Of

Table 4 Patient characteristics before treatment

Total number of patients	42
Age (years), median (range)	46 (26–63)
Clinical stage	
IIIA	21
IIIB	21
Inflammatory breast cancer	11
Tumour diameter (cm) median (range)	9 (5–15)
Axillary lymph node involvement (clinical)	35
Number of chemotherapy cycles	
≤ 4	5
5	13
6	24
Primary tumour histology[a]	
Ductal	27
Lobular	3
Medullary	1
Papillary/mucinous	1
Ductal/mucinous	1

[a]Nine cases only preoperative cytology.

Table 5 Clinical and pathological response

Pathological response	Number of patients	CCR	CPR	CSD
No residual tumour	6	5	1	
Minimal microscopic tumour[a]	17	11	6	
Diffuse microscopic tumour	6	1	5	
Macroscopic tumour	13	4	8	1
Axillary lymph nodes				
Negative	18	14	4	
1–3 positive	8	4	3	1
4–10 positive	15	3	12	
>10 positive	1	0	1	
Apical node positive	8	1	7	

[a]Three patients had only very few tumour cells in one lymph node. CCR, clinical complete response; CPR, clinical partial response; CSD, clinical stable disease.

the pretreatment factors only one variable appeared to predict either DFS and/or OS (co-expression of P-gp and p53, $P = 0.006$ for DFS and $P = 0.003$ for OS) and only two had a P-value ≤ 0.1 for OS (age, $P = 0.09$; oestrogen receptor, $P = 0.08$). An analysis of postchemotherapy variables revealed that patients who had received more chemotherapy cycles ($P = 0.0007$), those with MRD ($P = 0.03$), co-expression of P-gp/p53 ($P = 0.03$), low Ki-67 staining ($P = 0.03$), and negative axillary lymph nodes ($P = 0.05$) had a more favourable DFS, whereas only the first three factors were predictive for a better 2 year OS ($P = 0.009$, $P = 0.05$ and $P = 0.04$ respectively). There was also a significant trend for better DFS ($P = 0.02$) and better OS ($P = 0.04$) in patients with fewer positive lymph nodes at pathological examination. There was no difference in DFS and OS for patients with PCR compared with patients who had MPR, and these two factors were, therefore, combined in the prognostic factor analysis.

Multivariate analysis

The Cox regression analysis revealed the number of chemotherapy cycles ($P = 0.003$), pathological response ($P = 0.04$), co-expression of P-gp and p53 pre- and post-chemotherapy ($P = 0.04$ and $P = 0.05$ respectively) and lymph node status at pathological examination ($P = 0.05$) to be independent prognostic factors for DFS, whereas only the first three variables were independent predictors for OS ($P = 0.03$, $P = 0.04$ and $P = 0.04$ respectively). When multivariate analysis was carried out with the actual number of positive lymph nodes at pathological examination, this was an independent prognostic factor for DFS as well as for OS. A multivariate analysis was carried out both with and without Ki-67 staining post chemotherapy and coexpression of P-gp/p53 post chemotherapy because these factors were only measurable in patients with residual disease after chemotherapy. When included in this analysis, Ki-67 staining post-chemotherapy ($P = 0.008$), co-expression of P-gp/p53 post-chemotherapy ($P = 0.05$) and the number of chemotherapy cycles proved to be the most discriminant prognostic factors for DFS but the former was not an independent prognostic factor for OS.

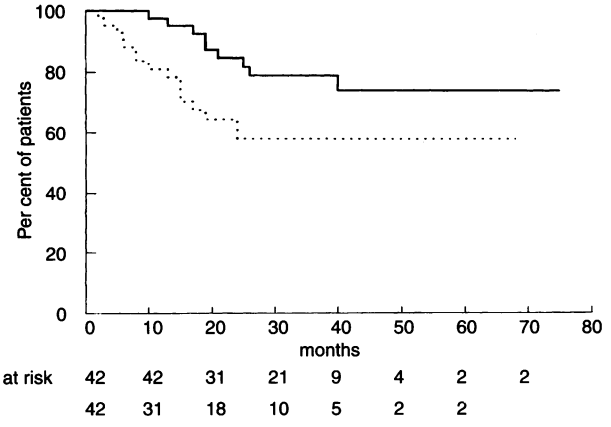

Figure 1 Overall survival (—) and disease-free survival (...) for all patients

DISCUSSION

The aim of this study was to identify clinical and biological factors with prognostic significance for DFS and OS in patients with LABC treated with a multidisciplinary approach. The follow-up time is approaching 3 years, and the median time to progression is 17 months, so we believe that this is long enough to allow sufficient events to have occurred to make a preliminary analysis of putative prognostic factors valid. An important prognostic factor was the number of chemotherapy cycles administered. Because the groups of patients receiving different numbers of cycles were not randomized, these results should be interpreted with caution, but the differences in OS and DFS were highly significant and this at least suggests that the duration of chemotherapy is important. This underlines the need for further investigation of optimal treatment duration in this disease setting. The magnitude of pathological response, and the presence of involved axillary lymph nodes at pathological examination were also important prognostic factors. Earlier, Feldman and colleagues (1986) reported on the prognostic significance of pathological response after neoadjuvant chemotherapy in LABC patients, whereas others (McGready et al, 1989; Gardin et al, 1995) have stressed the importance of lymph node metastases after neoadjuvant chemotherapy in LABC patients. Certainly, the attainment of a pathological complete remission is an important measure of the efficacy of neoadjuvant chemotherapy, as attested by data from patients with osteosarcoma (Rosen et al,

1982). Proliferation as measured by Ki-67 staining did not have prognostic significance in the pretreatment biopsies, which is in contrast to most studies in earlier breast cancer (Railo et al, 1993; Gasparini et al, 1994*b*). Patients with a higher proliferation after chemotherapy as measured by Ki-67 staining had shorter DFS and OS. This may indicate that this primary tumour variable reflects the proliferative capacity of the micrometastases that ultimately determines the prognosis. Other biological variables such as nuclear accumulation of p53 (Isola et al, 1992; Allred et al, 1993; Gasparini et al, 1994*a*; Rosen et al 1995), MVD (Weidner et al, 1991; Gasparini et al, 1994*a*) or P-gp staining (Linn et al, 1995), which have been suggested to be of importance in stages I and II breast cancer, were not significant in this study (P-values all > 0.1). Riou et al (1993) studied the prognostic significance of nuclear accumulation of p53 in 24 patients with inflammatory breast cancer, and they observed a worse prognosis for patients with nuclear accumulation of p53. Earlier we have reported that co-expression of P-gp and p53 were indicative of a worse prognosis in LABC patients (Linn et al, 1996), and this remained so in this slightly larger group of patients. Expression of one of these factors did not have prognostic significance. The failure of MVD to be of significance may well be related to the fact that these tumours are of a more advanced stage compared with stage I breast cancer.

In conclusion, biological markers such as p53, P-gp, MVD and Ki-67, determined in this small group of LABC patients treated with multidisciplinary therapy, did not seem to have the prognostic importance that they possess in early-stage breast cancer. The co-expression of P-gp and p53 was, however, predictive of a poor prognosis. It is possible that with a larger sample size significant differences might become apparent. It is, however, also possible that because of the advanced stage of these tumours micrometastases had already occurred in the majority, if not all, of these patients. In this situation, the outcome may well be determined by biological characteristics of tumour cells in the metastases and be less influenced by the characteristics of the primary tumour. Only with a larger series of patients would it be possible to resolve this question. Furthermore, it appears that attainment of a complete pathological response is of importance in this disease. The probability that this will occur might be enhanced by extending the duration of chemotherapy, as this was an important factor in this study. Alternatively, regimens with greater efficacy in breast cancer, such as those containing taxanes, vinorelbine or dose intensification, would be worth exploring in this setting. A larger study with longer follow-up is needed to confirm these conclusions.

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