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Linking survival of HER2-positive breast carcinoma patients with surgical invasiveness

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ABSTRACT

The early peak of relapse in patients with breast carcinomas that overexpress HER2 oncoprotein and dissemination to the axillary lymph nodes might be related to proliferation of micrometastatic lesions induced by EGF family growth factors released at the time of surgery. If the levels of these growth factors have an impact on relapse, the survival of patients with positive nodes and HER2-positive tumours should be dependent on surgery wideness. To test this hypothesis, HER2 status of primary tumours from patients included in a randomized clinical trial addressing conservative quadrantectomy versus radical mastectomy was retrospectively analyzed. In HER2-negative patients, independently of node infiltration, and in HER2-positive patients without node infiltration, no differences in survival according to the type of surgery were observed. In patients with positive nodes and HER2-positive tumours the estimation of the time-dependent log-hazard ratios showed that radical mastectomy significantly increased early death rates ($P = 0.037$).

1. Introduction

Amplification and overexpression of the *HER2* oncogene, encoding a transmembrane tyrosine kinase receptor of the epidermal growth factor (EGF) receptor family, has been identified in about 25% of breast carcinomas.[1] Several data[2–4] have shown that HER2-positive breast carcinomas represent a particularly aggressive tumour subset with increased proliferation and metastatic potential. While there is no consensus on the prognostic value of *HER2* amplification/overexpression in node-negative cases, its value in node-positive patients has been widely demonstrated.[5–8] Patients with breast carcinomas that overexpress *HER2* oncogene and disseminate to the axillary lymph nodes display an early peak of relapse in the first years after surgery.[2] A simple increase in the proliferation potential of HER2-positive tumours does not satisfactorily explain the poor prognosis associated with HER2 positivity in node-positive patients and certainly cannot explain the confinement of the poor prognosis in HER2-positive/node-positive patients to the first 3–4 years after surgery. It could be possible that processes triggered by surgery itself stimulate recurrence. Some data point to the role of growth factors released during healing after surgery in preferentially stimulating HER2-positive micrometastatic lesions, which are more likely to be present in node-positive patients. Indeed, we found that growth factors of the EGF family, released during the wound healing process, stimulate the growth of HER2-positive carcinomas.[9] These factors are released by degranulation of platelets during blood coagulation, and their serum levels correlate with the invasiveness of surgery, measured as levels of creatine

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phosphokinase.[9] We reasoned that if the levels of growth factors released at surgery have an impact on early relapse, then survival of patients with node-positive and HER2-positive tumours should be poorer after mastectomy than after the less invasive quadrantectomy. However, no differences are expected in node-negative cases due to the low probability of disseminated micrometastatic foci that might be stimulated by surgically-induced growth factors. This hypothesis was tested on archival material from the Milano 1 randomized clinical trial that compared Halsted radical mastectomy with quadrantectomy, axillary dissection, and radiotherapy in patients with small cancers of the breast.

2. Patients and methods

2.1. *Patients*

Of the 701 patients entered into the Milano 1 trial (comparing Halsted radical mastectomy with quadrantectomy, axillary dissection, and radiotherapy in patients with small cancers of the breast,[10]) all node-positive cases (172) and an equivalent number of node-negative cases (162) belonging to the same series were retrieved from archived specimens and included in this study. All patients had primary unilateral breast and tumour characteristics and were well matched in the mastectomy (167 pts.) and quadrantectomy (167 pts.) arms (Table 1). Node-positive patients were post-surgically treated with 12-cycles of CMF. No women had reconstructive surgery at time of mastectomy. Data on reconstructions done years after surgery are not present in the database. No differences in survival for the two types of surgery in the total series were reported.[10]

2.2. *Immunohistochemistry*

Immunohistochemical staining for HER2 protein was performed on samples, using 4 μm-thick sections from formalin fixed-paraffin embedded material using the DAKO HercepTest™ immunocytochemical assay. The staining procedure was performed according to the DAKO HercepTest™ for the DAKO Autostainer manual. Immunostaining was assessed using light microscopy and the determination of HER2 protein overexpression was interpreted according to DAKO's instructions for interpretation of staining of HercepTest™. Briefly, a score was given according to the intensity and pattern of cell membrane staining as follows: 0 or 1+, no staining or membrane staining in <10% or barely perceptible incomplete membrane staining in >10% of the tumour cells; 2+, weak to moderate complete membrane staining in >10% of tumour cells; 3+, strong complete membrane staining in >10% of tumour cells. Scores of 0 and 1+ are interpreted as negative.

Table 1 – Characteristics of patients and tumours according to surgery

	Radical mastectomy (167 pts.) (%)	Quadrantectomy (167 pts.) (%)
Age < 55	64	73
Infiltrated lymph nodes		
1	28	29
2–3	8	15
>3	12	10
Tumour diameter < 1 cm	31	28

2.3. *Statistical analysis*

Overall survival was defined as the time elapsed from the date of surgery and the date of death from any cause or the date of last follow-up. Survival curves were obtained with the Kaplan–Meier method and compared using Cox regression models. Preliminary checks of the proportional hazard assumption re-

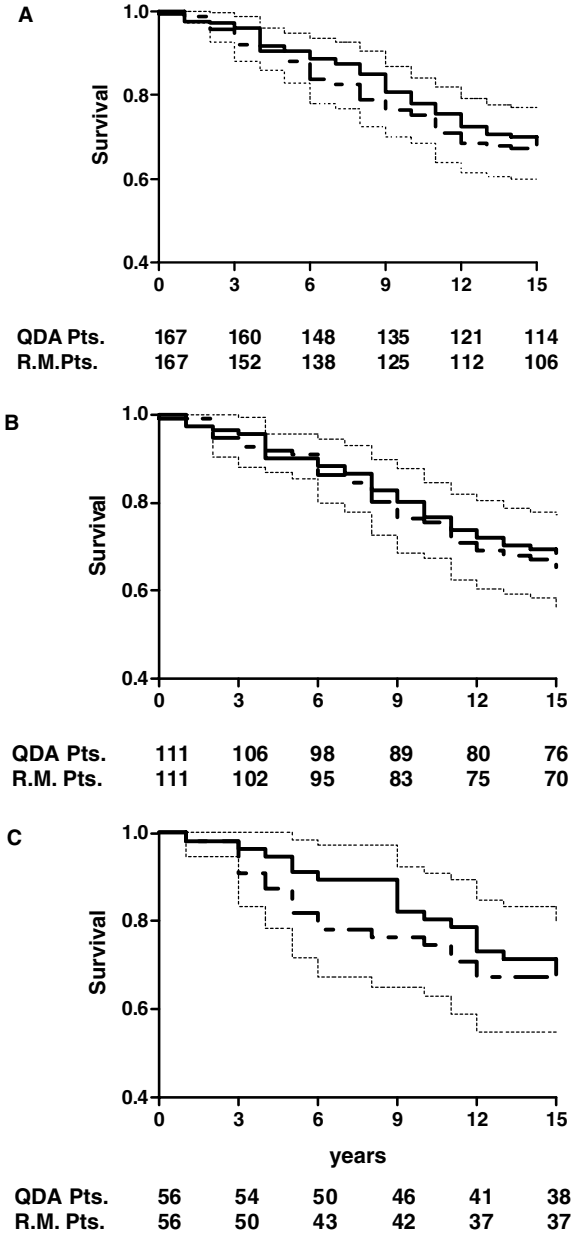

Fig. 1 – *Impact of surgical invasiveness on survival*. A: entire analyzed series; B: HER2-negative subgroup; and C: HER2-positive subgroup. Dashed line: radical mastectomy (RM); solid line: quadrantectomy (QDA). Hair dashed lines represent approximated 95% confidence interval.

lied on the analysis of scaled Shoenfeld's residuals.[11] All the analyses were conducted using SAS software (SAS Institute Inc., Cary, NC, USA). Two-sided *P* values below the conventional 5% thresholds were regarded as statistically significant.

3. Results

To test if early deaths of patients with positive nodes and HER2-positive tumours depend on the levels of growth factors released at surgery, HER2 status was evaluated by immunohistochemistry in all node-positive cases (172) and in an equivalent number of node-negative cases (162), from patients entered into the Milano 1 trial. One hundred and twelve were found to be HER2-positive; in particular, 54 scored 2+ and 58 scored 3+ according to the standard scoring system. Based on the finding that early peak of relapse in the first years after surgery was observed in 2+ and 3+ HER2-positive cases,[2] both HER2 scores were jointly classified as HER2-positive.

As previously reported in the entire series,[10] no difference in overall survival according to the type of surgery was found in our retrieved cases (Fig. 1A). When the analysis was conducted according to HER2 expression no significant differences were found in both HER2-negative and positive cases, even though in patients with HER2-positive tumours, the resulting curves separated slightly at the beginning of follow-up period (Fig. 1B and 1C).

On the basis of the aforementioned rationale, we plotted Kaplan–Meier overall survival curves according to the type of surgery across the strata defined by nodal and HER2 status. Considering node-negative women, overall survival curves according to the type of surgery were overlapping both in HER2-negative (Fig. 2A) and HER2-positive patients (Fig. 2B). Among node-positive women, overall survival curves were again overlapping for HER2-negative patients (Fig. 3A), but not for HER2-positive cases (Fig. 3B). Indeed in the latter, the curves were well separated in the early years after surgery, and tended to converge thereafter. This suggests a time-dependent effect of surgery in the patient subset, that was confirmed as statistically significant ($P = 0.037$) at the analysis of scaled Shoenfeld residuals from a Cox model. The effect of invasive surgery, radical mastectomy versus quadrantectomy, on 5-year survival has been confirmed also when 3+ and 2+ subsets were considered. The survival was 69% vs. 81% and 85% vs. 96%, respectively. No differences in survival were observed in HER2 negative patients (91% vs. 90%).

4. Discussion

These findings support the hypothesis that invasive surgery, such as radical mastectomy, by inducing the release of EGF-like growth factors, accelerates events already set to occur by virtue of prior micrometastatic seeding, thus increasing early death rates, as far as HER2-positive breast carcinoma patients with disseminated disease (node-positive cases) at diagnosis are considered. Indeed, the test of the proportional hazards assumption yielded a significant result ($P = 0.037$), indicating that in HER2-positive subgroup, there is an anticipation of metastases after mastectomy. These data are in keeping with the hypothesis that invasive surgery, by inducing the release of EGF-like growth factors, favours the onset of early relapses, and therefore increases early deaths for HER2-positive breast carcinomas. The lack of significance of the overall survival may rest in the finding that surgery only accelerates events that should have happened later on anyway because micrometastases were already seeded. These data represent a 'proof of principle' that surgery in some cases may enhance tumour burden. Thus, HER2 is not only indicative of the likelihood of tumour developing distant metastases, but also can be considered as a good marker of the rate at which this will occur, suggesting that tumour metastagenicity and virulence[12] depend on different biological pathways. Also in our series, according to previous studies,[13] patients with 3+ HER2-positive tumours presented worse outcome compared to patients scored as 2+. Therefore, the evidence of increase of early deaths for patients presenting both 3+ and 2+ HER2-positive tumours, may be indicative for a role of HER2 in tumour aggressiveness even when its

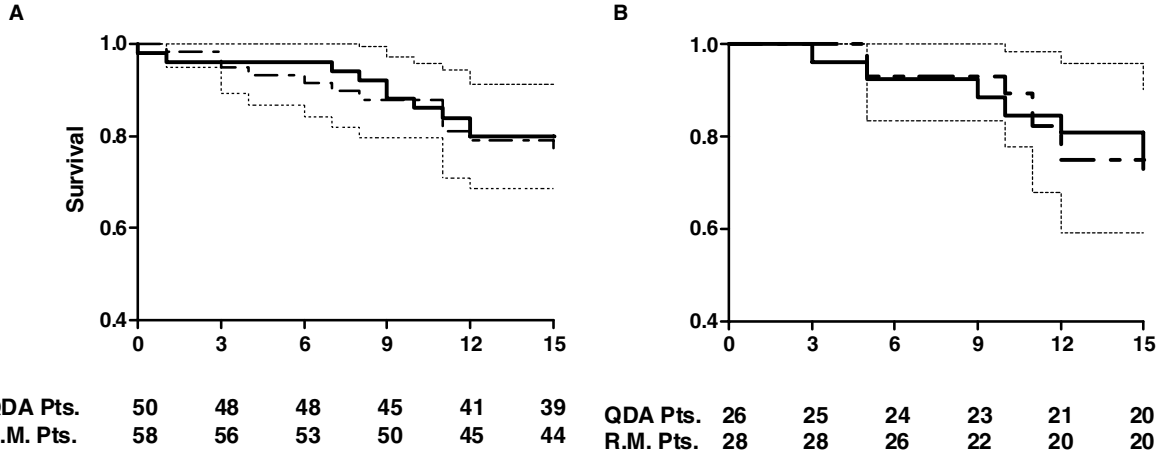

Fig. 2 – *Impact of surgical invasiveness on survival according to HER2 status in lymph node negative cases*. A: HER2-negative subgroup; and B: HER2-positive subgroup. Dashed line: radical mastectomy (RM); solid line: quadrantectomy (QDA). Hair dashed lines represent approximated 95% confidence interval.

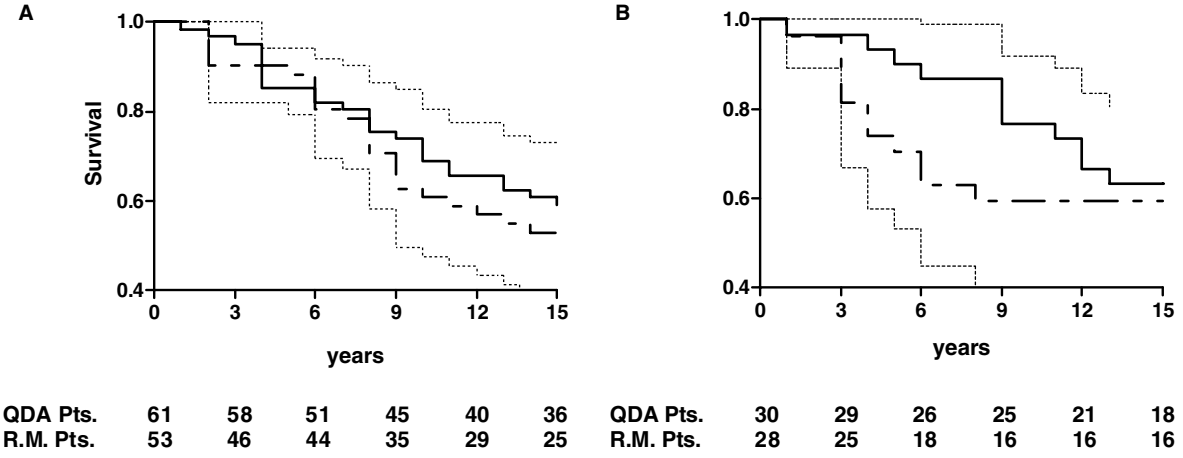

Fig. 3 – *Impact of surgical invasiveness on survival according to HER2 status in lymph node-positive cases*. A: HER2-negative subgroup; and B: HER2-positive subgroup. Dashed line: radical mastectomy (RM); solid line: quadrantectomy (QDA). Hair dashed lines represent approximated 95% confidence interval.

overexpression is not due to the gene amplification. This supports our previous results demonstrating that the peak of relapses in the first years from surgery was higher in both 3+ and 2+ HER2 positive groups.[2] Of course, these data would require a further validation on larger series, since HER2-positive subset is a small subset and the potency of the study was not planned for subset analysis. However, this can be done only by retrospective analysis of clinical trials since today, mastectomy and quadrantectomy are performed on patients at different stage of the disease and therefore the outcomes can no longer be compared.

The possibility remains that if we compared breast conserving surgery with modified radical mastectomy, the increase in early death rate might be smaller than that observed upon the Halsted procedure. Nevertheless, the obtained results will open the question concerning breast reconstruction which by increasing invasiveness of surgery when performed contemporary to modified radical mastectomy may increase the level of growth factors or may induce a second hit of proliferation when performed after.

Two options to avoid the induction of proliferation by surgery can be envisaged: a block of the HER2 receptor by trastuzumab, shown to block in vitro proliferation induced by post-surgical sera[9] or an early timing of chemotherapy, shown to greatly improve disease-free survival when delivered early after or at the time of surgery in estrogen receptor-negative subset,[14] which includes most of the HER2-positive tumours.

Conflict of interest statement

None declared.

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