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SERUM MARKERS AND PROGNOSIS IN LOCALLY ADVANCED BREAST CANCER

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Background: **Locally advanced breast cancer (LABC) represents a heterogeneous subgroup of breast cancer with an often dismal outcome. Identifying prognostic factors has acquired great significance for the selection of optimal treatment in individual patients.**
Methods: **Between January 1993 and December 1997, 103 patients were treated in our institution with multimodality treatment consisting of neoadjuvant chemotherapy followed by surgery, adjuvant chemotherapy and radiotherapy; tamoxifen was added in hormone receptor-positive cases. In the search for prognostic factors well-established parameters (clinical, pathological and treatment-related) as well as new features with potential value (c-erbB-2, baseline serum levels of CA 15.3 and CEA) were included in the univariate and multivariate analysis.**
Results: **At a median follow-up of 92 months (range, 8-130), the estimated five-year cancer-specific overall survival (OS) and disease-free survival (DFS) were 71.34% and 57.7%, respectively. Among the 22 different variables studied, only 10 were significantly correlated with OS and DFS. In multivariate analysis five retained independent prognostic value for both OS and DFS: tumor grade, serum markers, features of inflammatory breast cancer (IBC), response to neoadjuvant chemotherapy and lymph node status. With cutoff values of 35 U/mL for CA 15.3 and 5 ng/mL for CEA, the probability of five-year OS (Cox hazard ratio 3.91, *P* = 0.0009) and DFS (Cox hazard ratio 2.40, *P* = 0.02) decreased from 78% to 52% and from 68% to 47%, respectively, when at least one of these markers was abnormal.**
Conclusions: **Baseline serum levels of CEA and CA 15.3 emerged from this study as strong independent predictors of outcome in LABC, whose value adds to other established prognostic factors such as postoperative nodal status, IBC, histological grade and response to neoadjuvant chemotherapy.**

Key words: locally advanced breast cancer, prognostic factors, tumor markers.

Introduction

Locally advanced breast cancer (LABC) represents a heterogeneous group of tumors accounting for 10% to 30% of all breast carcinomas in Western countries[1]. Combined-modality treatment based on neoadjuvant chemotherapy followed by surgery and/or radiotherapy has improved its prognosis, with reported five-year disease-free survival (DFS) rates ranging between 30-70% and overall survival (OS) rates of 35-80%[2]. Nonetheless, the prognosis of this subgroup of patients remains dismal, showing no great survival benefit from the new multimodality approaches.

In order to define patient subgroups and select more appropriate treatment options, intensive efforts have been made to identify prognostic factors in LABC. In the adjuvant setting, clinical stage, number of involved axillary lymph nodes and hormone receptor status are currently the most determinant factors for treatment selection. The increasing use of neoadjuvant chemotherapy in LABC has introduced new features with their own prognostic meaning, such as the clinical and pathological response to chemotherapy, that may also change subsequent therapeutic decisions. Most data on neoadjuvant chemotherapy in breast cancer available from the literature are from single-institution studies where patient populations are often heterogeneous and response evaluation methods and chemotherapy schedules are also quite diverse.

Molecular characteristics (mainly HER2/neu and p53) have been widely assessed as prognostic factors in breast cancer but their potential role in neoadjuvant approaches in LABC has not yet been established. Biochemical factors such as the serum levels of tumor markers have been used mainly for response monitoring and follow-up, but there is little data on its up-front prognostic value, particularly in LABC.

We here present a prognostic factor analysis on a homogeneous group of 103 patients with LABC consecutively treated in our institution with uniform criteria during a period of five years.

Patients and methods

From all breast cancer patients treated in our institution between January 1993 and December 1997, we retrospectively selected a cohort that fulfilled the following criteria: cytologically or histologically confirmed stage II/III breast carcinoma, neoadjuvant chemotherapy given prior to surgery, minimum potential follow-up of five year since diagnosis, and histological tissue available for immunohistochemical analysis. All pa-

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tients were staged according to the UICC/AJCC classification.

Pretreatment evaluation

Patients had a detailed clinical history and physical examination. Diagnosis was usually obtained by biopsy. Steroid hormone receptor status was established by dextran-coated charcoal assay (threshold level for positivity 20 fmol/mg). Cytoprognostic grade was determined according to the Scarff-Bloom-Richardson histopathological grading system. HER2/neu status was determined by immunohistochemistry using the DAKO Herceptest® on paraffin-embedded tissue. Serum levels of CA 15.3 and carcinoembryonic antigen (CEA) were determined by enzymatic immunoanalysis (cutoff levels of 35 U/mL for CA 15.3 and 5 ng/mL for CEA). Patients were considered has having abnormal serum marker levels when at least one of them was abnormal.

Absence of clinical metastases was ascertained by a systematic workup including chest X-ray, bone scintigraphy, liver ultrasonography and bilateral mammography.

Treatment

Neoadjuvant chemotherapy was mostly based on anthracycline schedules. Different regimens were administered during these years: FEC-75 (epirubicin 75 mg/m^2, 5-FU 600 mg/m^2, cyclophosphamide 600 mg/m^2 all on day 1) given every 21 days; accelerated FEC-75 (an accelerated schedule giving the same doses every 14 days with filgrastim support); FEC-90 (FEC every 21 days with epirubicin 90 mg/m^2) and CMF (methotrexate 60 mg/m^2, 5-FU 600 mg/m^2, cyclophosphamide 600 mg/m^2 all on day 1, every 21 days). Independent of the scheme, four cycles were planned before surgery.

After primary treatment, the surgical procedure (modified radical mastectomy or conservative surgery) was chosen individually by the collaborating surgical team. Ipsilateral axillary node dissection was performed in all cases.

Postoperative treatment was planned individually, mainly based on the number of cycles administered during primary chemotherapy, the clinical-radiological response obtained, and the post-chemotherapy axillary status. Most patients were scheduled to receive two to three cycles of the same regimen they had received as primary treatment. The decision to recommend locoregional radiotherapy was based on axillary node status and extent of surgery. Tamoxifen was administered when the estrogen receptor (ER) or progesterone receptor (PgR) status was positive.

Evaluation of response

The clinical status of the patients was assessed before each chemotherapy cycle and before surgery as specified by the WHO guidelines. Mammography was performed at the beginning and at the end of induction treatment. Partial remission (PR) was defined as a greater than 50% reduction of the product of the largest perpendicular diameters of measurable lesions without the appearance of new lesions. Complete response (CR) was defined as complete disappearance of the initial tumor mass. Patients not fulfilling criteria for CR or PR and without evidence of increase in tumor size or new areas of involvement had stable disease (SD).

Pathological response was defined as complete when there was no evidence of residual tumor or only microscopic disease (invasive or intraductal), classified as minimal residual disease by Honkoop *et al.*[3]

Follow-up study

After completion of all treatment, patients were examined every three months for two years, every six months during the next three years and at least yearly thereafter. Clinical history and physical examination, blood count and serum chemistry including tumor markers were performed at every follow-up visit. Yearly mammography was mandatory.

Statistical analysis

Overall survival (OS) was defined as the time from diagnosis to death or last follow-up date. Disease-free survival (DFS) was defined as the time from the end of treatment to relapse (locoregional and/or distant metastases) or last follow-up date. Breast cancer-specific OS was defined as the time from diagnosis to death of breast cancer or last follow-up date. Follow-up time was defined only in alive patients as the time from the end of treatment to last follow-up date.

Estimates of OS and DFS were calculated from the date of diagnosis using the Kaplan-Meier method. Univariate comparisons of endpoints were made with the Mantel-Haentzel log-rank test, and a Cox proportional hazards model was used to estimate the hazard ratio of events by multivariate analysis, with a stepwise procedure and a P value to enter and remove variables from the model of 0.05 and 0.10, respectively. Since most authors agree that an increasing number of involved nodes has a linear effect on survival, the number of involved nodes, considered as a dichotomous variable in univariate analysis, was introduced as quantitative in the Cox model in order to preserve the broadest information. Taking advantage of the Cox regression model that allows the introduction of quantitative variables, we believe this results in a better understanding of the strength of the addition of each involved node on decreasing the probability of survival. Other models taking the number of nodes as a categorical variable in the Cox showed similar results. We have chosen the model that, as far as we know, best represents the specific weight of each variable. In all comparisons, whether in univariate or multivariate analysis, breast cancer-specific OS was the selected measure of survival, because it reflects more accurately the outcome in a study of prognostic factors, provided the existence of deaths not re-

lated to cancer. We thought that the risk of biased results would be greater if we had considered these deaths as events in the prognostic factors analysis.

Results

Patient characteristics

One hundred and nine consecutive breast cancer patients fulfilling the selection criteria of the study were treated with neoadjuvant chemotherapy at our hospital between January 1993 and December 1997. Six patients were excluded from the analysis because the available clinical data were incomplete. The median age of our patients was 55 years (range, 26-80). Table 1 summarizes their main characteristics.

Table 1 - Clinical characteristics at presentation

Characteristics	No. of patients (%)
Median age (years) (range)	55 (26-80)
Menopausal status	
Premenopausal	42 (40.8%)
Postmenopausal	61 (59.2%)
Family history	
Yes	29 (28.2%)
No	74 (71.8%)
Clinical T	
T1-2	29 (28.2%)
T3	35 (34%)
T4	39 (37.9%)
Clinical nodal status	
N0	31 (30.1%)
N1	38 (36.9%)
N2-3	34 (33%)
Clinical stage of disease	
IIA	7 (6.8%)
IIB	21(20.4%)
IIIA	36 (35%)
IIIB	39 (37.9%)
Cytoprognostic grade	
1	11(10.7%)
2	47 (45.6%)
3	36 (35%)
Not available	9 (8.7%)
Hormone receptor status	
ER- PgR-	53 (52%)
ER- PgR+	10 (9.8%)
ER+ PgR+	28 (27.5%)
ER+ PgR-	11 (10.8%)
Not available	1 (0.94%)
HER2/neu status	
+	12 (11.7%)
++	23 (22.3%)
+++	23 (22.3%)
Negative	42 (40.8%)
Not available	3 (2.9%)
CA 15.3 levels	*Median value: 20.4 ng/mL (range, 5.4-272)*
Normal (<35 ng/mL)	78 (75.7%)
Abnormal (>35 ng/mL)	24 (23.3%)
Not available	1 (1%)
CEA levels	*Median value: 1.6 ng/mL (range, 0.01-695)*
Normal (< 5 ng/mL)	77 (74.8%)
Abnormal (>5 ng/mL)	15 (14.6%)
Not available	11 (10.7%)
Inflammatory features	
Yes	8 (7.8%)
No	94 (91.3%)
Not available	1 (0.9%)
Vascular infiltration	
Yes	53 (51.5%)
No	49 (47.6%)
Not available	1 (1%)

Treatment

Patients received a median of four cycles (range, 2-6) of neoadjuvant chemotherapy. The schedules were distributed as follows: FEC-75 41 patients (39.8%), accelerated FEC-75 41 patients (39.8%), FEC-90 16 patients (15.5%) and CMF 5 patients (4.9%). The objective clinical response rate of the entire group was 68%, with 18.4% achieving clinical CR and 49.5% PR. Thirty-two percent presented SD or progression. All patients underwent surgery. In 27 patients (26.2%) a breast-conserving procedure was performed. Pathological examination of the primary tumor revealed a complete pathological response in 14 patients (13.6%), with 7 of them having microscopically invasive disease. The median number of sampled axillary lymph nodes was 22 (range, 4-58) with a median number of involved nodes of 4 (range, 0-57). None of the patients who achieved a complete pathological response in the breast had residual axillary lymph node involvement.

After surgery 100 patients (97.9%) received a median of four cycles (range, 0-7) of different schedules as adjuvant chemotherapy (82.5% FEC schedules). Twenty-six patients (25.3%) received also high-dose chemotherapy with autologous stem-cell support as part of an investigational protocol. Ninety-eight patients (95.1%) were treated with radiotherapy after completed chemotherapy. Fifty-six patients (57.3%) received adjuvant tamoxifen.

Overall and disease-free survival

At a median follow-up of 92 months (range, 68-130), 53 patients (51.5%) were alive with no evidence of disease and 2 patients were alive with recurrent disease (1.9%). Six patients (9.8%) had died without evidence of breast cancer (three after a cerebrovascular accident, 1 died from acute M4 myeloblastic leukemia, 1 died after advanced dementia and the last patient died from a second primary gastric tumor). Forty-two patients (40.8%) died of breast cancer. The estimated five-year overall survival (OS) was 67.9% (95% confidence interval, CI: 58.7-77.1). Median OS was 109 months. The estimated five-year breast cancer-specific OS was 71.34% (95% CI 62.3-80.3). The median specific OS was 112 months. The five-year disease-free survival (DFS) was 57.7% (95% CI 47.5-67.9).

Of the 47 women (45.6%) with disease recurrence, 2 presented only with locoregional relapse that was completely removed by salvage surgery and thereafter remained free of disease. Two further patients presented second primaries in the contralateral breast. One of them underwent surgical tumor removal and remained free of disease. Forty-four patients had systemic involvement; 14 of them showed both locoregional and distant metastases.

Prognostic factors

Univariate analysis

The univariate analysis of the pretreatment clinical prognostic factors is shown in Table 2. Three factors were significantly correlated with a poorer outcome (shorter OS and DFS): vascular invasion, elevated baseline CA 15.3, and features of inflammatory breast cancer (IBC). The latter variable had the strongest impact, decreasing the estimated five-year survival from 73% to 37%. Elevated baseline serum marker levels were associated with a decrease in estimated five-year OS from 78% to 52% (Figure 1). Some variables were only significantly correlated with either OS (grade) or DFS (age, stage).

The univariate analysis of treatment-related factors is shown in Table 3. All variables with a significant impact on OS and DFS were measures of response to neoadjuvant chemotherapy in different ways: presence of objective clinical response (Figure 2), presence of pathological residual disease, number of involved nodes (Figure 3) and type of surgery. Since patients were treated with different dose-intensity schedules of epirubicin, we analyzed factors related to chemotherapy dosing. None of them (number of cycles, total dose, dose intensity) proved to be significantly correlated with survival. The cutoff for the number of involved axillary lymph nodes clearly differentiating outcomes was nine. The estimated five-year OS decreased from 75% to 49%, and DFS from 30% to 66% when there were more than nine involved nodes. As we found a different behavior in the survival curves when patients were divided according to their hormone receptor status (survival plots crossing at the end of follow-up), univariate analysis with the Gehan-Breslow and Tarone-Ware tests was

Table 2 - Breast cancer-specific overall survival by pretreatment characteristics: univariate analysis

	5-year OS (%)	*P* value	5-year DFS (%)	*P* value
Age (years)				
<40	70	0.06	29	0.01
≥40	71		64	
Family history				
Yes	68	0.22	50	0.38
No	79		65	
Menopausal status				
premenopausal	78	0.40	59	0.67
postmenopausal	66		57	
Clinical T				
T1-2	80		72	
T3	61	0.08	61	0.08
T4	67		41	
Clinical nodal status				
N0	76		66	
N1	65	0.27	47	0.25
N2-3	72		63	
Stage				
II	82		74	
IIIA	66	0.10	58	0.03
IIIB	67		46	
Grade				
G1-2	78	0.02	64	0.06
G3	55		47	
Estrogen receptor				
Positive	81	0.28*	68	0.17°
Negative	64		51	
Progesterone receptor				
Positive	83	0.46**	64	0.32°°
Negative	63		54	
HER2/neu				
Positive	65	0.75	56	0.90
Negative	73		58	
Baseline marker levels				
Abnormal	52	0.007	43	0.02
Normal	78		64	
Vascular infiltration				
Yes	65	0.02	47	0.02
No	77		68	
Inflammatory features				
Yes	37	0.02	25	0.01
No	73		60	

*Gehan-Breslow *P* value 0.053; **Gehan-Breslow *P* value 0.047; °Gehan-Breslow *P* value 0.058; °°Gehan-Breslow *P* value 0.07.

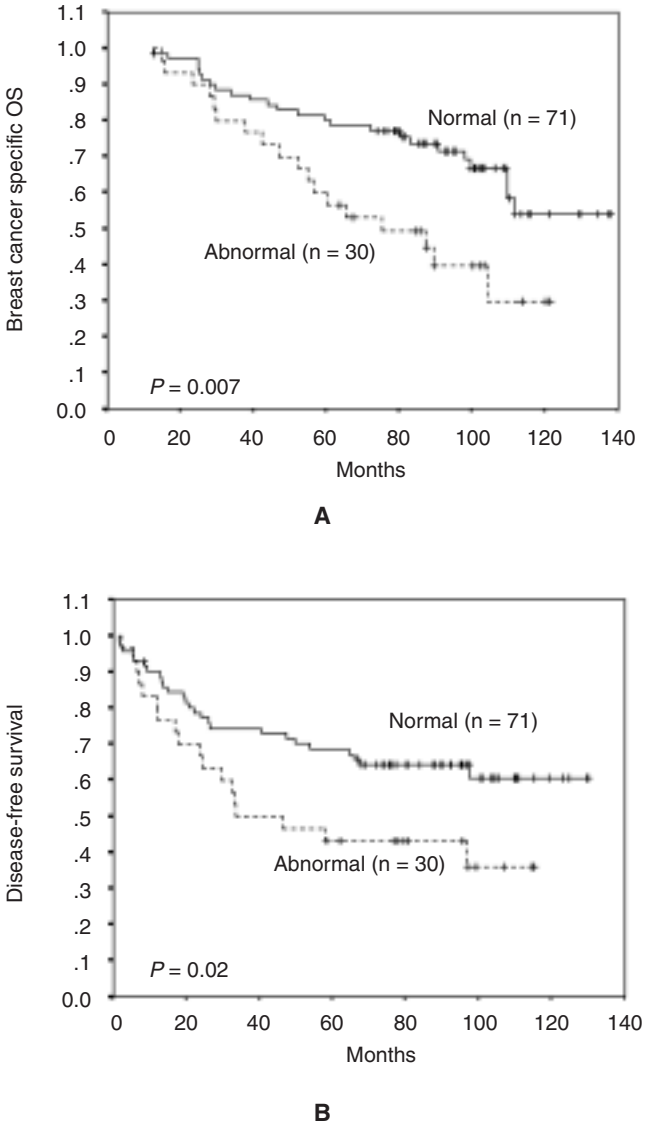

Figure 1 - Breast cancer-specific overall survival (A) and disease-free survival (B) by baseline tumor marker levels.

Table 3 - Breast cancer-specific overall survival by treatment-related factors: univariate analysis

	No.	5-year OS (%)	*P* value	5-year DFS (%)	*P* value
Neoadjuvant chemotherapy cycles					
≤3	34	76	0.78	55	0.74
>3	72	68		60	
Clinical response					
CR+PR	73	78	0.0002	69	0.003
Mr+SD+PD	33	57		35	
Pathological residual disease					
CR+microscopic	14	85	0.02	85	0.02
Macroscopic	92	69		54	
Pathological nodal status					
0	21	73	0.0001	85	0.0000
1-3	23	55		51	
4-9	30	47		37	
>9	32	19		22	
Surgery					
Mastectomy	79	66	0.006	50	0.004
Breast conserving	27	85		81	
Total CT cycles					
>6	24	69	0.23	59	0.36
≤6	79	68		45	
Total dose EPI					
>300 mg	35	71	0.60	59	0.68
< 300 mg	63	70		58	
Dose intensity					
>35 mg/week	48	72	0.98	56	0.80
<35 mg/week	49	70		62	

CR, complete response; PR, partial response; MR, minor response; SD, stable disease; CT, chemotherapy; EPI, epirubicin.

performed, which found borderline statistical significance (Figure 4).

Multivariate analysis

Factors with a significant or borderline impact on OS and/or DFS in univariate analysis were also introduced in a Cox model of multivariate analysis. Only clinical response and number of nodes were selected as related to the measurement of response to neoadjuvant chemotherapy because they correlated strongly with pathological response and type of surgery. Among all possible multivariate models, those depicted in Tables 4 and 5 were selected based on their clinical relevance and statistical power. According to this model, five factors independently seem to predict different outcomes (both OS and DFS): three of them were baseline characteristics (histological grade, elevated tumor marker levels and inflammatory features) and two were treatment-associated factors (clinical response to neoadjuvant chemotherapy and number of involved nodes).

Discussion

The study of prognostic factors for LABC treated with neoadjuvant chemotherapy has gained progressive relevance in the last decade. In this setting baseline biological factors can be combined with measures of response to chemotherapy, so features related to chemosensitivity and prognosis can be studied together. Neither the clinical baseline characteristics nor the survival results of our series differ essentially from those of most previous reports[4,5].

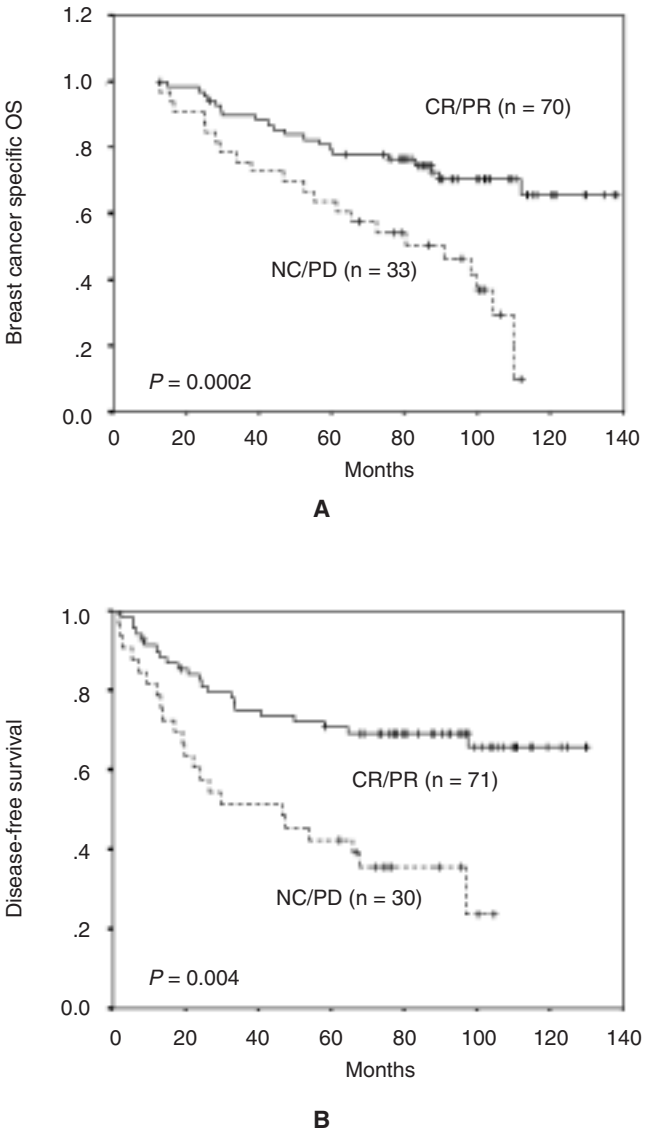

Figure 2 - Breast cancer-specific overall survival (A) and disease-free survival (B) by response to neoadjuvant chemotherapy.

The clinical characteristics, response to treatment and survival rates of our group of patients largely resemble those of previous reports on LABC[6-8]. Although we used different neoadjuvant chemotherapy schedules, more than 95% of the women received various types of FEC regimens. Rather than thinking that the use of different regimens could interfere with the analysis of prognostic factors, we believe it adds some variables such as total dose and dose intensity of epirubicin that may contribute to a more accurate analysis of the effect of chemotherapy on the prognosis of LABC.

We included in the univariate analysis most of the previously described clinical, histological and treatment-related variables that are currently considered to

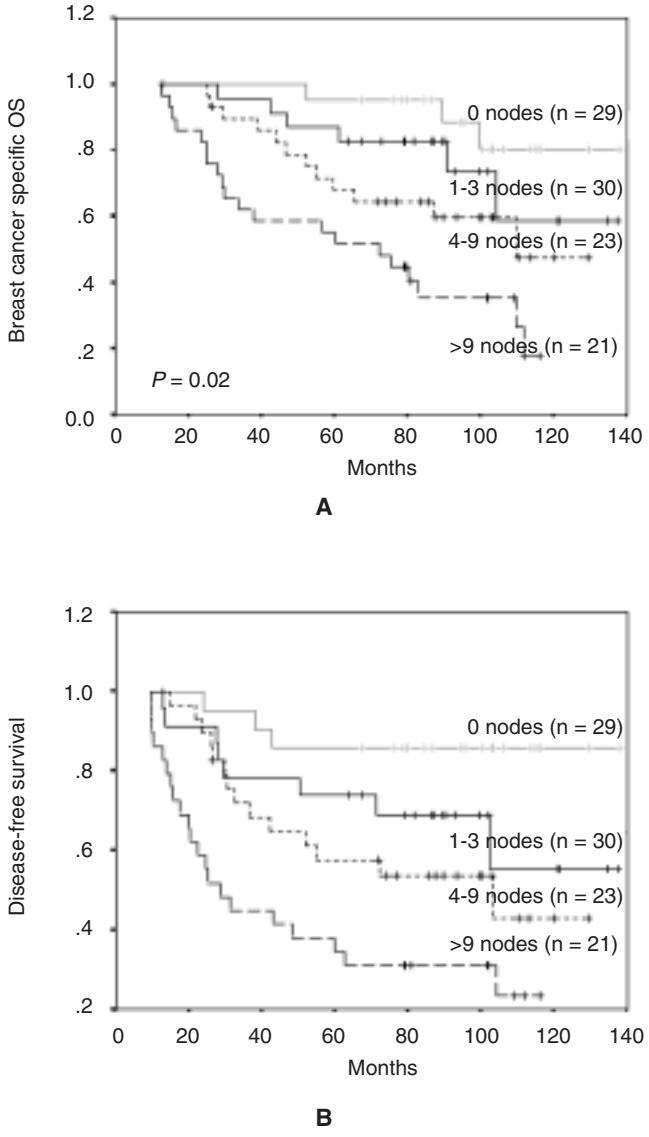

Figure 3 - Breast cancer-specific overall survival (A) and disease-free survival (B) by number of involved nodes.

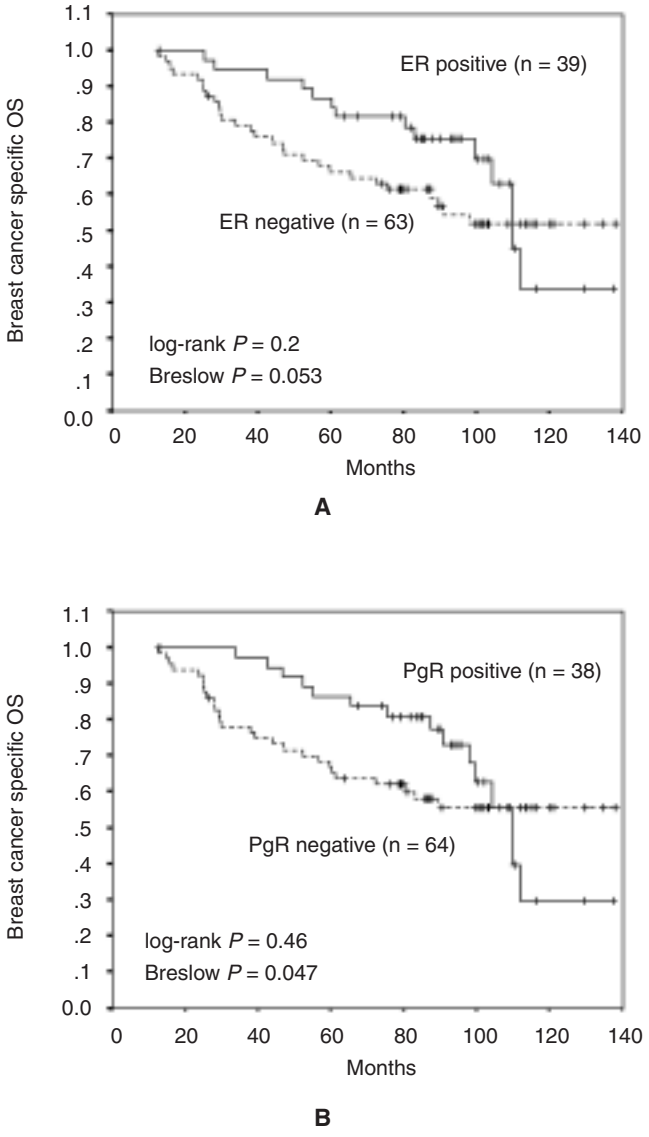

Figure 4 - Breast cancer-specific overall survival by hormone receptor status: estrogen receptor (A) and progesterone receptor (B).

be established prognostic factors in LABC. We observed statistical differences in OS and DFS that confirm previously reported results. Variables which retained their prognostic value in multivariate analysis were pathological nodal status, IBC, histological grade, response to neoadjuvant chemotherapy and baseline levels of serum biomarkers.

In order to preserve as much information as possible, the number of involved nodes was considered in the Cox model as a continuous variable. According to our results every added involved lymph node increased the risk of relapse by 6% and the risk of death of breast cancer by 5%. These data confirmed once more the value of pathological node status as the main prognostic factor also in LABC[9].

IBC constitutes an special entity with known aggressive behavior and a worse outcome. Our results also confirm the presence of inflammatory features as an independent prognostic factor for worse survival which, when present, decreased the five-year OS from 73% to 37%[10].

Histological grade is also a known independent prognostic factor in LABC and has been studied in populations quite similar to ours. According to our results the presence of grade III tumors increased the risk of death 2.5-fold and the risk of relapse twofold[11], although the latter only showed borderline significance.

Hormone receptor status has been described as a prognostic factor in breast cancer in several reports, although its impact on survival is relevant mainly in node-negative patients. Its prognostic value is time-dependent and decreases progressively with longer follow-up[12]. Some authors sustain the hypothesis that the

Table 4 - Multivariate analysis for breast cancer-specific overall survival

	P value	Risk ratio	95% CI
Number of involved nodes	0.0000	1.05	1.03-1.07
Response to neoadjuvant chemotherapy	0.0006	3.73	1.76-7.90
CA 15.3/CEA levels	0.0009	3.91	1.75-8.74
Inflammatory features	0.0037	6.09	1.79-20.67
Grade	0.014	2.57	1.20-5.48

Number of cases available, 90; number of events, 38; overall score, 47.51
Number of nodes introduced as quantitative variable.

Table 5 - Multivariate analysis for disease-free survival

	P value	Risk ratio	95%CI
Number of involved nodes	0.0000	1.06	1.03-1.08
Response to neoadjuvant chemotherapy	0.0006	3.49	1.70-7.16
CA 15.3/CEA levels	0.02	2.40	1.13-5.09
Inflammatory features	0.003	5.00	1.67-14.96
Grade	0.054	2.00	0.98-4.07

Number of cases available, 90; number of events, 40; overall score, 54.28
Number of nodes introduced as quantitative variable.

estrogen receptor could be responsible for late relapses, in relation to angiogenic mechanisms[13]. There is little data on this issue in multivariate analysis in LABC populations. As shown in our series, hormone receptor status only had prognostic relevance for survival during the early years of follow-up (as reflected in the significance in the Gehan-Breslow tests) (Figure 4). Given the time-dependent effect of hormone receptor status in univariate analysis, these variables were tested in multivariate models, which showed no evidence of independent prognostic relevance. However, we believe that studies with long follow-up periods such as ours will probably show that hormone receptor status has an important influence on late relapses. In the future this could make us change our follow-up strategies beyond five years in hormone receptor-positive patients.

CA 15.3 and CEA are the most widely studied serum markers in breast cancer. They appear to be useful tools for early detection of relapse during follow-up[14-16] and can serve as an adjuvant in monitoring response to treatment in advanced disease[17,18]. Several studies have found a correlation between elevated serum marker levels and other clinical parameters of aggressive biological behavior such as tumor stage, axillary node involvement, number and location of metastases, histological grade and hormone receptor status[19,20]. In stage III breast cancer abnormal levels of CA 15.3 have been found at diagnosis in 20% to 70% of patients[14].

Several studies aimed to assess the prognostic role of these tumor markers in breast cancer. Some of them included small groups of patients with short follow-up periods and only made univariate comparisons[21,22]. However, other studies that performed also multivariate analyses have been published recently (Table 6). Most of them focused on early breast cancer and evaluated the prognostic value of serum levels of CA 15.3 just before surgery. Three of these recent studies assessed the role of CEA together with CA 15.3[23-25]. To the best of our knowledge there is only one report on this marker combination in LABC treated with neoadjuvant chemotherapy[25].

Gion *et al.*[26] evaluated the prognostic value of serum CA 15.3 levels in 362 patients with node-negative primary breast cancer. They found an independent prognostic relationship when considering CA 15.3 levels as a continuous variable. Shering *et al.*[27] and Kumpulainen *et al.*[28] enrolled 368 and 272 patients, respectively. They both included node-negative and node-positive patients when evaluating this factor, which was shown to have independent prognostic value that was maintained in multivariate analysis. The cutoff points used in the last three studies based on statistical considerations (recursive partitioning technique) were close to 30 U/mL. All of them included patients at different disease stages and treated with therapeutic approaches in the adjuvant setting largely based on the authors' own practice.

Ebeling *et al.*[23] studied the prognostic value of CEA and CA 15.3 in 1046 breast cancer patients, 457 of

Table 6 - Reports on serum biomarkers as prognostic factors in breast cancer applying multivariate analysis

Authors	No.	Nodal status/Stage	Treatment	CA 15.3 cutoff (U/mL)	CEA cutoff (ng/mL)	Median follow-up (months)	Prognostic impact of baseline serum markers
Gion[26]	362	N (-)	S ± CT ± RT ± TAM	31	-	69	Yes
Shering[27]	368	184 N (+) 184 N (-)	S ± CT ± RT ± TAM	30	-	39	Yes
Ebeling[23]	1046	457 N (+) 558 N (-)	S ± CT ± RT ± TAM	25	2.5	36	No*
Kumpulainen[28]	272	All stages	S ± CT ± RT ± TAM	30	-	124	Yes
Canizares[24]	364	All stages	S ± CT ± RT ± TAM	40	6	-	No**
Molina[30]	503	All stages	S ± CT ± RT ± TAM	31	-	96	Yes°
Brenner[25]	104	LABC	NAC + S ± CT ± R ± TAM	35	5	57	Yes

*Decrease in marker levels during treatment had prognostic impact; **Positive in univariate analysis; °Positive only for CEA, CA 15.3 was significant only in univariate analysis; S ± CT ± RT ± TAM: Surgery ± adjuvant chemotherapy ± radiotherapy ± tamoxifen; NAC + S ± CT ± RT ± TAM: Neoadjuvant chemotherapy ± surgery ± adjuvant chemotherapy ± radiotherapy ± tamoxifen.

whom were node-positive. They reported an independent correlation between the percentage of decrease between pre- and postoperative serum CEA levels and survival. However, they did not observe any prognostic impact in multivariate analysis when baseline levels were analyzed as a prognostic factor. They selected lower cutoff points, choosing the 95% percentile for healthy individuals as described in previous reports[29].

Two other studies with 364 and 503 patients, respectively, have been published where both serum tumor markers were assessed in breast cancer[25,31]. Neither of them found a statistically significant relation between baseline levels of CA 15.3 and survival in multivariate analysis. Canizares *et al.*[24] used higher cutoff levels (CEA >6 ng/mL; CA 15.3 >40 U/mL). Molina *et al.*[30] did not find any independent prognostic impact when analyzing baseline levels of CEA.

Brenner *et al.*[25] analyzed as a single variable the prognostic value of baseline levels of CEA and CA 15.3 in 104 patients with LABC homogeneously treated with neoadjuvant chemotherapy, surgery, adjuvant chemotherapy, radiotherapy and hormonal treatment when indicated. The chosen cutoff levels of the serum markers were 35 U/mL for CA 15.3 and 5 ng/mL for CEA. When patients had abnormal levels of al least one of them, five-year survival was found to decrease from 76% to 45%, and this result was significant both in univariate and multivariate analysis, along with clinical tumor stage and pathological nodal status.

Our series shares several similarities with Brenner's study, confirming their results in another LABC population with different geographical features. According to our results, a woman diagnosed with LABC has an about four-fold increased risk of dying of breast cancer and an about 2.4-fold increased risk of relapse after multimodality treatment when her baseline levels of serum CA 15.3 are higher than 35 U/mL or her baseline CEA levels are higher than 5 ng/mL. We decided to choose the same cutoff levels as Brenner *et al.*[25] in order to be able to reproduce their results since our populations had similar clinical characteristics. In patients with abnormal serum marker levels the five-year disease-specific OS decreased from 78% to 52% and the five-year DFS from 64% to 43%. This corroborates the value of serum biomarkers as an independent prognostic factor in LABC. In this specific subgroup of patients the presence of micrometastatic disease is more likely than in any other stage of primary breast cancer. Serum biomarkers, especially CA 15.3, could be a sign of the presence of micrometastatic disease that cannot be detected by conventional staging procedures.

Moreover, our study has produced some additional data. First, the follow-up time of our series (median 92 months) was longer. Based on the characteristics of our series we were able to analyze the impact of different doses and dose intensities of chemotherapy in relation to the other prognostic variables. Since the received dose and dose intensity of epirubicin were analyzed patient by patient, we can conclude that they had no prognostic implications. Another specific feature of our series is that clinical objective response retained its independent prognostic impact on OS and DFS also in multivariate analysis. In fact, when there was a CR or PR, the five-year OS increased from 57% to 78% and DFS from 35% to 69% and the risk ratios were almost equivalent to those obtained with the serum markers. It must be pointed out that the possible interaction effect between response and other biological variables was analyzed and taken into account when the proposed model was made. Although we adopted similar response criteria to the ones used in Brenner's study, our results differed from theirs because the response to neoadjuvant chemotherapy was not found to be an independent prognostic factor in their study. However, this has been previously described in LABC[31,32]. Measurement of response in LABC is a rather complex issue and often the results cannot be reproduced in different institutions. Although some authors agree that pathological response is a better predictor of survival than clinical response[33], in our series clinical response predicted survival better than pathological response in multivariate analysis. Larger series will probably be needed to properly address this issue.

In conclusion, we found in a homogeneously treated population of LABC that, besides the well-known prognostic factors (nodal status, histological grade and IBC), additional variables may provide very useful information to predict outcome. Response to neoadjuvant chemotherapy and baseline serum biomarkers (CEA and CA 15.3) behaved as independent prognostic factors in LABC treated with neoadjuvant chemotherapy. Tests for serum biomarkers are objective, easy to reproduce, comparable and do not require tumor tissue. They are therefore useful tools to determine which patients are likely to have a worse outcome and should be routinely included in investigational protocols.

References

1. Valero V, Buzdar A, Hortobagyi G: Locally advanced breast cancer. Oncologist, 1: 8-17, 1996.
2. Hortobagyi GN, Singletary SE, Strom EA: Treatment of locally advanced and inflammatory breast cancer. In: Diseases of the breast, 2nd ed, Harris JR, Lippman ME, Morrow M, Osborne CK (Eds), pp 585-599, Lippincott, Williams & Wilkins, Philadelphia, 2000.
3. Honkoop AH, Pinedo HM, De Jong JS, Verheul HM, Linn SC, Hoekman K, Wagstaff J, Van Diest PJ: Effects of chemotherapy on pathologic and biologic characteristics of locally advanced breast cancer. Am J Clin Pathol, 107: 211-218, 1997.
4. Jacquillat C, Baillet F, Weil M Auclerc G, Housset M, Auclerc M, Sellami M, Jindani A, Thill L, Soubrane C: Results of a conservative treatment combining induction (neoadjuvant) and consolidation chemotherapy, hormonotherapy, and external and interstitial irradiation in 98 patients with locally advanced breast cancer (III-A, III-B). Cancer, 61: 1977-1982, 1988.
5. Fisher B, Maumonas EP: Preoperative chemotherapy: a mod-

el for studying the biology and therapy of primary breast cancer. J Clin Oncol, 13: 537-540, 1995.
6. Hortobagyi GN, Singletary SE, Strom EA: Treatment of locally advanced and inflammatory breast cancer. In: Diseases of the breast, 2nd ed, Harris JR, Lippman ME, Morrow M, Osborne CK (Eds), pp 645-668, Lippincott, Williams & Wilkins, Philadelphia, 2000.
7. Fisher B, Bryant J, Wolmark N, Mamounas E, Brown A, Fisher ER, Wickerham DL, Begovic M, DeCillis A, Robidoux A, Margolese RG, Cruz AB Jr, Hoehn JL, Lees AW, Dimitrov NV, Bear HD: Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. J Clin Oncol, 16: 2672-2685, 1998.
8. DeLena M, Varini M, Zucali R, Rovini D, Viganotti G, Valagussa P, Veronesi U, Bonadonna G: Multimodal treatment for locally advanced breast cancer: results of chemotherapy-radiotherapy versus chemotherapy-surgery. Cancer Clin Trials, 4: 229-236, 1981.
9. Early Breast Cancer Trialists' Collaborative Group: Systemic treatment of early breast cancer by hormonal, cytotoxic, or immune therapy. 133 randomised trials involving 31,000 recurrences and 24,000 deaths among 75,000 women. Lancet, 339: 71-85, 1992.
10. Koh EH, Buzdar AU, Ames FC, Singletary SE, McNeese MD, Frye D, Holmes FA, Fraschini G, Hug V, Theriault RL: Inflammatory carcinoma of the breast: results of combined modality approach: MD Anderson Cancer Center Experience. Cancer Chemother Pharmacol, 27: 94-100, 1990.
11. Brain E, Garrino C, Misset JL, Carbonero IG, Itzhaki M, Cvitkovik E, Goldschmidt E, Burki F, Regensberg C, Pappo E, Hagipantelli R, Musset M: Long-term prognostic and predictive factors in 107 stage II/III breast cancer patients treated with anthracycline-based neoadjuvant chemotherapy. Br J Cancer, 75: 1360-1367, 1997.
12. Hilsenbeck SG, Ravdin PM, De Moor CA, Chamness GC, Osborne CK, Clark GM: Time-dependence of hazard-ratios for prognostic factors in primary breast cancer. Breast Cancer Res Treat, 52: 227-237, 1998.
13. Coradini D, Daidone MG, Boracchi P, Biganzoli E, Oriana S, Bresciani G, Pellizzaro C, Tomasic G, Di Fronzo G, Marubini E: Time-dependent relevance of steroid receptors in breast cancer. J Clin Oncol, 18: 2702-2709, 2000.
14. Bon GG, Kenemans P, Yedema CA, Van Kamp G, Nigmen H, Hilgers I: Clinical relevance of the tumor marker Ca 15.3 in the management of cancer patients. In: From clone to clinic, Crommelin DJA, Schellekens H (Eds), pp 111-122, Kluwer Academic Publishers, Amsterdam, 1990.
15. Vizcarra E, Lluch A, Cibrian R, Jarque F, García Conde J: CA 15.3, CEA and TPA tumor markers in the early diagnosis of breast cancer relapse. Oncology, 51: 491-496, 1994.
16. Lamerz R: Role of tumour markers, cytogenetics. Ann Oncol, 10 (Suppl 4): 145-149, 1999.
17. Robertson JFR, Pearson D, Price MR, Selby C, Blamey RW, Howell A: Objective measurement of therapeutic response in breast cancer using tumor markers. Br J Cancer, 64: 757-763, 1991.
18. Tondini C, Hayes DF, Gelman R, Henderson IC, Kufe DW: Comparison of CA 15-3 and CEA in monitoring the clinical course of patients with metastasic breast cancer. Cancer Res, 48: 4107-4112, 1988.
19. Wojtacki J, Dziewulska-Bokiniec A, Kowalski DM, Zóltowska A, Ciesielski D, Suszko M: Pretreatment values of serum CA 15.3 antigen related to prognostic factors in breast cancer patients. Neoplasma, 43: 225-229,1996.
20. Colomer R, Ruibal A, Salvador L: Circulating tumor marker levels in advanced breast cancer correlate with the extent of metastasic disease. Cancer, 64: 1674-1681, 1989.
21. Kallioniemi OP, Oksa H, Aaran RK, Hietanen T, Lehtinen M, Koivula T: Serum CA 15-3 assay in the diagnosis and follow-up of breast cancer. Br J Cancer, 58: 213-215, 1988.
22. O'Hanlon DM, Kerin MJ, Kent P, Maher D, Grimes H, Given HF: An evaluation of preoperative CA 15-3 measurement in primary breast carcinoma. Br J Cancer, 71: 1288-1291, 1995.
23. Ebeling FC, Schmitt UM, Untch M, Nagel D, Fateh-Moghadam A, Stieber P, Seidel D: Tumour markers CEA and CA 15-3 as prognostic factors in breast cancer-univariate and multivariate analysis. Anticancer Res, 19: 2545-2550, 1999.
24. Canizares F, Sola J, Perez M, Tovar I, De Las Heras M, Salinas J, Penafiel R, Martinez P: Preoperative values of CA 15-3 and CEA as prognostic factors in breast cancer: a multivariate analysis. Tumour Biol, 22: 273-281, 2001.
25. Brenner B, Siris N, Rakowsky E, Fenig E, Sulkes A, Lurie H: Prediction of outcome in locally advanced breast cancer by post-chemotherapy nodal status and baseline serum tumour markers. Br J Cancer, 87: 1404-1410, 2002.
26. Gion M, Boracchi P, Dittadi R, Biganzoli E, Peloso L, Mione R, Gatti C, Paccagnella A, Marubini E: Prognostic role of serum CA15.3 in 362 node-negative breast cancers. An old player for a new game. Eur J Cancer, 38: 1181-1188, 2002.
27. Shering SG, Sherry F, McDermott EW, O'Higgins NJ, Duffy MJ: Preoperative CA 15-3 concentrations predict outcome of patients with breast carcinoma. Cancer, 83: 2521-2527, 1998.
28. Kumpulainen EJ, Keskikuru RJ, Johansson RT: Serum tumor marker CA 15.3 and stage are the two most powerful predictors of survival in primary breast cancer. Breast Cancer Res Treat, 76: 95-102, 2002.
29. Stieber P, Nagel D, Ritzke C, Rossler N, Kirch CM, Eierman W, Fateh-Moghadam A: Significance of bone alkaline phosphatase, CA 15-3 and CEA in the detection of bone metastases during the follow-up of patients suffering from breast carcinoma. Eur J Clin Chem Clin Biochem, 30: 809-814, 1992.
30. Molina R, Jo J, Filella X, Zanon G, Pahisa J, Monoz M, Latre ML, Pahisa J, Velasco M, Fernandez P, Estape J, Ballesta AM: C-erb-b2 oncoprotein, CEA and CA 15.3 in patients with breast cancer: prognostic value. Breast Cancer Res Treat, 51: 109-119, 1998.
31. Kuerer HM, Newman LA, Smith TL, Ames FC, Hunt KK, Dhingra K, Theriault RL, Singh G, Binkley SM, Sneige N, Buchholz TA, Ross MI, McNeese MD, Buzdar AU, Hortobagyi GN, Singletary SE: Clinical course of breast cancer patients with complete pathologic primary tumor and axillary lymph node response to doxorubicin-based neoadjuvant chemotherapy. J Clin Oncol, 17: 441-444, 1999.
32. Scholl SM, Pierga JY, Asselain B, Beuzeboc P, Dorval T, Garcia-Giralt E, Jouve M, Palangie T, Remvikos Y, Durand JC: Breast tumour response to primary chemotherapy predicts local and distant control as well as survival. Eur J Cancer, 31A: 1969-1975, 1995.
33. Honkoop AH, van Diest PJ, de Jong JS, Linn SC, Giaccone G, Hoekman K, Wagstaff J, Pinedo HM: Prognostic role of clinical, pathological and biological characteristics in patients with locally advanced breast cancer. Br J Cancer, 77: 621-626, 1998.