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CLINICAL TRIAL

Local recurrence after mastectomy for breast cancer: analysis of clinicopathological, biological and prognostic characteristics

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Abstract
Background Despite the increasing use of breast-conserving therapy, modified radical mastectomy retains an important role in primary as well as in salvage treatment of breast cancer. Nevertheless, a significant number of patients will eventually develop a local recurrence (LR).
Aims To identify the potential prognostic factors at the time of the first isolated LR, and to compare the expression of several parameters of the molecular biology of breast carcinomas by primary tumors and paired isolated LRs.
Methods We analyzed the medical records from 1,087 women who underwent mastectomy for breast cancer, out of which 98 developed LRs as the first manifestation of tumor progression. We investigated the prognostic value of various classical prognostic factors, at the time of mastectomy as well as when the diagnosis of LR was made. In addition, by using tissue microarrays and immunohistochemical techniques, we analyzed the expression of estrogen (ER), progesterone (PR) and androgen receptors (AR), ki67, p53, c-erbB-2 and apolipoprotein D in primary tumors and paired isolated LRs from a subset of patients ($n = 25$).
Results Patients who developed distant metastases as well as patients with local recurrent disease showed a significantly higher percentage of larger tumors, node-positive status and higher tumoral grade than patients without evidence of tumoral recurrence. Furthermore, patients with LR had a better outcome compared with those with distant metastases, although the former received less frequently adjuvant systemic therapy and/or radiotherapy. Tumor size, histological grade, ER and PR status, and a shorter disease-free interval (< 12 months) were significantly associated with overall survival amongst mastectomized patients that developed isolated LR. There was a significant concordance between primary tumors and LRs regarding the expression of the following factors: ER, PR and p53. However, we were not able to demonstrate similar findings for AR, c-erbB-2 and ki67. In addition, ER, PR and p53 status in the LRs were significantly associated with a poorer overall survival.
Conclusions Based on classical clinicopathological factors as well as on some new biological parameters we have been able to identify subgroups of mastectomized patients with LR differing in their prognosis. Thus, at the

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present time it would be possible to select group of patients candidates for further and individualized therapeutic strategies.

Keywords Breast cancer · Local recurrence · p53 · Estrogen receptors · Tissue microarrays

Introduction

Despite the increasing use of breast-conserving therapy, modified radical mastectomy remains an important surgical technique in primary as well as in salvage treatment for breast cancer. Nevertheless, despite the achievement of negative surgical margins, followed by systemic therapy and the appropriate use of postmastectomy radiation, 5–40% of patients will eventually develop a locoregional recurrence [1–7].

Locoregional recurrence following mastectomy is defined as the development of adenocarcinoma, confirmed by biopsy and similar to the initially resected tumor, in one or more of the following locations: skin, subcutaneous tissues or muscles of the ipsilateral chest wall, axilla, supraclavicular fossa or internal mammary chain. However, the prognosis varies with regard to these different locations. Thus, the poorer prognosis for patients with supraclavicular metastases confirms that recurrence in this location may be considered as distant disease [8, 9]. LR is the most frequent form of presentation for locoregional breast cancer recurrence after mastectomy, developing in about 10–18% of patients [10–12]. Experimental studies have shown a preference for tumor cells to grow in scar tissue, being either surgical scars [13–17] or radiated tissues [18, 19]. Presumably, scar tissue is a preferential site for circulating tumor cells to lodge and grow. Known parameters of the primary tumors directly related with the development of LR are: tumor size, nodal status, estrogen receptor status and tumor grade [5, 6, 8, 9, 20–25].

The basic principle of the treatment of LRs consists of complete excision of gross disease followed by local radiation [2], while adjuvant hormonal therapies are often used in the treatment of primary tumors, and chemotherapy being generally reserved for advanced disease. However, isolated LR is very often associated with distant metastases [26] while survival after a postmastectomy local recurrence (LR) is diagnosed remains very poor, with 5-year survival rates ranging from 40% to 70% and disease-free survival ranging from 13% to 50% [27–32]. Hence, it would be extremely helpful to be able to identify breast cancer patients at a higher risk of loco-regional recurrence and therefore most likely to benefit from a more aggressive treatment, such as postmastectomy radiotherapy.

The aims of the present study performed on women who had undergone mastectomy for breast cancer were: (i) to analyze the differences between characteristics of the primary tumor in patients who developed a local lesion as the first event of tumoral recurrence, in patients without evidence of tumoral recurrence, and in patients whose first sign of disease recurrence is the appearance of distant metastases; (ii) to identify the possible prognostic factors related to time to the first isolated LR; and (iii) to compare the expression between primary tumors and paired isolated LRs of several parameters of the molecular biology of breast carcinomas.

Materials and methods

Patient characteristics

We analyzed the records of 1,087 women who were treated of breast cancer at Hospital de Jove (Gijón, Spain), Hospital de Cabueñes (Gijón, Spain) and at Hospital Central de Asturias (Oviedo, Spain), between 1990 and 2002. All patients underwent a modified radical mastectomy. Three groups of patients were considered: 98 patients with a first isolated LR of breast cancer following primary mastectomy, 143 patients who develop distant metastases as the first event of tumor recurrence, and 846 patients without evidence of recurrence. Patients with concomitant distant metastases at the time of the initial diagnosis were excluded from the study. The median age was 61 years (range, 27–92 years). None of the patients showed evidence of any other malignant tumor at the time of diagnosis. Patient characteristics with respect to age, menopausal status, clinical tumoral stage, histological grade, hormonal receptor status, adjuvant radiotherapy and/or systemic therapy, are listed in Table 1. Histological grade was determined according to criteria reported by Bloom and Richardson, whereas nodal status was assessed histopathologically. LR was defined as any reappearance of tumor on the ipsilateral chest wall or mastectomy scar.

Patients underwent a modified radical mastectomy with level I and II axillary lymph nodes dissection. Postoperative radiotherapy was given to 311 patients (28.6%). The criteria for systemic adjuvant therapy were as follows: (i) node-negative patients with ER and/or PgR positive tumors received tamoxifen (20 mg per day during 5 years); (ii) node-negative patients with ER and PR negative tumors received six cycles of

Table 1 Breast carcinoma patients: demographic and tumor characteristics

Patient and tumor characteristics Total	LR 98	Distant metastasis 143	No. recurrence 846	*P*
Menopausal status				**0.459**
Premenopausal	32 (32.7)	38 (26.6)	230 (27.2)	
Postmenopausal	66 (67.3)	105 (73.4)	616 (72.8)	
Size				**0.0001**
T1	13 (13.3)	30 (21)	385 (45.5)	
T2	71 (72.4)	68 (47.6)	349 (41.3)	
T3	4 (4.1)	21 (14.7)	56 (6.6)	
T4	10 (10.2)	24 (16.8)	56 (6.6)	
Nodal status				**0.0001**
N(–)	43 (44.3)	42 (29.4)	539 (63.7)	
N(+)	54 (54)	99 (69.2)	295 (34.9)	
Unknown	1 (1)	2 (1.4)	12 (1.4)	
Histological grade				**0.0001**
Well Dif.	8 (8.2)	17 (11.9)	239 (28.3)	
Mod. Dif.	38 (38.8)	70 (49)	383 (45.3)	
Poorly Dif.	21 (21.4)	42 (29.4)	162 (191)	
Unknown	31 (31.6)	14 (9.8)	62 (7.3)	
ER				**0.039**
Negative	28 (28.6)	67 (46.9)	297 (35.1)	
Positive	44 (44.9)	56 (39.2)	394 (46.6)	
Unknown	26 (26.5)	20 (14)	155 (18.3)	
PR				**0.219**
Negative	35 (35.7)	73 (51)	369 (43.6)	
Positive	26 (26.5)	40 (28)	292 (34.5)	
Unknown	37 (37.8)	30 (21)	185 (21.9)	
Radiotherapy				**0.0001**
Yes	21 (21.4)	72 (50.3)	235 (27.8)	
No	77 (78.6)	71 (49.7)	611 (72.2)	
Chemotherapy				**0.0001**
Yes	34 (34.7)	89 (62.2)	346 (40.9)	
No	64 (65.3)	54 (37.8)	500 (59.1)	
Tamoxifen				**0.027**
Yes	29 (29.6)	57 (39.9)	368 (43.5)	
No	69 (70.4)	86 (60.1)	478 (56.5)	

intravenous CMF (cyclophosphamide, methotrexate and 5-fluorouracil) every 3 weeks, if their tumors were either larger than one centimeter, moderately or poorly differentiated, or if patients were younger than 35 years old; (iii) node-positive patients received six cycles of intravenous FEC (5-fluorouracil, epirubicin and cyclophosphamide) every 3 weeks, plus sequential tamoxifen if they had ER and/or PgR-positive tumors. Overall, 431 patients received chemotherapy, 434 patients received tamoxifen, and 107 patients received both types of systemic therapy.

All patients were followed for disease recurrence and survival status by clinical and biochemical studies every 3 months for the first 2 years and then yearly. Radiological studies were performed yearly, or when considered necessary. The median follow-up period was of 39 months (range, 6–217 months) for patients with LR, of 83 months (range, 12–301 months) for patients with distant metastasis as the first event of tumoral progression, and of 44 months (range, 12–245 months) for patients without tumoral recurrence. The end-point was death secondary to tumor progression. Two hundred and forty one (22.1%) out of the 1,087 patients developed tumor recurrence, and 116 died from it.

Breast carcinoma tissue samples were obtained at the time of surgery. Immediately after surgical resection, samples were processed for pathological examination while the remainder tissue was washed with cold saline solution, divided in aliquots, rapidly transported on ice to the laboratory and stored at –70°C pending biochemical studies. Tissue samples were obtained prior informed consent from the patients. Estrogen (ER) and progesterone (PR) receptor measurements were

performed on cytosol extracts by using a solid phase enzyme immunoassay based on the ''sandwich'' principle (ER-EIA and PR-EIA from Abbot Laboratories, Diagnostics Division, Wiesbaden, Germany). ER and PR values were expressed as fentomols per milligram of protein. Protein concentration was quantified according to the elsewhere described Bradford method. For data analysis, a value higher than 10 fmol/mg total protein was considered as positive for ER and PR.

Tissue microarrays

Specimens from the primary tumors as well as from their corresponding recurrences, obtained at the time of diagnosis, were obtained from 25 out of the 98 patients included in this study. Routinely fixed (performed overnight in 10% buffered formalin), paraffin-embedded tumor samples stored in the files of our Pathology laboratories were examined. Histopathologically representative tumor areas were defined from the haematoxylin and eosin-stained sections and marked on the slide. Tumor tissue array blocks were obtained by punching a tissue cylinder (core) with a diameter of 1 mm through a histologically representative area of each ''donor'' tumor block, wich was then inserted into an empty ''recipient'' tissue array paraffin block using a manual arrayer (Beecher Instruments, Sun Prairie, Wisconsin, USA). Two cores (double redundancy) were employed for each case, as this method has been shown to correlate well with conventional immunohistochemical staining [33]. From the 50 tumor samples available, 2 tissue array blocks were prepared each containing 25 primary and secondary tumors samples. Five-millimeter sections were cut and processed for immunohistochemistry. Consecutive sections of each sample from primary tumors and paired isolated LRs were used for ER, PR, AR, ki67, p53, c-erbB-2 and apolipoprotein D determination.

Immunohistochemical assays

A Dako TechMate TM50 autostainer (Dako, Glostrup, Denmark) was used to immunostain 5 μm thick sections that were then incubated with the following (all from Dako): mouse anti-ER clone 1D5, anti-PR clone PgR 636, anti-AR clone AR 441at a dilution of 1/50, anti-Ki67 clone MIB-1 at a dilution of 1/50, anti-p53 clone DO-7 at a dilution of 1/75, rabbit policlonal anti-HER-2/neu oncoprotein at a dilution of 1/250, and anti-Apo-D clone 8CD6 (Signet, Dedham, MA, USA) at a dilution of 1/50; all the dilutions were made in Antibody Diluent, (Dako) for 30 min at room temperature.

To enhance antigen retrival all the antibody sections were microwave-treated in citrate buffer (Citra Plus Solution, BioGenex, USA) at 99°C for 15 min. Endogenous peroxidase activity was bloqued by incubating the slides in peroxidase-blocking solution (Dako ChemMate^{TM}) for 5 min. EnVision^{TM} Detection kit, peroxidase/DAB was used as the detection staining system. The sections were counterstained with haematoxilin, dehydrated with ethanol, and permanently coverslipped. After the staining was completed the slides were evaluated by two pathologists. For each tumor, the mean of score of two different core biopsies was calculated.

Staining of the TMA sections with antibody against Apo D was scored semiquantitatively taking into account both the intensity of the staining (1: diffuse, weak immunoreactivity; 2: intermediate intensity staining; 3: strong intensity staining of the tumor cell) and the proportion of the cells stained (percentage).

Staining for ERs, PgRs and AR was scored according to the method described by Allred et al. [34], and c-erbB-2 staining according to the criteria used for the Herceptest. Ki-67 (MIB-1) was assessed by the number of positively stained nuclei, with a greater than 10% of cells staining indicating a positive result. The p53 was assessed by the number of positively stained nuclei, with greater than 25% of cells staining indicating a positive result. Controls included breast cancer tissue with known immunoreactivity for each antibody used in the study. Negative controls had the primary antibody omitted and replaced by Antibody Diluent (Dako, Glostrup, Denmark).

Statistical analysis

Differences in percentages were calculated with the chi-square test. Probabilities of survival were calculated with the Kaplan–Meier method. Differences between curves were evaluated with the log rank test. To establish a cut-off point in order to convert the time interval to the appearance of the first LR into a categorical variable by combining patients into two groups, we followed the ''minimum *P*-value approach''. The Cox's regression model was also used to examine several combinations and interactions of different prognostic factors in a multivariate analysis. The following variables were considered in this analysis: age, menopausal status, tumor size, nodal status, histological grade, adjuvant radiotherapy and adjuvant systemic therapy. In the multivariate analysis only parameters that achieve statistical significance for relapse-free survival or overall survival in the log rank test were included. The SPSS 11.5 program was used for all

calculations. Statistical significance was considered at 5% probability level ($P < 0.05$).

Results

Patients with LR, distant metastases and patients without recurrence had a mean age at the time of surgery for their primary tumors of 57.7, 58.9 and 60.6 years, respectively. Table 1 shows both patients' and tumors' characteristics in the group of patients with isolated LR, with distant metastases as the first event of tumor recurrence and in the group of patients without tumoral recurrence. As it can be seen in this table, there are significant differences in the percentage distribution according to tumor size, nodal status, histological grade or ER status. Thus, patients who developed distant metastases and patients with LR showed higher percentage of tumors of large size ($P = 0.0001$), node-positive status ($P = 0.0001$) and higher tumoral grade ($P = 0.0001$) compared with patients without any evidence of tumoral recurrence. However, patients who develop LR had a lower percentage of ER-negative tumors then either patients with distant metastases or patients without recurrence ($P = 0.039$). Moreover, there were also significant differences between groups according to the adjuvant therapy received. Thus, patients with distant metastasis received more frequently, at the time of the initial treatment, either adjuvant radiotherapy or chemotherapy, whereas patients who developed LR received less frequently any of the three types of adjuvant therapy (Table 1).

Figure 1 shows the distribution of patients with LR with regard to the disease-free interval after the initial mastectomy. As it can be seen, there was a wide variability in the time of recurrence presentation, being the mean time (± standard error) of 48 ± 4.3 months (range, 3–256 months). A total of 15 patients (15.3%) developed LR during the first year after mastectomy, 19 (19.4%) during the second year, 18 (18.4%) during the third year, 7 (7.2%) during the fourth year, and the remainder 39 patients (39.8%) past the fourth postoperative year.

In the present study patients were analyzed during a prolonged follow-up period. First, we compared the overall survival curves determined for patients with LR and for patients with distant metastases, in both cases being the first event indicating tumoral progression after mastectomy. As it can be seen in Fig. 2, there were significant differences between the two survival curves for those two groups ($P < 0.00001$), showing a better outcome for those patients with LR. Second, we

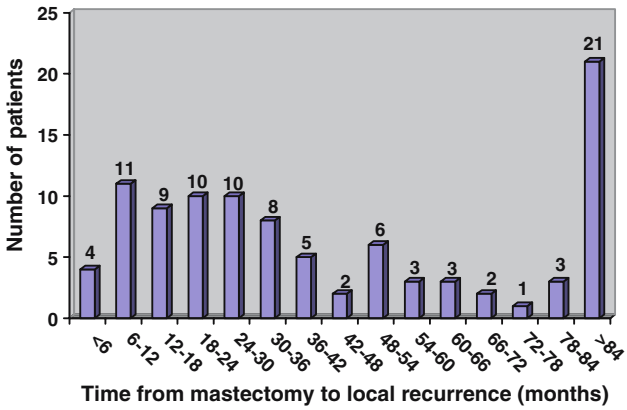

Fig. 1 Distribution of patients with local recurrence after mastectomy as a function of the length of the disease-free interval

analyzed the classical factors possibly associated with prognosis in this latter group of patients. Table 2 shows the relationship between the clinical-pathological parameters of primary tumors and relapse-free survival after mastectomy and also overall survival in patients with LR. As can be seen in this table, tumor size, histological grade, ER and PR status, adjuvant radiotherapy and adjuvant tamoxifen, were classical factors of breast cancer significantly associated with both relapse-free and overall survival after mastectomy in the patients who develop isolated LR.

To examine the possibility that those prognostic factors determined by the recurrence itself could more accurately predict patients' outcome, we analyzed the

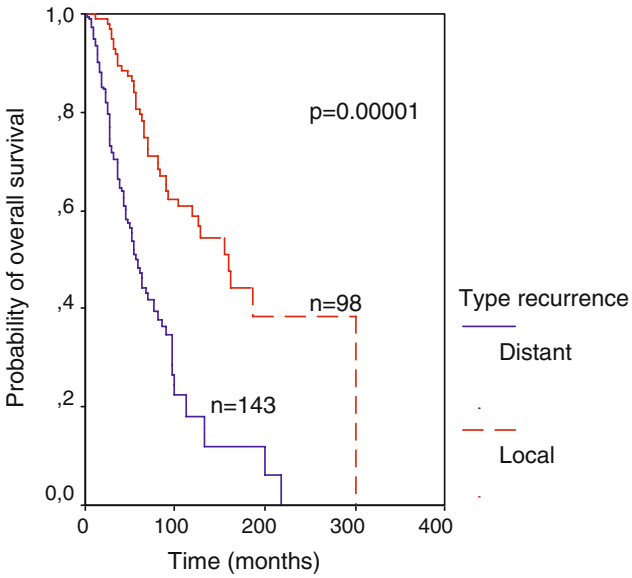

Fig. 2 Overall survival as a function of the type or tumoral recurrence (local or distant metastasis) after mastectomy in patients with breast carcinoma

Table 2 Univariate analysis of the relationship between the clinicopathological and biological characteristics of primary tumors and both relapse-free and overall survival in patients with LR after mastectomy

Patient and tumor characteristics	No	Relapse-free survival			Overall survival		
	98	2 years % ± ES	8 years % ± ES	*P*	2 years % ± ES	8 years % ± ES	*P*
Menopausal status				**0.6841**			**0.3321**
Premenopausal	32	59 ± 8	12 ± 5		75 ± 8	0	
Postmenopausal	66	66 ± 5	12 ± 4		82 ± 4	51 ± 7	
Tumor size				**0.0004**			0.0024
T1	13	76 ± 11	0		–	–	
T2	71	64 ± 5	16 ± 4		74 ± 5	35 ± 11	
T3	4	50 ± 25	0		-	50 ± 35	
T4	10	30 ± 14	0		80 ± 12	0	
Nodal status				**0.0007**			0.1635
N (–)	43	71 ± 6	45 ± 9		80 ± 6	44 ± 12	
N (+)	55	33 ± 6	22 ± 6		79 ± 5	43 ± 8	
Histological grade				**0.00001**			**0.0091**
Well dif.	8	75 ± 15	37 ± 17		87 ± 11	48 ± 22	
Mod. dif.	38	68 ± 7	10 ± 4		85 ± 5	25 ± 19	
Poorly dif.	21	28 ± 9	0		59 ± 11	11 ± 9	
Location (quadrants)				0.9199			0.2863
Medial	39	64 ± 7	15 ± 5		80 ± 5	34 ± 12	
Lateral	55	58 ± 6	9 ± 3		76 ± 7	48 ± 12	
Neoadyuvant treatment				**0.0002**			0.1889
No	92	68 ± 4	15 ± 3		85 ± 3	44 ± 8	
Yes	6	16 ± 15	0		–	33 ± 25	
ER*				**0.0069**			**0.04**
Negative	28	35 ± 9	7 ± 4		73 ± 8	21 ± 16	
Positive	44	81 ± 5	11 ± 4		92 ± 4	60 ± 9	
PR*				**0.0306**			**0.0169**
Negative	35	48 ± 8	2 ± 2		70 ± 8	24 ± 12	
Positive	26	65 ± 9	11 ± 6		91 ± 5	61 ± 15	
Ki67*				0.0589			**0.0097**
Negative	13	66 ± 13	8 ± 7		0	60 ± 18	
Positive	12	41 ± 14	0		57 ± 14	35 ± 15	
P53*				**0.00001**			**0.0017**
Negative	20	65 ± 10	5 ± 4		95 ± 4	46 ± 15	
Positive	5	0	0		20 ± 17	20 ± 17	
C-erbB-2*				**0.0244**			0.2640
Negative	19	63 ± 11	5 ± 5		83 ± 8	50 ± 14	
Positive	6	33 ± 19	0		66 ± 19	0	
AR*				0.7121			0.6241
Negative	16	43 ± 12	6 ± 6		75 ± 10	44 ± 15	
Positive	9	66 ± 15	0		87 ± 11	46 ± 18	
ApoD*				0.5775			0.9986
Negative	23	52 ± 10	8 ± 5		81 ± 8	35 ± 14	
Positive	2	50 ± 3	0		50 ± 35	50 ± 35	
Stromal desmoplastic reaction*				**0.001**			0.6072
Yes	14	28 ± 12	0		70 ± 12	40 ± 15	
No	11	81 ± 11	18 ± 11		90 ± 8	44 ± 21	
Peritumoral inflammatory reaction*				0.1452			0.2394
No	15	60 ± 12	13 ± 8		86 ± 8	46 ± 15	
Slight	9	44 ± 16	0		63 ± 16	33 ± 18	
Intense	1	0	0		0	0	

Table 2 continued

Patient and tumor characteristics	No	Relapse-free survival			Overall survival		
	98	2 years % ± ES	8 years % ± ES	*P*	2 years % ± ES	8 years % ± ES	*P*
Tumor edge*				0.0699			0.8413
Expansive	11	12 ± 13	18 ± 11		90 ± 9	25 ± 21	
Infiltrative	14	38 ± 13	0		76 ± 11	49 ± 14	
Angiolymphatic Invasion*				**0.03**			0.07639
Yes	13	46 ± 13	0		76 ± 12	17 ± 14	
No	12	15 ± 14	16 ± 10		83 ± 10	66 ± 17	
Perineural Invasion*				0.2956			0.7639
Yes	5	40 ± 21	0		80 ± 17	26 ± 22	
No	20	55 ± 11	10 ± 6		78 ± 9	47 ± 14	
Nuclear grade*				0.1069			0.4191
I	8	62 ± 17	25 ± 15		87 ± 11	65 ± 20	
II	11	45 ± 15	0		71 ± 13	32 ± 17	
III	6	50 ± 20	0		83 ± 15	31 ± 24	
Tumoral necrosis*				**0.00001**			**0.0012**
No	20	60 ± 10	10 ± 6		89 ± 6	46 ± 15	
Focal (<5%)	3	33 ± 27	0		66 ± 27	33 ± 27	
Diffuse (>5%)	2	50 ± 35	0		0	0	
No. of mitosis*				**0.0228**			**0.0076**
≤8	14	57 ± 13	14 ± 9		92 ± 6	66 ± 17	
>8	11	45 ± 15	0		62 ± 15	15 ± 13	

*Data were not available in all cases

influence of several classical factors of LR on outcome, such as number of recurrent nodules, size of the lesion, histological grade, tumoral invasion of the surgical margins, as well as the disease-free interval to the first recurrence. However, as it is shown in Table 3, out of all these factors only ER and PR-negative status in LRs ($P = 0.00005$ and $P = 0.00001$, respectively) as well as a short disease-free interval were significantly associated with overall survival. With regard to the latter factor, we analyzed all the possible cut-off points of time to the first recurrence for predicting overall survival, and we found a value of 12 months as the optimal cut-off for overall survival rate ($\chi^2 = 51.83$; $P = 0.00001$) (Fig. 3), with the possibility to identify a subgroup of patients (16.3%) developing LRs within the first 12 months after mastectomy and at a high risk of death by tumoral progression. In addition, as Table 4 show, multivariate analysis demonstrated that the length of the disease-free interval to the first recurrence was significantly and independently associated with overall survival (>12 months: RR = 0.033 (CI = 0.008–0.135); $P = 0.0001$).

Considering there are few studies evaluating changes in the morphological or biological characteristics between primary tumors and metastases or recurrences, in the present study we also decided to compare the histological grade and the expression of several parameters of the molecular biology of breast carcinomas, between primary tumors and paired isolated LRs, in the largest number of patients possible ($n = 25$) (Fig. 4). Our data showed that there is a significant parallelism regarding histological grade between primary tumors and paired LRs ($P = 0.001$) (Table 5). Likewise, we found a significant concordance between primary tumors and LRs in the expression of the following factors: ER ($P = 0.007$), PR ($P = 0.0001$) and p53 ($P = 0.007$) (Table 6). However, we could not demonstrate these findings for AR, c-erbB-2, ki67 and apolipoprotein D (Table 6). In addition, despite the reduced number of cases analyzed for these biological markers, our data showed that p53 status, in primary tumors as well as in LRs was significantly associated with a poorer overall survival in our study population ($P = 0.0017$ and $P = 0.0331$, respectively) (Tables 2 and 3, respectively). Likewise, high tumoral expression of ki67 was also significantly related with a short overall survival of patients with LR ($P = 0.0097$).

Discussion

The outcome of patients with local or regional breast cancer recurrence after mastectomy is often described as fatal [35], because many patients develop distant

Table 3 Univariant analysis of the relationship between the clinicopathological and biological characteristics of LRs and overall survival

Patient and tumor characteristics	No	Overall survival			
	98	2 years % ± ES	5 years % ± ES	8 years % ± ES	*P*
Age (years)					0.1258
<50	28	95 ± 4	68 ± 10	38 ± 14	
≥50	70	97 ± 1	82 ± 4	66 ± 5	
Menopausal status					0.4654
Premenopausal	32	96 ± 3	71 ± 8	51 ± 10	
Postmenopausal	66	96 ± 2	83 ± 4	67 ± 6	
Size (median)					0.9362
<2 cm	27	96 ± 3	77 ± 8	59 ± 11	
>2 cm	71	98 ± 1	80 ± 4	62 ± 6	
No. recurrences					0.1249
Multiple	25	96 ± 3	72 ± 9	39 ± 11	
Single	73	97 ± 1	81 ± 4	67 ± 5	
Location					0.9423
Surgical scar	69	97 ± 2	79 ± 4	60 ± 6	
Chest wall	29	96 ± 3	78 ± 7	65 ± 9	
Histological grade					0.0870
Well dif.	5	80 ± 17	80 ± 17	60 ± 21	
Mod. dif.	21	95 ± 4	80 ± 8	61 ± 11	
Poorly dif.	15	–	46 ± 12	23 ± 11	
Time to recurrence					**0.0002**
<12 months	16	75 ± 10	31 ± 12	7 ± 7	
≥12 months	82	88 ± 3	62 ± 6	58 ± 6	
Surgical margin					0.326
Positive	34	79 ± 6	46 ± 9	29 ± 11	
Negative	44	86 ± 5	60 ± 9	60 ± 9	
ER					**0.00005**
Negative	21	90 ± 6	71 ± 9	40 ± 11	
Positive	31	100	93 ± 4	81 ± 7	
PR					**0.00001**
Negative	22	90 ± 6	90 ± 6	43 ± 11	
Positive	29	100	100	79 ± 8	
Ki67					0.3241
Negative	9	88 ± 10	66 ± 15	50 ± 18	
Positive	16	74 ± 11	59 ± 12	39 ± 18	
P53					**0.0331**
Negative	19	94 ± 5	69 ± 11	46 ± 15	
Positive	6	33 ± 19	33 ± 19	33 ± 19	
c-erbB-2					0.8880
Negative	17	83 ± 8	59 ± 12	47 ± 14	
Positive	8	12 ± 16	72 ± 16	36 ± 27	
AR					0.0680
Negative	9	66 ± 15	53 ± 17	17 ± 15	
Positive	16	87 ± 8	66 ± 12	66 ± 12	
ApoD					0.6343
Negative	23	81 ± 3	59 ± 11	39 ± 13	
Positive	2	50 ± 35	50 ± 35	50 ± 35	

metastases within a short period of time [2, 36]. However, certain subgroups with more favorable prognoses are believed to exist. To identify subsets of patients differing in the clinical course of the disease, we considered different well-known prognostic factors for primary tumors and/or locoregional tumoral

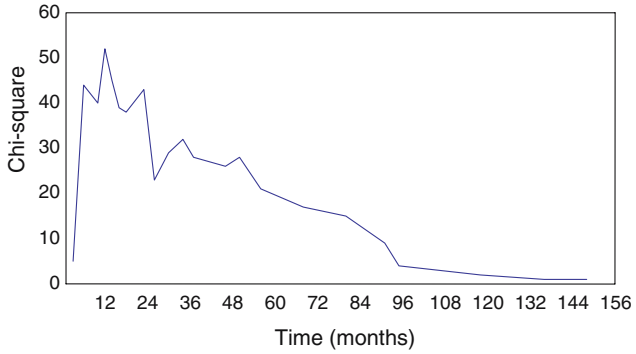

Fig. 3 Maximum likelihood determination of the cut-off value of the length of the disease-free interval after mastectomy for predicting overall survival in 98 patients with LR. *P*-values obtained for each cut-off value are plotted against the value itself

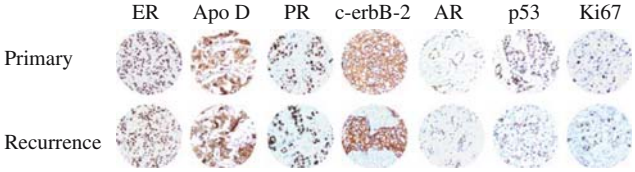

Fig. 4 Representative images of a tissue microarray spot of primary tumor and LR, positive for ER, ApoD, PR, c-erbB-2, AR, p53, and Ki67 (200×)

recurrences. Our results demonstrate a higher percentage of primary tumors of larger size, node-positive status, and poorly differentiated grade in women with LR than among those without it. These results are in accordance with prior studies showing that these clinicopathological parameters are strongly associated with the risk of locoregional recurrence [5–9, 20–25]. Nevertheless, it is remarkable that the very same parameters are unable to differentiate between the risk of LR or distant metastasis after mastectomy for invasive breast cancer. We also consider noteworthy the findings on our study population indicating that patients with LR received less frequently either adjuvant radiotherapy, chemotherapy and, particularly, tamoxifen, when compared with patients who developed distant metastasis as the first event of tumoral

Table 4 Multivariate analysis of the association of clinico-pathological characteristics of breast tumors with relapse-free and overall survival

Tumor characteristics	Relapse-free survival			Overall survival		
	RR	CI (95%)	*P*	RR	CI (95%)	*P*
Primary tumors						
Tumor stage			**0.033**			–
I	1	–		–	–	
II	8.8	1.2–65.7		–	–	
II	15.2	1.5–149.5		–	–	
IV	20.8	2.5–175.2		–	–	
Nodal status			**0.001**			–
N (–)	1	–		–	–	
N (+)	3.7	1.7–7.9		-	-	
Histological grade			0.0001			0.0001
Well dif.	1	-		1	-	
Mod. dif.	4.6	1.3–17.0		0.4	0.1–1.8	
Poorly dif.	21.1	4.9–91.7		3.9	1.0–14.8	
Tamoxifen			0.001			–
Yes	1	–		–	–	
No	3.3	1.6–6.8		–	–	
Chemotherapy			**0.016**			–
Yes	1	–		–	–	
No	2.6	1.2–5.5		–	–	
Radiotherapy			**0.039**			–
Yes	1	–		–	–	
No	2.3	1.0–5.2		–	–	
LRs						
Histological grade			–			**0.015**
Well dif.	–	–		1	–	
Mod. dif.	–	–		0.4	0.1–1.7	
Poorly dif.	–	–		2.0	0.5–8.0	
Cut-off			–			**0.0001**
<12 months	–	–		1	–	
>12 months	–	–		0.0	0.0–0.1	

Abbreviations: RR, relative risk; CI, confidence interval

Table 5 Relationship between the histological grade in the primary tumor and in the first LR in 25 patients who developed LR as the first event of tumoral progression

SBR in primary tumor	SBR in the first LR		
	I	II	III
I	2 (40)	3 (60)	0
II	1 (6.7)	11 (73.3)	3 (20)
III	1 (7.1)	2 (14.3)	11 (78.6)

Chi-square: $P = 0.001$

Table 6 Relationship between the expression of biological factors in the primary tumor and in the first LR in 25 patients who developed LR as the first event of tumoral progression

	N	PT + /LR + No. (%)	PT + /LR – No. (%)	PT – /LR + No. (%)	PT – /LR – No. (%)	*P**
ER	25	15 (60)	2 (8)	2 (8)	6 (24)	**0.007**
PR	25	12 (48)	3 (12)	0	10 (40)	**0.0001**
AR	25	8 (32)	1 (4)	8 (32)	8 (32)	0.131
Ki 67	25	10 (40)	3 (12)	7 (28)	5 (20)	0.369
c-erbB-2	25	4 (16)	2 (8,3)	4 (16)	15 (60)	0.113
P53	25	4 (16)	1 (4)	2 (8)	18 (72)	**0.007**
ApoD	25	2 (8)	1 (4)	1 (4)	21 (84)	0.373

*Chi-square tests

Abbreviations: PT, primary tumor; LR, local recurrence

progression or with patients without any tumoral recurrence. This finding seems to indicate that adjuvant undertreatment is related with the developement of LR in primary breast cancer.

There are two main hypotheses on the origin and significance of LRs: The first one is that LR is caused by an incomplete initial removal of the tumor [27, 37–39]; the second one is that LR is a sign (the first one) of the disease being already disseminated [40–45]. Unfortunately, at the present time it is not possible to determine the cause of an isolated LR. In addition, it is remarkable that, as is shown in the present study, some of the prognostic factors that had a strong effect on prognosis after the primary diagnosis, such as tumor size or tumoral grade, retained their effect after LR as well. Likewise, it has also been reported that other primary tumoral factors, such as histological grade, node status, hormonal receptor status or tumoral necrosis have been identified as prognostic factors for post-recurrence outcome [9, 46, 47]

It seems reasonable to consider the possibility that prognostic factors determined by the recurrence itself might predict the final outcome in a more accurately way. This way, factors such as the number of recurrent nodules, the size of the lesion (the two reflecting the extent of tumor burden and growth pattern) and the disease-free interval [30–32, 48–50], have been shown to correlate with distant relapse and survival [2, 9, 36, 46]. Our data indicate that the interval to the first LR seems to be the most important of the classical factors. In addition, our results show that a disease-free interval of 12 months from original diagnosis to chest wall recurrence was the optimal cut-off value to predict the ultimate outcome in patients with LR. Thus, it appears that the early happening of LR after mastectomy is a sign of the biological aggressiveness of the breast cancer.

There are also a few studies evaluating modifications in the morphological or biological characteristics between primary tumors and metastasis or recurrences. Therefore, in the present study we also investigated in a subset of study patients as large as possible the hypothetical changes in histological grade as well as in several other biological markers in LRs with regard to the ones in the primary tumors.

On the basis of the reduction of mortality seen in breast cancer screening programs, and the histological evaluation of breast cancer detected by mammographic screening, it has been suggested that there is a progression of tumor grade with time. It has been found as well that screen-detected carcinomas are smaller and of lower grade when compared with those found in non-screened women [51–53]. Although several authors have reported a high concordance in grading between the primary tumor and its metastases [54, 55] or subsequent locally recurrent and metastatic lesions [55], it has also been described that low and intermediate grade carcinomas often recur as higher grade tumors, although the opposite phenomenon—

higher grade tumors recurring as better differentiated ones—has been seen but only rarely [56]. Whether or not breast cancer becomes less differentiated with time remains unanswered, but our data demonstrate that there is a significant relation between the histological grade of primary tumors and paired LRs. In addition, our data revealed that the histological grade in primary tumors have a significant value in predicting overall survival in patients developing LR after mastectomy.

There are limited available data regarding the value of molecular markers at the time of the primary tumor to predict LR and at the time of loco-regional recurrence to predict the final outcome. In the present study we have also analyzed the expression of several parameters of the molecular biology of breast carcinomas in primary tumors and in paired isolated LRs in a subset of patients as large as possible, and we have compared their differential expression between these two groups and their prognostic significance. These well-known biological parameters included: steroid receptors (ER, PR and AR), the oncoprotein c-erbB-2, the tumoral suppressor p53 protein, the proliferative marker ki67, and the apolipoprotein D, which is the major protein component of breast secretions in non-lactating women and a marker associated with a favorable prognosis in breast cancer [57]. Our results demonstrate a significant concordance in the expression of the following factors between primary tumors and their LRs: ER, PR and p53. However, we found no significant concordance for AR, c-erbB-2 and ki67. Thus, these data suggest that LRs do not maintain all of the biological characteristics of their corresponding primary tumors. In relation with the prognostic significance of the expression of these molecular markers in LRs, our data coincide with previous reports indicating that a positive ER and PR status implies a longer disease-free survival as well as a longer overall survival [47, 58], whereas p53 status was associated with a poorer outcome in our study population. The loss of p53 function is a recognized adverse prognostic factor in invasive breast cancer. The tumor suppressor gene p53 is currently the focus of much attention in breast cancer research. The presence of an altered p53 has been identified in 50% of cases of invasive disease. Because loss of p53 function leads to higher proliferative and lower apoptosis rates, altered p53 should therefore be associated with a worse clinical outcome. Thus, different studies have shown that altered function of this gene is associated with decreased disease-free and overall survival [59–61].

It is also remarkable that despite the few cases analyzed for the different biological factors in the present study, and in accordance with prior data, the expression in primary tumors of either ER, PR [62] or p53 [63], was associated with both relapse-free survival until LR development and overall survival, whereas c-erbB-2 expression was associated with a shorter relapse-free survival [58]. Thus, these latter data indicate that certain biological characteristics from primary tumors also retain prognostic importance when LR occurs.

The data presented in this study suggest that classical clinicopathological factors as well as new biological parameters may identify subgroups of patients with LR after mastectomy differing in their prognosis. Thus, despite the role of systemic therapy for LR is still unclear [64], it is feasible at the present time to select patients potentially candidates for further therapeutic strategies.

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