HER-2-positive Breast Carcinomas As a Particular Subset with Peculiar Clinical Behaviors

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ABSTRACT

Purpose: The association between HER-2-positivity, and prognostic variables and survival have been addressed in many studies with still controversial results because of the small series analyzed.

Experimental Design: A series of 1928 primary breast carcinomas was analyzed for the prognostic potential of HER-2 overexpression.

Results: In our series, HER-2-positivity was not associated with nodal status, unless the number of infiltrated nodes was considered, whereas it was strongly associated with large tumors (P < 10^-4), grade III tumors (P < 10^-4), lymphoid infiltration (P < 10^-4), and absence of hormone receptor expression (P < 10^-4). HER-2 overexpression was a strong prognostic indicator in N+ patients (P < 10^-7), whereas its prognostic impact was weak and not statistically significant in the N- patients. Analysis of the hazard ratio of relapse in relation to time from surgery indicates that the poor prognosis associated with HER-2 positivity in N+ patients was found to be attributable to a peak of relapses in the first 3–4 years from surgery. Multivariate analysis of different prognostic factors in HER-2+ and HER-2– subsets indicated that grade is the most important factor followed by nodal status, lymphoid infiltration, and tumor size in HER-2-negative breast carcinomas, whereas nodal status was the most important prognostic factor, with tumor size showing only borderline significance, in the HER-2-positive group.

Conclusions: Together, the results indicate that HER-2-positive breast carcinomas represent a particular subset of tumors with peculiar clinical and pathological behaviors. Thus, conclusions drawn from clinical trials, which serve as the basis for clinical management of breast carcinomas, might not always be valid for this low-frequency subset.

INTRODUCTION

Amplification and overexpression of the HER-2/neu oncoprotein, which encodes a transmembrane tyrosine kinase receptor of the epidermal growth factor receptor family, has been identified in ~25% of breast carcinomas (1). A variety of clinical studies have evaluated the relationship between HER-2 and breast cancer outcome (2), and most have shown that women with HER-2-positive tumors have a worse prognosis than women with HER-2-negative tumors. However, whereas the prognostic value of amplification/overexpression in node-positive patients has been widely demonstrated, there is no consensus on its value in node-negative cases (3). Moreover, in some studies, the prognostic value of HER-2 was not always independent of cell proliferation rate or other markers of tumor aggressiveness (4). However, these disparate results might well rest in the relatively small number of patients in each study. Indeed HER-2-positive tumors represent a small subset of breast carcinomas so that conclusions drawn from studies performed on a small patient series are necessarily suspect.

Here we report our analysis of HER-2 overexpression and its prognostic potential in a very large series of primary breast carcinomas. The large sample size allowed consideration of HER-2-positive tumors as an independent subset of breast carcinomas and demonstrated that this subset displays peculiar clinical behaviors.

PATIENTS AND METHODS

Patients. Two different series of consecutive patients treated at the Istituto Nazionale Tumori in Milan, Italy, for primary breast carcinoma were considered: the first series consisted of 1211 patients who underwent surgery in 1968–1969 (5) and the second series consisted of 717 patients operated in 1978–1979 (6). In the first series, no postsurgical therapy was given, whereas in the second, women with histological evidence of involvement of one or more axillary nodes treated with adjuvant CMF chemotherapy (cyclophosphamide per os 100 mg/m^2 days 1–14, methotrexate i.v. 40 mg/m^2 days 1 and 8; fluorouracil i.v. 600 mg/m^2 days 1 and 8) for 12 monthly cycles. Relapse was defined based on the first documented evidence of new manifestations of disease in distant sites. For survival evaluation, causes of death were ascertained through medical records, death certificates, family doctors, and, when available, autopsy records.

Pathological Parameters. Primary tumor diameter and axillary nodal status were obtained from histopathological reports. H&E-stained slides of all of the patients were retrospectively reviewed for diagnostic reassessment of the tumor histotype, histological grade, and presence of lymphocytic infiltration. Histological grade was determined and scored as

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2 To whom requests for reprints should be addressed, at Molecular Targeting Unit, Department of Experimental Oncology, Istituto Nazionale Tumori, Via Venezian 1, 20133 Milan, Italy. Phone: 39-02-23902571; Fax: 39-02-23903073; E-mail: menard@istitutotumori.mi.it.
Recurrence was defined as the first event (local recurrence, was defined as the time elapsed from the date of surgery and the survival curves drawn based on the life-table method; statistical SE), whereas the effect on the risk of death was expressed as /H11006 40 magnification). The latter parameter was scored as /H11003 fields (phism (nuclear grade), and the mitotic count/10 high-power Briefly, this method involves semiquantitative evaluation of the described, and assigned a Nottingham/Tenovus grade (7). Briefly, this method involves semiquantitative evaluation of the percentage of tubule formation, the degree of nuclear pleomorphism (nuclear grade), and the mitotic count/10 high-power fields (×40 magnification). The latter parameter was scored as “+” for a mean of >1 mitosis per high-power field and “−” for ≤1 mitosis per high-power field after analysis of ≥30 microscopic fields. Lymphocyte infiltration was scored as present when a moderate or marked infiltrate was observed and absent when a low number of infiltrating cells was found.

Biological Parameters. At the same time of retrospective pathological review, immunocytochemical staining was carried out on Bouin-fixed, paraffin-embedded tissue using a polyclonal antibody against HER-2-specific peptide (kindly provided by Dennis J. Slamon, Los Angeles, CA; Ref. 5) for the first series, anti-HER-2 CB11 (1:10 dilution; Ylem; Avezzano, Aquila, Italy) for the second series, and antigenprostrogen receptor MAb1A6 (1:100 dilution; DBA; Segrè, Milan, Italy) for both series. Consecutive sections were processed (cut, deparaffinized, and rehydrated), washed in PBS, and immunostained by a sensitive peroxidase-streptavidin method using an automated immunostainer (TechMate 1000; Dako). Negative controls were incubated with nonimmune serum from the same species in which the primary antibody was produced. Appropriate cases with known reactivity for each antibody applied were used as positive controls. Sections were scored progesterone receptor-positive when more than ~10% of the tumor cells were labeled in the nucleus. HER-2 was scored as positive when intense membrane labeling was observed in >50% of the tumor cells.

As shown in Fig. 2A, HER-2 was a strong prognostic factor (P = 2.10−6); however, whereas the prognostic value of HER-2 was unquestionable in N+ patients (P < 10−7), it was not statistically significant in N− patients (Fig. 2B). Analysis of the

RESULTS

Table 1 lists the characteristics of the 1211 cases of the first series (1968–1969) and of the 717 cases of the second series (1978–1979). Tumors from the second series were in general less aggressive (28% versus 47% grade III), although the frequency of nodal positivity was the same, attributable probably to more accurate nodal staging at the three axillary levels in the later series. No difference in the frequency of HER-2 expression, as determined by immunohistochemistry (23% in both series), was observed. Survival of patients was 6–9% higher in the second series than in the first. Analysis of survival in patients grouped according to nodal status (N− and N+) revealed improvement in both subgroups (Fig. 1).

Because HER-2-positive tumors represent a small subset of breast carcinoma, additional analyses were carried out with the total number of patients grouped according to HER-2 status. Table 2 lists the distribution of clinical and pathological parameters of the HER-2-positive and HER-2-negative subsets. Nodal status was not associated with HER-2 unless the number of infiltrated nodes was considered; the frequency of N+ was similar in the HER-2-positive and HER-2-negative cases, but the frequency of cases with more than three infiltrated nodes as compared with cases with one to three positive nodes was higher in the HER-2-positive than in the HER-2-negative subset (P = 8.10−4). HER-2 positivity was somewhat more frequent in premenopausal women (P = 0.03), whereas it was strongly associated with large tumors (P < 10−4), grade III tumors (P < 10−4), lymphoid infiltration (P < 10−4), and absence of progesterone receptor expression (P < 10−4).
hazard ratio of relapse in relation to time from surgery (Fig. 3) showed that the poor prognosis associated with HER-2 positivity in N+ patients reflects mostly the peak of relapses in the first 3–4 years from surgery (Fig. 3D). On the other hand, this early peak of relapses was lower in the HER-2-negative N+ group (Fig. 3C) and not evidenced at all in the N− group, independent of HER-2 status (Fig. 3, A and B).

Disease outcome was analyzed according to different prognostic factors in the total series and in the two subsets divided according for HER-2 expression. Univariate analysis of the total series indicated that nodal status, tumor size, grade, and lymphoid infiltration were prognostic factors (Table 3). These parameters remained associated with prognosis even in multivariate analysis (Table 4). Menopausal status, which was weakly associated with prognosis, lost its significance in multivariate analysis, whereas progesterone receptor expression was associated with prognosis only in the first 5–6 years from surgery because only the univariate Gehan’s test revealed a significant $P (P = 0.003; Table 3)$ in keeping with previous data (10). All of these factors maintained their prognostic impact in univariate and multivariate analysis in HER-2-negative breast carcinomas; tumor grade was the most important factor considering grade I tumors (RR$^3$ = 0.384) followed by lymphoid infiltration (RR = 0.474), nodal status (RR = 0.577), and tumor size (RR = 0.745). By contrast, nodal status was the most important prognostic factor in the HER-2-positive group in univariate and multivariate analysis (RR = 0.372), whereas tumor size had borderline significance with the RR of 0.0724 in multivariate analysis as in the HER-2-negative subgroup. Tumor grade provided no prognostic information, because grade II and grade III showed exactly the same survival rates, and grade I was not considered because only 12 cases (1%) occurred among the HER-2-positive tumors. Lymphoid infiltration also gave no prognostic information in either univariate or multivariate analysis. Neither progesterone receptor nor menopausal status was associated with survival in the HER-2 subsets, suggesting that part of their prognostic relevance in the global series was attributable to their association with HER-2.

\begin{table}
\centering
\begin{tabular}{|l|c|c|}
\hline
 & HER−2-negative & HER−2-positive & $P^a$ \\
\hline
No. of cases & 1490 & 438 & \\
Node-positive & 55% & 57% & n.s.$^b$ \\
N1-3 & 44% & 37% & 8.10$^-4$ \\
N>3 & 11% & 20% & \\
Tumor $>2$ cm & 55% & 68% & $<10^-4$ \\
Premenopause & 39% & 45% & 0.03 \\
Grade III & 33% & 59% & $<10^-4$ \\
Mitosis+ & 55% & 79% & $<10^-4$ \\
Lymphoid infiltration & 13% & 27% & $<10^-4$ \\
Progesterone receptor- & 69% & 32% & $<10^-4$ \\
positive & & & \\
\hline
\end{tabular}
\caption{Characteristics of the total number of patients grouped according to HER−2 status}
\end{table}

\(^a P \) by $\chi^2$ test.

\(^b n.s., \) not significant.

DISCUSSION

Our present analysis of two series of breast carcinomas patients, considering HER-2-positive tumors as a special subset, indicates that HER-2 positivity is strongly associated with grade, lymphoid infiltration, and tumor size, weakly associated with menopausal status, and not at all associated with nodal status unless the number of infiltrated nodes is considered. This analysis of the largest series examined to date might help in resolving the controversy surrounding the associations of HER-2 positivity with tumor size and lymph node infiltration. The data indicating the association of HER-2 positivity with large tumors and with the high number of mitosis but not
directly with nodal status, which are consistent with previous studies, suggest that HER-2 confers a high proliferative capability that is not necessarily associated with high metastatic potential (4).

In regard to the prognostic value of HER-2 according to nodal status, we found a very strong prognostic impact of HER-2 status in node-positive cases but not in node-negative patients. This latter finding, in accord with some but not all of the previous reports (2, 11–14) indicating that HER-2 has no prognostic impact for node-negative patients, considerably de-

**Table 3** Prognostic factors in the total series and the two subgroups according to HER-2 status: Univariate analysis

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Total series</th>
<th>HER 2-negative</th>
<th>HER 2-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodal status</td>
<td>$&lt;10^{-7}$</td>
<td>$2.10^{-3}$</td>
<td>$&lt;10^{-7}$</td>
</tr>
<tr>
<td>Tumor size</td>
<td>$&lt;10^{-7}$</td>
<td>$3.10^{-3}$</td>
<td>$2.10^{-3}$</td>
</tr>
<tr>
<td>Menopausal status</td>
<td>0.005</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Grade</td>
<td>$&lt;10^{-7}$</td>
<td>5.10^{-5}</td>
<td>n.s.</td>
</tr>
<tr>
<td>Lymphoid infiltration</td>
<td>0.009</td>
<td>1.10^{-3}</td>
<td>n.s.</td>
</tr>
<tr>
<td>Progesterone receptor-negative</td>
<td>0.003c</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

* $P$ by $\chi^2$ test.
* n.s., not significant.
* $P$ by Gehan’s test.

**Table 4** Prognostic factors in the total series and the two subgroups according to HER-2 status: Multivariate analysis

<table>
<thead>
<tr>
<th>Criterion (adjusted by the others)</th>
<th>Total series</th>
<th>HER 2-negative subgroup</th>
<th>HER 2-positive subgroup</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$</td>
<td>RR</td>
<td>$P$</td>
</tr>
<tr>
<td>Grading</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>0.469</td>
<td>0.391</td>
<td>$&lt;10^{-11}$</td>
</tr>
<tr>
<td>II</td>
<td>0.032</td>
<td>1.066</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N−</td>
<td>-0.329</td>
<td>0.517</td>
<td>-0.477</td>
</tr>
<tr>
<td>N+</td>
<td></td>
<td></td>
<td>0.384</td>
</tr>
<tr>
<td>LI*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPL1+</td>
<td>-0.240</td>
<td>0.618</td>
<td>-0.373</td>
</tr>
<tr>
<td>IPL1−</td>
<td></td>
<td></td>
<td>0.474</td>
</tr>
<tr>
<td>Size*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\leq$2 cm</td>
<td>-0.159</td>
<td>0.727</td>
<td>-0.147</td>
</tr>
<tr>
<td>$&gt;2$ cm</td>
<td></td>
<td></td>
<td>0.745</td>
</tr>
<tr>
<td>$&lt;2$ cm</td>
<td></td>
<td></td>
<td>0.160</td>
</tr>
</tbody>
</table>

* Nodal status.
* Lymphoid infiltration.
* Primary tumor size.

**Fig. 3** Frequency of relapses from the time of surgery.
The differences between HER-2-positive and HER-2-negative tumors in clinical behaviors can be related to their different biology. In fact, a recent biomolecular study of breast carcinomas using microarray technology indicates that HER-2-positive tumors share some markers specific for basal epithelial cells, whereas HER-2-negative/hormone receptor-expressing tumors appear to be of luminal cell origin (18).

Our data identify the HER-2-positive breast carcinoma as a particular subset of tumors with peculiar clinical and pathological behaviors. This finding points to the need for re-evaluation of conclusions from clinical trials on which clinical management of breast carcinoma is based, because these conclusions might not apply to this low-frequency subset of tumors.

**ACKNOWLEDGMENTS**

We thank Laura Mameli for manuscript preparation.

**REFERENCES**


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**Table 5** Prognostic factors in the node-negative, HER 2-negative subgroup: Multivariate analysis

<table>
<thead>
<tr>
<th>Criterion (adjusted by the others)</th>
<th>β</th>
<th>RR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grading</td>
<td></td>
<td></td>
<td>&lt;10⁻³</td>
</tr>
<tr>
<td>I</td>
<td>−0.550</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>0.106</td>
<td>1.23</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>−</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Size 🅗</td>
<td>−0.187</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>≤2 cm</td>
<td>−</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>&gt;2 cm</td>
<td>−</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>LI 🅗</td>
<td>−0.263</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>−</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

* Primary tumor size.
* Lymphoid infiltration.

creases the relevance of HER-2 status for prognostic purposes, because the node-positive cases have a poor prognosis even when HER-2 negative. The difference in HER-2 prognostic value does not appear to be related to the lower number of events in the node-negative group than in the node-positive group, as suggested previously (15). Indeed, our analysis of the death risk from breast carcinoma, which considers the different baseline risks of the node-positive and node-negative groups, indicates that at 10 years, the increase risk attributable to HER-2 positivity in comparison to HER-2-negative cases is notably higher in the node-negative group (43%) than in the node-negative group (7%). A simple increase in the proliferation potential of HER-2-positive tumors does not satisfactorily explain the poor prognosis associated with HER-2 positivity in node-positive patients and certainly cannot explain the confinement of the poor prognosis in HER-2-positive/node-positive patients to the first 3–4 years after surgery. Instead, some data point to the role of growth factors released during healing after surgery in preferentially stimulating HER-2-positive micrometastatic lesions, which are more likely to be present in node-positive patients. This hypothesis, now under investigation in vitro, suggests the rescue of HER-2-positive breast carcinoma cells from dormancy by growth factors present in the postsurgical sera of breast carcinoma patients.

Our analysis also shows that the HER-2-positive and HER-2-negative subgroups differ greatly in regard to prognostic factors: i.e., nodal status, tumor grade, lymphoid infiltration, and, to a lesser extent, tumor size, are significant and independent factors in HER-2-negative breast carcinomas, whereas only nodal status is important for prognosis in the HER-2-positive group. It must be noticed that the COX regression model for multivariate analyses might be not statistically corrected considering the lack of proportional hazard observed in the HER-2-positive group. Nevertheless, the univariate analysis shows a different prognostic impact of the variables in the two subgroups according to HER-2, which is a clinically important finding that raises concerns regarding the therapeutic management of HER-2-positive and HER-2-negative breast carcinoma patients.

Indeed, if the two subsets divided according to HER-2 substantially differ for prognostic factors, they might differ for other behaviors, and therapeutic strategies established for breast carcinomas in general might not be valid for some subsets that are poorly represented in the total series and, therefore, have little weight on the final outcome.

A substantial improvement in prognosis in the 1978–1979 series in comparison to the 1968–1969 one was observed both in the postsurgically untreated (node-negative) and CMF-treated (node-positive) groups in the second patient series recruited 10 years after the first. Thus, the impact of adjuvant therapy in this series appears to be modest, as suggested previously (16). It is noteworthy that in 1978–1979, chemotherapy was just introduced recently in the clinical management of breast carcinoma patients, and the institutional database reported only the “intent to treat,” with no information on the actual drug delivery or actual doses administered. A differential impact of adjuvant treatment according to HER-2 status is unlikely, because none of the patients received a doxorubicin-containing regimen suggested to be more active on HER-2-positive tumors (17).

The differences between HER-2-positive and HER-2-negative tumors in clinical behaviors can be related to their different biology. In fact, a recent biomolecular study of breast carcinomas using microarray technology indicates that HER-2-positive tumors share some markers specific for basal epithelial cells, whereas HER-2-negative/hormone receptor-expressing tumors appear to be of luminal cell origin (18).

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