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HER2 amplification in recurrent breast cancer following breast-conserving therapy correlates with distant metastasis and poor survival

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The authors analyzed the *HER2* status in early-stage nonrecurrent and recurrent breast cancer groups following breast-conserving treatment. Retrospective analyses of a group of 36 invasive early breast cancer (IBC) patients who developed a local recurrence as a first event and of a random control group of 69 IBC patients were made. *HER2* status was assessed by the HercepTest® (Dako Corp., Carpinteria, CA) and fluorescence *in situ* hybridization. The Kaplan–Meier proportional log-rank test was used to study the impact of the biological factors on the metastasis-free interval (MFI) and the overall survival (OS). The Cox proportional hazards model, using stepwise selection was performed to identify the independent predictors of poor outcome. The median time of follow-up was 156 months (range: 22–230) for the nonrecurrent group of patients and 119 months (range: 36–228) for the recurrent group. No significant differences between either group were observed in terms of either patient or tumor characteristics, or of HER2 expression. However, a higher proportion of *HER2* amplified cases were found in the recurrent group, in contrast to a higher proportion of hormonal receptor positive cases in the nonrecurrent group. After univariate and multivariate analyses, *HER2* amplification was found to be an independent predictive factor for distant metastasis (HR = 10.75; $p = 0.00008$) and for survival (HR = 4.22; $p = 0.004$). In conclusion, *HER2* amplification constitutes an independent poor prognostic factor for the MFI and OS in patients with recurrent breast cancer. The clinical implications are discussed.

Key words: breast carcinoma; local recurrence; *ERBB2/HER2*; FISH; trastuzumab

Breast cancer is the most common worldwide malignancy in women, making up 18% of all cancers in women.[1] Several attempts have been made to improve the outcome and care for these patients. Breast conserving therapy (BCT), including tumorectomy or quadrantectomy, axillary dissection and radiotherapy, has become a standard in early-stage invasive breast cancer (IBC), providing survival rates equivalent to those achieved with mastectomy.[2,3] However, following BCT, a small proportion of patients (1.0–1.5% per year) develop a local recurrence (LR) in the treated breast.[4,5] LR represents a failure of breast preservation, requiring a mastectomy as rescue treatment.[6] Although, the impact of such LR on survival remains in debate,[7] minimizing the risk of LR is therefore an essential goal in the context of curative treatment.

A large number of factors have been correlated to the development of LR, the status of the microscopic margins of excision being the most useful in clinical practice.[8] The analysis of biological and molecular markers in the primary tumor with tumor-free margins were able to identify patterns associated with a higher risk for LR.[8] Haffty and co-workers reported higher LR rates in patients whose tumors expressed HER2 oncoprotein than in those that didn't,[9] although this observation has not always been confirmed.[8]

Amplification and/or overexpression of *HER2* is an early event in some breast tumors, and has been shown to have a detrimental effect on prognosis,[10,11] it may also predict the sensitivity to certain types of cytotoxic[11–13] or endocrine agents.[10,11,13] In addition, preclinical studies have shown that monoclonal antibodies directed against HER2 are capable of inhibiting both the *in vitro* proliferation of HER2 overexpressing tumor cells, and the *in vivo* growth of HER2 overexpressing human breast cancer xenografts in nude mice.[10,14] This has opened up a new approach to HER2-targeted monoclonal antibody therapy of breast cancer with trastuzumab (Herceptin®), which has been shown to be active in the treatment of patients with HER2-expressing metastatic breast cancer.[10,14,15]

The aim of this study is to establish whether the status of the *HER2* gene is an indicator of poor prognosis in a series of recurrent breast cancer compared with a nonrecurrent group of IBC that were treated with BCT. In addition, we compare the primary tumor and the LR from the recurrent breast cancer group so as to establish if any significant difference exists that might reveal a higher aggressivity of the LR. We demonstrate that *HER2* amplification constitutes an independent poor prognostic factor for the metastasis-free interval (MFI) and overall survival (OS) in recurrent breast cancer and discuss its clinical implication.

Material and methods

Patient selection

Paraffin blocks were selected from a group of 36 recurrent IBC patients, with their respective recurrences, and from a random control group of 69 IBC patients, with no evidence of LR. All cases had been subjected to conserving surgery and postoperative radiotherapy (a total of 60 Gy over 5 weeks to the tumor bed: 50 Gy on the whole breast volume and 10 Gy on the wound) between May 1982 and September 1993 at the Fundación Instituto Valenciano de Oncología (Valencia, Spain). No patient received adjuvant systemic therapy. Patients in the recurrent group underwent successful removal of a local relapse and had no evidence of distant metastasis. The breast tumors were graded according to the modified Bloom and Richardson score on H&E-stained slides.[16]

Assessment of the HER2 *status*

Immunohistochemistry. Four-micrometer sections from embedded blocks were cut on poly-L-lysine-coated slides and dewaxed, and endogenous peroxidase was inhibited with 3% hydrogen peroxide for 30 min. Immunoreactivity was enhanced with antigen retrieval treatment by heating the slides in a microwave oven for 10 min (700 W) in 10 mM sodium citrate buffer pH 7, followed by cooling for 20 min at room temperature. Sections were blocked with 20% horse serum in phosphate-buffer saline (PBS) and

Abbreviations: BCT, Breast-conserving treatment; FISH, fluorescence *in situ* hybridization; IBC, invasive early breast cancer; LR, local recurrence; MFI, metastasis-free interval; OS, overall survival; PBS, phosphate-buffer saline.

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incubated with primary antibody for 1 hr at room temperature. The incubation time for the secondary antibody and avidin–biotin complexes was 30 min at room temperature. Sections were extensively washed and the immunoreactions developed using DAB (0.05% 3′3′ diamino-benzidine in 0.1% hydrogen peroxide). Negative controls included substitution of the primary antibody by mouse ascitis or PBS. Slides were counterstained in Mayer hematoxylin, dehydrated and mounted.

HER2 protein expression was immunohistochemically evaluated using the HercepTest Kit (Dako Corp., Carpinteria, CA) according to the manufacturer's guide. The scoring system was the following: 0, tumors with no or weak staining in less than 10% of the cells; +, tumors with a faint or barely perceptible membrane staining in more than 10% of cells or with noncircumferential staining; ++, moderate circumferential membrane staining; +++, strong circumferential membrane staining. Scores 0 and + were considered as negative; scores ++ and +++ were considered as positive for HER2 overexpression.[17]

Fluorescence in situ hybridization. Fluorescence in situ hybridization (FISH) was performed on formalin-fixed paraffin-embedded tissue using a *HER2* DNA probe (Oncor Inc.) with an α-satellite centromere probe as control for aneuploidy of the chromosome 17 (D17Z1, Oncor Inc.), on which the *HER2* is located.

Five-micrometer sections were deparaffinized using a heating plate (56°C) for 30 min, xylene (3 changes for 10 min each) and methanol (2 changes for 5 min each). After a first incubation with 1 M sodium thiocyanate-solution for between 3 and 15 min on a heating plate at 80°C, further digestion with 0.2% Proteinase K (Sigma) or with Pepsin (4 mg pepsin in 1 ml 0.2 N HCl) (Sigma) at 37°C for between 5 and 30 min was required. The preparations were washed and dehydrated in an ethanol series before simultaneous denaturation of probes and target DNA sequences. The slides and probe combination were incubated simultaneously on a stable hotplate at 78–80°C for 10 min. After overnight incubation at 37°C, the slides were washed at 42°C in 50% formamide/2× SSC for 15 min followed by 40 ml of 2× SSC for 2 washes each of 7 min. Detection and amplification was carried out according to standard protocols. Biotinylated sequences were detected and amplified with fluorescein-labeled avidin and antiavidin antibody (Oncor). Digoxigenin probes were detected and amplified with rhodamine-labeled antidigoxigenin, rabbit anti-sheep and rhodamine/anti-rabbit (Oncor). Slides were counterstained with DAPI and were viewed using a Zeiss Axioplan 2 microscope with appropriate filters (Jena, Germany). Images were captured using a video camera (IMAC_CCD S30) coupled to a personal computer with software (ISIS 2.85) from MetaSystems (Althlusseim, Germany). The number of signals was analyzed in at least 500 nuclei. A ratio of more than 2 oncogene signals/chromosome 17 centromere control signals was used to define amplification.[18,19]

Statistical analysis

For the statistical analysis we used binary variables reflecting the positivity status of the measures (yes or no). The association between *HER2* overexpression and amplification as assessed by the different techniques was tested using a χ^2-test for homogeneity. Association with histopathological parameters, all categorical, was also assessed using a χ^2-test to determine homogeneity or linear trend for ordinal variables. The significance level was set at 5%. To study the impact of the biological factors on the MFI and OS, the Kaplan–Meier proportional risk test (log rank) was used.[20,21] Univariate predictors of metastasis-free survival and OS were entered into a Cox proportional hazards model using stepwise selection to identify the independent predictors of poor outcome.[22] All tests used are included in the SPSS statistical package (version 12.0).

Results

Patient characteristics

In total, 134 IBC were studied, including a random control group of 69 patients with an IBC with no evidence of LR, and a cohort of 36 recurrent IBC patients, from which the primary tumor and 31 LR were analyzed. The median follow-up period was of 156 months (range: 22–230 months) and of 119 months (range: 36–228 months) for the nonrecurrent and recurrent group, respectively. Histologically, all cases had tumor-free margins of at least 2 mm. The histopathological and clinical features of both groups are listed in Table I. No statistically significant differences between either group were observed regarding age, stage, histological grade and histological type, the majority of cases being infiltrating ductal carcinomas. However, the frequency of positive cases for the hormonal receptor status, determined by immunohistochemistry against estrogen and progesterone receptors, was higher in the control group; whereas the incidence of metastases and cancer specific deaths during follow-up were higher in the recurrent group of patients (Table I).

TABLE I – PATIENT CHARACTERISTICS

Variable	Group		*p*-value
	Nonrecurrent	Recurrent	
Age (years)	54 (32–74)	49 (28–76)	NS
Diagnosis			
IDC	66	34	
ILC	3	1	NS
IPC		1	
T			
T1	42	20	NS
T2	27	15	
N			
N0	45	25	NS
N+	24	10	
Stage			
I	36	17	NS
II	33	18	
Dedifferentiation			
I	2		NS
II	19	14	
III	48	22	
Polymorphism			
I	11	2	
II	37	24	NS
III	21	10	
Mitosis			
I	48	29	
II	12	5	NS
III	9	2	
Histological grade			
I	18	11	
II	33	17	NS
III	18	8	
Hormonal status[1]			
Negative	20	19	0.017
Positive	49	17	
Metastasis during follow-up			
M0	51	18	0.022
M+	18	17	
State			
Alive	55	17	0.001
Exitus	14	18	
Follow-up (months)	156 (22–230)	119 (36–228)	0.058
Total	69	36	

NS, not significant; IDC, infiltrating ductal carcinoma; ILC, infiltrating lobular carcinoma; IPC, infiltrating papilar carcinoma.
[1]Hormonal status was considered as negative when either oestrogen and progesterone receptors were negative, and was considered as positive when at least one of the receptors were positive.

HER2 status

No differences between the recurrent and nonrecurrent group of IBC were observed in relation to the HER2 expression, both groups containing 18% of positive cases (Table II). However, a higher proportion of *HER2* amplified cases were present in the recurrent group (Table II).

TABLE II – IMMUNOHISTOCHEMICAL AND GENETIC ANALYSIS FOR THE *HER2* STATUS

Variable	Group (%)		*p*-value	Recurrent group (%)		*p*-value
	NonRecurrent	Recurrent		PT	R	
Hercep test						
Negative	80	83	NS	83	77	NS
Positive	20	17		17	23	
HER2 (FISH)						
Nonnamplified	86	67	0.024	67	57	NS
Amplified	14	33		33	43	

NS, not significant; PT, primary tumor; R, recurrence.

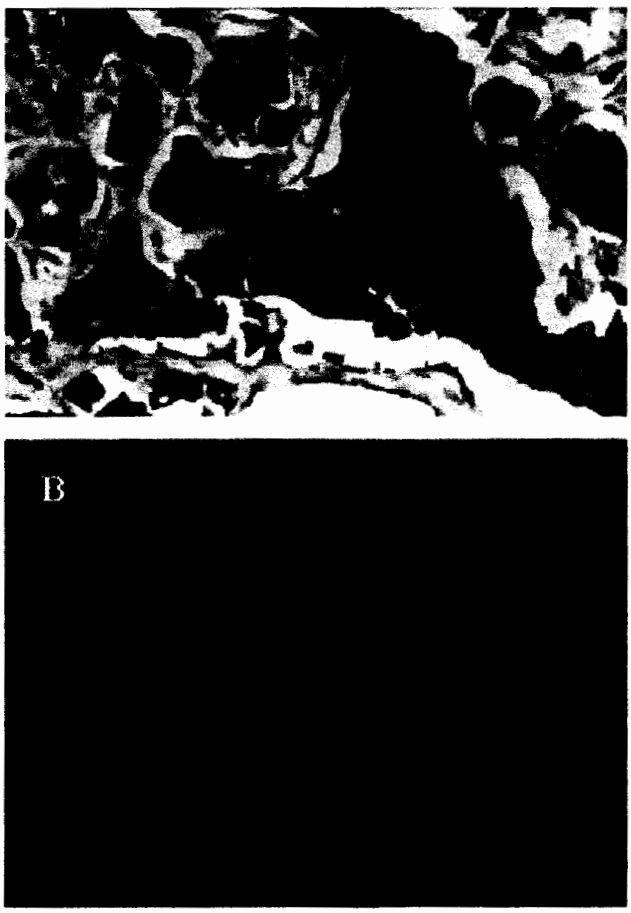

FIGURE 1 – Determining of the *ERBB2/HER2* status on invasive breast cancer paraffin-embedded sections. A, HercepTest immunostaining showing an intense membranous staining (score, 3+). B, representative FISH analysis showing an amplified case.

A highly significant association between HER2 overexpression and *HER2* gene amplification was observed (Fig. 1). Eighty-four percent of HercepTest negative cases were also nonamplified by FISH, whereas 67% of HercepTest positive tumors presented an amplified *HER2* gene ($p < 0.001$).

No differences regarding the *HER2* status were observed between the primary tumor and the LR from the recurrent group of patients. Interestingly, when *HER2* amplification was present in the primary tumor, it was also present in the corresponding matched LR.

Association of the *HER2* status and the histopathological parameters showed that *HER2* gene amplification and hyperexpression are directly associated with the histological grade of the tumors ($p = 0.033$ and $p = 0.000015$, respectively). In the nonrecurrent group of patients, an association between *HER2* amplification/hyperexpression and the development of systemic metastasis was observed: 5 out of 18 tumors (28%) that developed metastasis had *HER2* amplification ($p = 0.063$). In the same way, 11 out of 12 cases (92%) with *HER2* amplification in the recurrent group developed metastasis, *vs.* 6 cases with metastasis in the 23 cases (26%) with normal gene status ($p = 0.00023$). *HER2* amplification was inversely associated with the HR status; only 3 out of 17 (18%) HR positive cases in the recurrent group were *HER2* amplified *vs.* 9 out of 19 (47%) cases HR negative ($p = 0.059$). HER2 amplification was also associated with both lymph node involvement and tumor stage in the recurrent group; 6 out of 12 (50%) *HER2* amplified cases *vs.* 4 out of 23 (17%) cases with no gene amplification presented lymph node involvement ($p = 0.043$), whereas 9 out of 18 (50%) stage II tumors *vs.* 3 out of 17 (17%) stage I tumors presented *HER2* amplification ($p = 0.044$).

Univariate and multivariate analysis for the MFI

The median follow-up for the 69 nonrecurrent patients was 156 months (range: 22–230 months). Eighteen patients from this group (26%) developed systemic metastasis. Of the 36 recurrent cases, clinical monitoring was available for 34 patients, median follow-up was 119 months (range: 36–228 months), with 17 cases (50%) developing systemic metastasis. A log-rank test for each study group was performed for the association between histopathological, immunohistochemical and genetic findings for the MFI. The principal findings are listed in Table III.

For the nonrecurrent group, only lymph node involvement was associated with a shorter MFI. Hyperexpression and gene amplification of *HER2* (Fig. 2*a*) showed a trend toward shorter MFI, although this was not statistically significant (Table IV). In the recurrent group, stage and *HER2* amplification (Fig. 2b) were statistically associated with shorter MFI (Table III). Furthermore, tumor size and lymph node involvement showed a strong trend toward statistical significance for a short MFI in the recurrent patients (Table III).

Tumor size, lymph node involvement, stage, histological grade, hormonal receptor status, HER2 expression and *HER2* amplification were entered into a multivariate model to identify independent predictors of metastasis-free survival in the nonrecurrent and recurrent groups. After Cox regression analysis, the factors significantly associated with metastasis were lymph node involvement in the nonrecurrent group and *HER2* amplification in the recurrent group (Table V).

Univariate and multivariate analysis for OS

Fourteen out of 69, and 18 out of 35 patients, from the nonrecurrent and recurrent groups, respectively, died during the follow-up period. The log-rank test for the OS showed that neither clinico-pathological nor immunohistochemical and genetic variables were significantly indicative of poor outcome in the nonrecurrent group (Table IV; Fig. 3*a*). However, in the recurrent group, stage II tumors and *HER2* amplification were statistically indicative of a poor prognosis (Table IV; Fig. 3*b*).

TABLE III – KAPLAN-MEIER ANALYSIS FOR METASTASIS-FREE INTERVAL (MFI) IN GROUPS UNDER STUDY

Variable	NonRecurrent		*p*-value	Recurrent		*p*-value
	Events	%MFI		Events	%MFI	
T						
T1	9	79	NS	8	60	0.101
T2	9	67		9	40	
N						
N0	8	82	0.0327	11	56	0.128
N+	10	58		6	40	
Stage						
I	8	78	NS	6	65	0.041
II	10	70		11	39	
Histological grade						
I	5	72	NS	4	64	NS
II	9	73		10	38	
III	4	78		3	63	
HR						
Negative	5	75	NS	10	44	NS
Positive	13	73		7	59	
HercepTest						
Negative	12	78	0.1261	13	55	NS
Positive	6	57		4	33	
HER2 (FISH)						
Nonamplified	13	78	0.0644	6	86	<0.00001
Amplified	5	50		11	27	

NS, not significant.

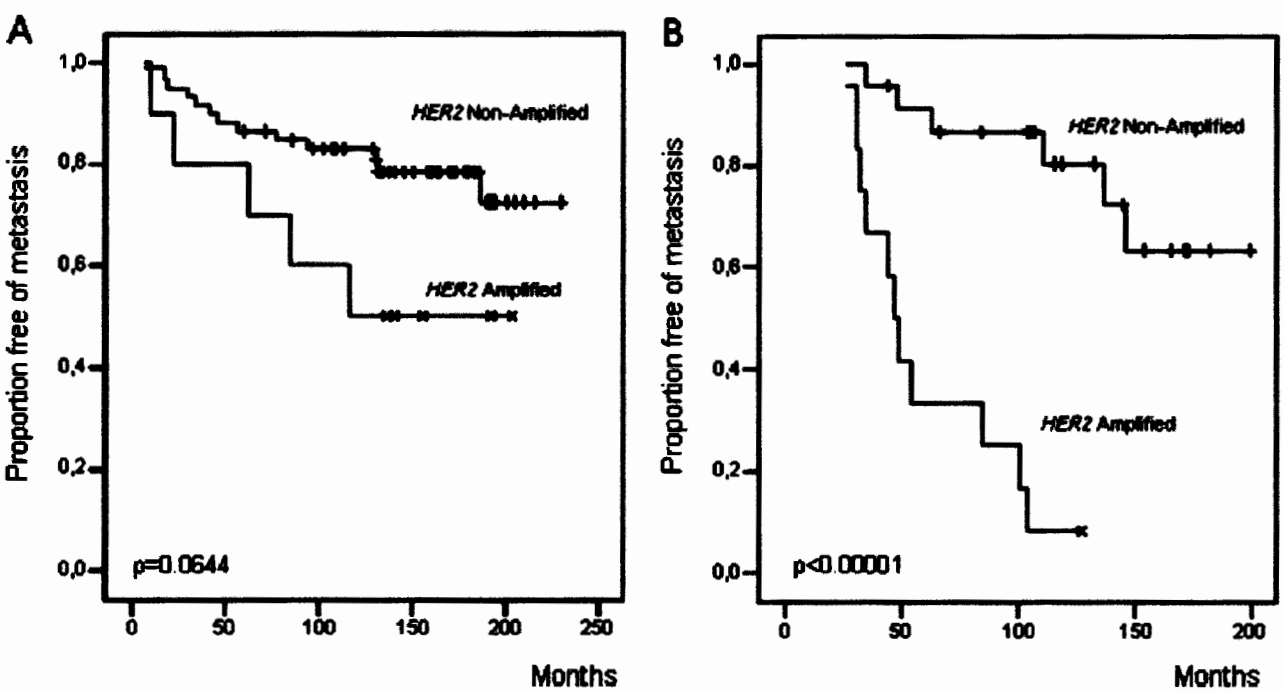

FIGURE 2 – Significant Kaplan–Meier plots showing the shorter MFI for those cases with *ERBB2/HER2* amplification in the nonrecurrent (A) and recurrent (B) groups of patients.

TABLE IV – INDEPENDENT PREDICTORS OF METASTASIS-FREE SURVIVAL FOR NONRECURRENT AND RECURRENT IBC PATIENTS

Group	Prognostic factor	Hazard ratio (HR)	95% CI for HR	*p*-value
Nonrecurrent ($N = 69$; $\chi^2 = 4{,}564$)	N	2.65	1.04–6.76	0.040
Recurrent[1] ($N = 34$; $\chi^2 = 22{,}946$)	*HER2* amplification	10.75	3.3–34.48	0.00008

[1]Follow-up data in the recurrent group was available in 34 out of 36 patients.

TABLE V – KAPLAN-MEIER ANALYSIS FOR OVERALL SURVIVAL (OS) BETWEEN GROUPS UNDER STUDY

Variable	NonRecurrent		*p*-value	Recurrent		*p*-value
	Events	%OS		Events	%OS	
T						
T1	6	86	0.1397	8	60	0.070
T2	8	70		10	33	
N						
N0	6	87	0.055	11	56	0.083
N+	8	67		7	30	
Stage						
I	6	83	NS	6	65	0.026
II	8	76		12	33	
Histological grade						
I	3	83	NS	4	64	0.1407
II	7	79		9	44	
III	4	78		5	37	
HR						
Negative	5	75	NS	11	39	NS
Positive	9	82		7	59	
HercepTest						
Negative	83	83	0.1079	13	55	0.0585
Positive	64	64		5	16	
HER2 (FISH)						
Nonamplified	10	83	0.0978	8	65	0.0018
Amplified	4	60		10	16	

NS, not significant.

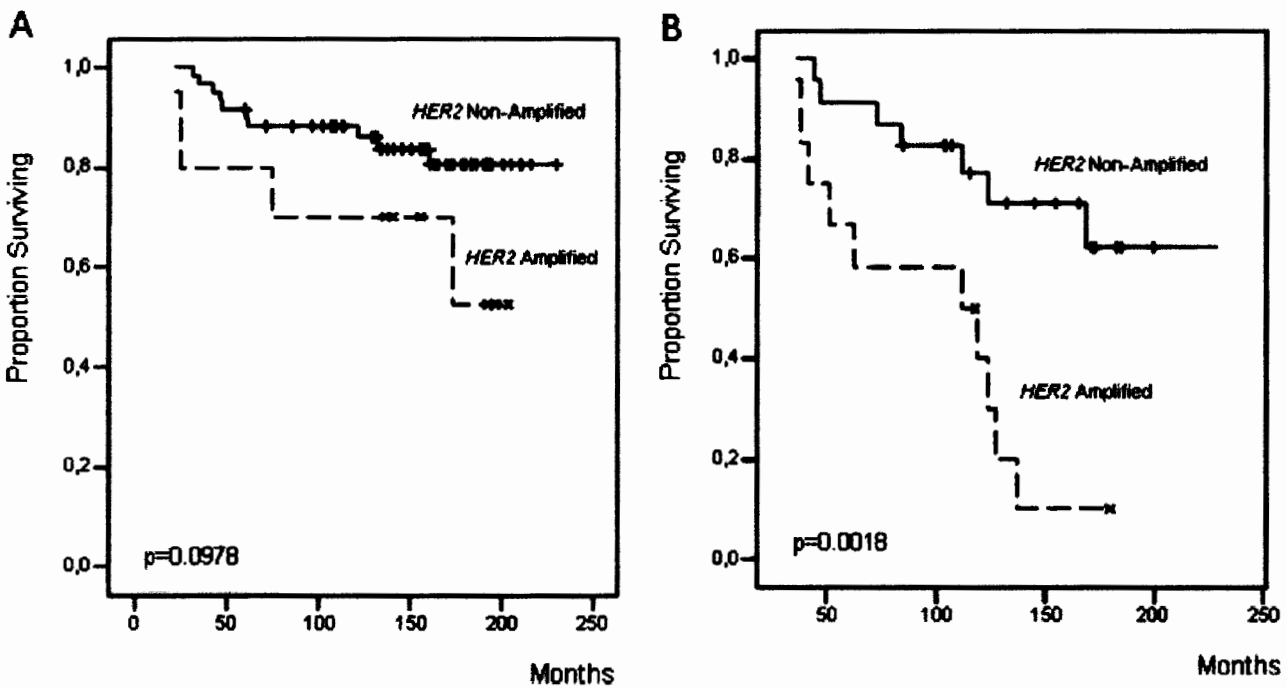

FIGURE 3 – Significant Kaplan–Meier plots showing the shorter OS period for those cases with *ERBB2/HER2* amplification in the nonrecurrent (A) and recurrent (B) groups of patients.

The Cox regression analysis demonstrated that no independent predictor of cancer-specific death was found in the nonrecurrent group; whereas in the recurrent group HER2 amplification indicated poor survival with an HR of 4.22 (1.58–11.24) (Table VI).

Discussion

The aim of BCT is to achieve maximum cosmesis without compromising local control and OS. Exclusive breast conserving surgery is associated with an excessive 6–43% incidence of LR,[5] with postoperative radiotherapy reducing LR rates by about 70%.[23] Therefore, following adequate BCT the risk of LR is ~1.5–2% per year and appears to stabilize at around 10–20% at 10–15 years of follow-up.[5] Randomized trials have shown that this treatment does not modify overall or recurrence-free survival compared with radical or modified radical mastectomy.[24]

LR has been considered as a predictor of distant metastasis,[25,26] and several efforts have been made to find factors that predict for LR. Among these are included increasing tumor size, axillary lymph node involvement, multifocality of primary tumor, the

TABLE VI - INDEPENDENT PREDICTORS FOR OVERALL SURVIVAL IN THE RECURRENT IBC PATIENTS

Group	Prognostic factor	Hazard ratio	95% CI for HR	*p*-value
Recurrent[1] ($N = 34$; $\chi^2 = 9,676$)	*HER2* amplification	4.22	1.58–11.24	0.004

[1]Follow-up data in the recurrent group was available in 34 out of 36 patients.

presence and the extent of intraductal component, positive tumor margins, adjuvant therapies (radiotherapy, hormone therapy and chemotherapy), family history of breast cancer and gene mutation status of the *BRCA1* and *BRCA2* genes.[5,8,27,28] Histological type and grade, the presence of tumor emboli, endolymphatic invasion, peritumoral vascular invasion, estrogen receptor negativity, hyperexpression of HER2,[9] p53 positivity[5,27] and colony-stimulating factor receptor expression[29] have all variably been found to be associated with the risk of LR.

Preclinical data suggest that overexpression of HER2 confers cellular radioresistance[30] and it could constitute a predictor of risk for LR in those patients treated with BCT. In this regard, Haffty and co-workers, in a matched case-control study, demonstrated a higher expression of the HER2 oncoprotein in a group of recurrent breast cancer patients when compared with that in a control group with no evidence of LR, indicating that HER2 overexpression is a predictive factor for LR.[9] However, other authors have not confirmed this finding in a larger cohort of patients.[31]

In the present matched case-control study, we have compared 2 groups of early IBC patients who were subjected to BCT at the same hospital, with a median follow-up of more than 10 years. A recurrent group and a random group of nonrecurrent patients were selected with the aim of establishing whether the status of the *HER2* oncogene is an indicator of poor prognosis in the recurrent group of patients.

No significant differences between either group were observed regarding age, histological type and grade, stage and follow-up period. HR status showed clear differences between recurrent and nonrecurrent groups, the frequency of HR+ being higher in the latter group. This observation has already been described by others and is a direct consequence of the immunophenotypical differences between both the groups.[5,27] The HR status did not reveal any influence on the MFI and OS of the nonrecurrent group. In our series, none of these patients received any form of adjuvant systemic therapy on the basis of the HR status due to the treatment period (before 1993). On the contrary, the incidence of metastases (49% *vs.* 26%) and cancer-specific deaths (51% *vs.* 20%) was significantly higher in the recurrent group of patients. These results are in agreement with those observations that consider LR to be both a failure of BCT[5,6] and a predictor of distant metastasis.[25,26,32,33] In the same way, a higher proportion of *HER2* amplified cases (33%) were present in the recurrent group when compared with that in the nonrecurrent breast cancers (18%). All these incidences are concordant with the frequencies reported for the *HER2* in primary breast cancers,[11,13,18] and other matched case-control studies.[9]

Interestingly, when analyzing the primary tumor and the paired LR from the recurrent group of patients, no differences regarding *HER2* status were observed. Those primary tumors where *HER2* amplification was observed also displayed an oncogenic amplification in the LR. From these observations we can confirm the hypotheses that in our series of recurrent IBC patients the LR is the consequence of a microscopic focus of tumor cells from the primary tumor and that the possibility of a probable contamination by an incidence of secondary tumors can be discarded. In this regard, some authors have emphasized that the importance of LR within a conserved breast depends on the micrometastatic environment at the time of initial clinical presentation. Therefore, in the absence of micrometastases, LR would be a determinant of distant disease; however, in the presence of micrometastases, it would represent a marker of distant relapse. In this regard, the clinical management would be different, depending on the case. Maximum locoregional treatment at primary diagnosis would be appropriate in the former group, whereas minimum treatment would be sufficient in the latter group, with full treatment prescribed at the time of LR.[24,34,35]

We observed that *HER2* amplification/hyperexpression was associated with the histological tumor grade in both the groups, and with lymph node involvement and tumor stage in the recurrent patients. Similar observations have previously been reported.[11,13] However, the most interesting finding was the association between the *HER2* amplification and the development of systemic metastasis. In the nonrecurrent group, 50% of cases with *HER2* amplification developed distant metastasis, compared to 92% in the recurrent group. The association between *HER2* amplification and metastasis was more distinct in the survival analysis. Multivariate analyses for the MFI found that only *HER2* amplification in the recurrent group was independently associated with a shorter MFI (HR = 10.75), in contrast with the nonrecurrent group where the lymph node involvement was the predictor of short MFI (HR = 2.65). Likewise, for OS, we observed that only in the recurrent group was *HER2* amplification (HR = 4.22) an independent predictive factor of poor survival. These associations have extensively been reported by several authors (see Ross *et al.* for review).[13]

HER2-targeted monoclonal antibody therapy of breast cancer with trastuzumab has a major impact on the survival of HER2-positive metastatic breast cancer patients.[10,14,15,36] In addition to chemotherapy, trastuzumab provides a significant clinical benefit in terms of higher response rates and increased survival in patients with HER2-positive advanced breast cancer.[10,15,37] Trastuzumab also has therapeutic action as a monotherapy in the management of overexpressed HER2 or amplified *HER2* metastatic breast cancer.[15] Very early results confirm the benefit of trastuzumab both in combination or in sequential use with chemotherapy in early-stage breast cancer. Three trials in the adjuvant setting have provided a 48–52% risk reduction, with a median follow up of 1–2.4 years, with the addition of trastuzumab to standard chemotherapy regimens. Even though the results are not mature enough, it stresses the ability of trastuzumab to modify the natural history of the disease, showing very early benefits even in OS.[37–39]

Therefore, in spite of the retrospective nature of our study and the number of patients in our series being low, we found in the multivariate analysis an association between HER2 amplification and impaired survival in the recurrent group. In our series, for 92% of patients (11 out of 12) with HER2 amplification, the local relapse precluded distant metastasis. This correlation, together with the presence of HER2 amplification both in the primary tumor and in the LR, suggests a very strong probability of micrometastasic disease being present at the time of primary tumor. One interpretation of the results is that LR for *HER2* amplified tumors is a first evidence of extended disease and not the primary cause itself of distant widespread. In favor of this, the HERA trial testing trastuzumab sequentially with chemotherapy has observed a similar gain in risk reduction for LR and distant metastasis (ASCO 2005, unpublished results).

The evidence of LR as a primary metastatic event in HER2 amplified tumors strongly justifies the use of systemic therapy, including trastuzumab, so as to delay or avoid new metastatic events. In the future, the introduction of trastuzumab in early-stage breast cancer therapy will change the patterns of relapse for both local and distant disease in HER2 amplified tumors. However,

larger retrospective studies together with more mature results from trastuzumab randomized trials in early stages[39] will confirm the utility of HER2-based therapies in the clinical management of IBC patients treated with BCT.

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