original article

*Annals of Oncology* 19: 1706–1712, 2008
doi:10.1093/annonc/mdn369
Published online 9 June 2008

# Biology, prognosis and response to therapy of breast carcinomas according to HER2 score

S. Ménard[1]*, A. Balsari[1,26], E. Tagliabue[1], T. Camerini[2], P. Casalini[1], R. Bufalino[2], F. Castiglioni[1], M. L. Carcangiu[1], A. Gloghini[4], S. Scalone[5], P. Querzoli[6], M. Lunardi[6], A. Molino[7], M. Mandarà[7], M. Mottolese[8], F. Marandino[8], M. Venturini[9], C. Bighin[9], G. Cancello[10], E. Montagna[10], F. Perrone[11], A. De Matteis[12], A. Sapino[13], M. Donadio[14], N. Battelli[15], A. Santinelli[16], L. Pavesi[17], A. Lanza[17], F. A. Zito[18], A. Labriola[18], R. A. Aiello[19], M. Caruso[19], F. Zanconati[20], G. Mustacchi[21], M. Barbareschi[22], M. Frisinghelli[23], R. Russo[24] & G. Carrillo[25] On the behalf of the OMERO group

[1]*Department of Experimental Oncology,* [2]*Scientific Directorate,* [3]*Department of Pathology, Fondazione IRCCS 'Istituto Nazionale dei Tumori', Milano;* [4]*Department of Pathology,* [5]*Division of Medical Oncology, Istituto CRO, Aviano;* [6]*Department of Pathology, Università di Ferrara, Ferrara;* [7]*Department of Medical Oncology, Università di Verona, Verona;* [8]*Department of Pathology, Istituto Tumori Regina Elena, Roma;* [9]*Department of Oncology, Istituto Nazionale Tumori, Genova;* [10]*Division of Medical Oncology, Università degli Studi Federico II, Napoli;* [11]*Clinical Trails Unit,* [12]*Medical Oncology, Istituto Nazionale Tumori, Pascale, Napoli;* [13]*Department of Biomedical Sciences and Human Oncology;* [14]*UO Medical Oncology, Az. Osp. Molinette, Torino;* [15]*OU Medical Oncology,* [16]*Division of Pathology, Ospedale Regionale Torrette, Ancona;* [17]*Division of Medical Oncology, Fondazione Maugeri, Pavia;* [18]*Department of Pathology, Istituto Oncologico, Bari;* [19]*UO Medical Oncology, Humanitas, Centro Catanese di Oncologia, Catania;* [20]*Department of Clinical Sciences,* [21]*Department of Medical Oncology, Ospedale Maggiore, Trieste;* [22]*Department of Pathology,* [23]*Division of Medical Oncology, Ospedale S.Chiara, Trento;* [24]*Department of Pathology, A.O.R.N. 'San Giovanni di Dio e Ruggi D'Aragona', Salerno;* [25]*Department of Pathology, Ospedale Cardarelli, Napoli;* [26]*Chair od Immunology, Institute of Pathology, University of Milan, Italy*

*Received 25 February 2008; revised 22 April 2008; accepted 28 April 2008*

**Background:** The standardization of the HER2 score and recent changes in therapeutic modalities points to the need for a reevaluation of the role of HER2 in recently diagnosed breast carcinoma.
**Patients and methods:** A multicenter, retrospective study of 1794 primary breast carcinomas diagnosed in Italy in 2000/2001 and scored in HER2 four categories according to immunohistochemistry was conducted.
**Results:** Ductal histotype, vascular invasion, grade, MIB1 positivity, estrogen and progesterone receptor expression differed significantly in HER2 3+ tumors compared with the other categories. HER2 2+ tumors almost showed values intermediate between those of the negative and the 3+ subgroups. The characteristics of HER2 1+ tumors were found to be in between those of HER2 0 and 2+ tumors. With a median follow-up of 54 months, HER2 3+ status was associated with higher relapse rates in node-positive and node-negative subgroups, while HER2 2+ only in node positive. Analysis of relapses according to type of therapy provided evidence of responsiveness of HER2-positive tumors to chemotherapy, especially taxanes.
**Conclusions:** The present prognostic significance of HER2 is correlated to receptor expression level and points to the need to consider HER2 2+ and HER2 3+ tumors as distinct diseases with different outcomes and specific features.
**Key words:** HER2, HeceptTest, prognosis, therapy

## introduction

HER2 is a member of the HER family of transmembrane receptor tyrosine kinases and its overexpression or gene amplification in breast tumors has been associated with poor patient outcome [1, 2]. Most studies on the role of HER2 in outcome of primary breast carcinoma patients have been retrospective and suffer from the low frequency (~20%) of this tumor subset. Moreover, those studies were usually conducted on the basis of the tumors classified as HER2 positive or negative instead of the four categories more recently used in the HercepTest to score the extent of HER2 expression. The high variation in frequency of HER2 positivity in previous studies, ranging from 10% [3] to 50% [4], reflects the inclusion of only strongly positive tumors in some studies and the additional inclusion of lower level positive cases in others. Finally, most previous analyses of the prognostic value of HER2 expression were carried out on patients treated >15 years ago, when mastectomy and now-outdated adjuvant treatments predominated. Notwithstanding these limitations, the majority of the studies concur on the poor prognosis associated with HER2 positivity. While the prognostic value of HER2

*Correspondence to:* Dr S. Ménard, Fondazione IRCCS 'Istituto Nazionale dei Tumori', via G. Venezian, 1, 20133 Milan, Italy. Tel: +39-02-23902571; Fax: +39-02-23903073; E-mail: sylvie.menard@istitutotumori.mi.it

amplification/overexpression in node-positive patients has been widely demonstrated, its value in node-negative cases remains controversial, with some studies arguing for a prognostic impact of HER2 positivity in node-negative patients, while others, including our study [1, 2, 5–8], do not. Consistent with the latter view, HER2 was not among 70 prognostic genes associated with poor and good molecular prognostic signatures in node-negative patients [9]. The prognostic impact of HER2 in node-negative patients assumes particular current relevance in light of adjuvant treatment with trastuzumab.

The standardization of the HER2 score and recent changes in therapeutic modalities in both the surgical and pharmaceutical adjuvant arenas point to the need for a reevaluation of the role of HER2 in recently diagnosed breast carcinomas. Such analyses are possible even with a relatively short follow-up period since the prognostic impact of HER2 positivity is related to the first 3–4 years after surgery [8].

We report the results of a national multicentric study designed to evaluate the biopathologic and prognostic predictive role of HER2 expression score in four categories according to immunohistochemical tests.

## patients and methods

Cases consisted of consecutive HER2-positive primary breast carcinomas surgically treated in 2000–2001 classified as HER2 3+ (485 patients) or 2+ (433 patients by immunohistochemistry). As controls a case successive to HER2 3+ or 2+ was enrolled for a total of 280 patients scored 1+ and 596 scored 0. Case number from each of the 20 participating oncologic centers, including eight National Cancer Institutes, ranged from 52 to 459. Median follow-up was 54 months. To ensure rapid and homogeneous data gathering for patients enrolled in the study, a Web-based system was developed with structured forms for data entry using the Microsoft SQL server. Each participating center had Internet access via exclusive login and password to insert and update all data. The database was accessible to a single administrator who has supervised and monitored data accrual in each center since the beginning of the project.

At the end of the data entry period, access to the system was restricted for all participating centers and the data were analyzed with standard statistical software (STATA-8). A Web site dedicated to the 'Omero Study' is available at: www.progettomero.it, where newsletters compiled during the project are also available.

### statistical analyses

Contingency tables were evaluated by the chi-squared test. Relapse-free survival rates were calculated by the Kaplan–Meier method, considering recurrences of all types (local, regional, controlateral, and distant) as events and date of surgery of the primary tumor as time zero. Survival curves were compared using the log-rank test. The Cox proportional hazards regression analysis mode was used to assess the association with prognosis at the multivariate level for each prognostic factor found significant at the univariate level. All statistical tests were two sided at the conventional 5% significance level. Analyses were carried out using STATA 8 software (Stata Corporation, College Station, TX).

## results

HER2 status was determined using: HercepTest (12% of the cases), polyclonal anti-p185 antibody utilized in HercepTest (19% of the cases), Tab 200 (10% of the cases), or CB11 (20% of the cases); the reagent used was not reported in 39% of the cases. All participating centers were included in the Italian quality control trial for HER2 determination. An internal quality control was also provided by a parallel program of telepathology, in which 120 randomly selected cases of this series were scanned and scored for HER2 status by five pathologists of the network. Overall agreement was 73.15% (kappa 0.6404 $\pm$ 0.055; $Z = 11.54$; $P < 0.00001$). This relative low concordance might be related to a staining decrease during the about 4-year period between the first and the reevaluation of HER2 status. Data concerning FISH in HER2+ cases were only available for 22 carcinomas which resulted amplified in six cases.

Analysis of HER2 status according to patient age, height, weight, blood group and smoking revealed no significant differences among the four HER2 subgroups (Supplementary Table S1, available online).

Table 1 lists the patients' pathologic and biologic parameters recorded in the database according to HER2 score. Several pathologic and biologic parameters differed significantly among the four HER2 categories, with the highest or lowest expression in patients with HER2 3+ tumors, including frequency of ductal histotype ($P < 0.0001$), vascular invasion ($P = 0.002$), grade ($P < 0.0001$), MIB1 positivity ($P < 0.0001$), and both estrogen and progesterone receptor expression ($P < 0.0001$). For p53 positivity in immunohistochemistry and presence of necrosis within tumor mass, HER2 3+ tumors differed from the other three categories but the differences were less significant ($P = 0.05$ and $P = 0.019$, respectively). An association between HER2 expression level and lymph node infiltration was also observed ($P < 0.0001$), despite the highest frequency of positive lymph nodes occurring in the HER2 1+ cases (63.7% versus 47.3%, 56.8% and 59.1% in HER2 0, 2+ and 3+ cases, respectively).

Clinical data included type of surgery, therapy, and follow-up. Mastectomy was carried out in 38% of the cases, axillary dissection in 89%, and sentinel node biopsy in 11%. For adjuvant therapy, 32% of patients received hormone therapy (HT) alone (25% in the mastectomy group and 35% in the quadrantectomy group), whereas the remaining patients received chemotherapy with or without HT depending on hormone receptor expression (14% received anthracyclines, taxanes, or both; 28% received cyclophosphamide, methotrexate and fluorouracil (CMF) and 26% received anthracycline and/or taxanes plus CMF; the frequency of these treatments in mastectomized and quadrantectomized patients was superimposable).

At 54 months, a total of 310 neoplastic events were observed, including 46 local relapses, 24 controlateral tumors, 28 regional relapses, and 174 distant relapses. Event details were not reported for 38 cases.

Analysis of relapse-free survival (RFS) according to the HER2 categories (Figure 1A) indicated worst prognosis for the 3+ cases ($P = 0.0002$). The 2+ cases showed an RFS similar to that of the 3+ group in the first 18 months, eventually reaching the RFS of the negative cases thereafter. RFS values for 0 and 1+ cases were superimposable. Analysis of RFS according to nodal status (Figure 1B and C) indicated that both 3+ and 2+ tumors were associated with higher relapse rates, the former in both node-positive and node-negative subgroups and the

**Table 1.** Pathobiological tumor characteristics according to HER2 score

| Parameters | No. of patients (1794) | HER 2 score | | | | *P* value[a] |
|---|---|---|---|---|---|---|
| | | 0 | 1+ | 2+ | 3+ | |
| | | *N* = 596 | *N* = 280 | *N* = 433 | *N* = 485 | |
| Pathologic | | | | | | |
| Histotype | | | | | | <0.0001 |
| Ductal histotype | 1354 | 428 (72.1) | 197 (70.6) | 322 (75.6) | 407 (87.5) | |
| Lobular histotype | 208 | 84 (14.1) | 46 (16.5) | 58 (13.6) | 20 (4.3) | |
| Mixed | 72 | 31 (5.2) | 16 (5.7) | 9 (2.1) | 16 (3.5) | |
| Other | 130 | 51 (8.6) | 20 (7.2) | 37 (8.7) | 22 (4.7) | |
| Missing | 30 | 2 | 1 | 7 | 20 | |
| Necrosis | | | | | | 0.019 |
| Yes | 198 | 61 (18.5) | 27 (13.8) | 41 (15.8) | 69 (24.0) | |
| No | 874 | 269 (81.5) | 168 (86.2) | 219 (84.2) | 218 (76.0) | |
| Missing | 722 | 266 | 85 | 173 | 198 | |
| Vascular invasion | | | | | | 0.002 |
| Yes | 282 | 89 (20.0) | 28 (12.6) | 71 (21.3) | 94 (25.7) | |
| No | 1084 | 356 (80.0) | 194 (87.4) | 263 (78.7) | 271 (74.3) | |
| Missing | 428 | 151 | 58 | 99 | 120 | |
| Grade | | | | | | <0.0001 |
| I | 152 | 84 (14.7) | 23 (8.6) | 31 (7.9) | 14 (3.1) | |
| II | 796 | 315 (55.3) | 135 (50.4) | 198 (50.1) | 148 (33.1) | |
| III | 732 | 171 (30.0) | 110 (41.0) | 166 (42.0) | 285 (63.8) | |
| Missing | 114 | 26 | 12 | 38 | 38 | |
| Biologic | | | | | | |
| Estrogen receptor | | | | | | <0.0001 |
| Negative | 445 | 110 (20.1) | 36 (14.3) | 86 (20.6) | 213 (46.9) | |
| Positive | 1225 | 437 (79.9) | 216 (85.7) | 331 (79.4) | 241 (53.1) | |
| Missing | 124 | 49 | 28 | 16 | 31 | |
| Progesterone receptor | | | | | | <0.0001 |
| Negative | 599 | 149 (27.4) | 59 (23.4) | 137 (32.8) | 254 (56.7) | |
| Positive | 1061 | 394 (72.6) | 193 (76.6) | 280 (67.2) | 194 (43.3) | |
| Missing | 134 | 53 | 28 | 16 | 37 | |
| P53 | | | | | | 0.05 |
| Positive | 345 | 120 (49.6) | 42 (49.4) | 84 (53.9) | 99 (63.1) | |
| Negative | 295 | 122 (50.4) | 43 (50.6) | 72 (46.1) | 58 (36.9) | |
| Missing | 1154 | 354 | 195 | 277 | 328 | |
| MIB-1 | | | | | | <0.0001 |
| Positive | 675 | 234 (56.4) | 75 (68.2) | 155 (68.6) | 211 (84.7) | |
| Negative | 325 | 181 (43.6) | 35 (31.8) | 71 (31.4) | 38 (15.3) | |
| Missing | 794 | 181 | 170 | 207 | 236 | |
| Stage | | | | | | 0.201 |
| T | | | | | | |
| T1 | 1049 | 364 (61.5) | 169 (61.7) | 264 (62.0) | 252 (53.9) | |
| T2 | 532 | 172 (29.1) | 79 (28.8) | 117 (27.4) | 164 (35.0) | |
| T3 | 53 | 21 (3.5) | 6 (2.2) | 11 (2.6) | 15 (3.2) | |
| T4 | 126 | 35 (5.9) | 20 (7.3) | 34 (8.0) | 37 (7.9) | |
| Missing | 34 | 4 | 6 | 7 | 17 | |
| N | | | | | | <0.0001 |
| N+ | 761 | 227 (47.3) | 130 (63.7) | 184 (56.8) | 220 (59.1) | |
| N− | 619 | 253 (52.7) | 74 (36.3) | 140 (43.2) | 152 (40.9) | |
| Missing | 414 | 116 | 76 | 109 | 113 | |

[a]Chi-squared *P* value calculated for evaluable data only.

latter only in the node-positive subgroup. Multivariate analysis, carried out inserting the category 'missing', identified HER2 3+ as an independent prognostic factor (Table 2). The parameter dictating prognosis was tumor size in every considered category, with nodal status, vascular invasion, and MIB1 positivity also significantly associated with prognosis. Progesterone receptor expression represented a protective factor with a hazard ratio (HR) of 0.70. Unexpectedly, patient's height >160 cm was significantly associated with decreased HR. Missing category of T, N, and age, representing the mean of

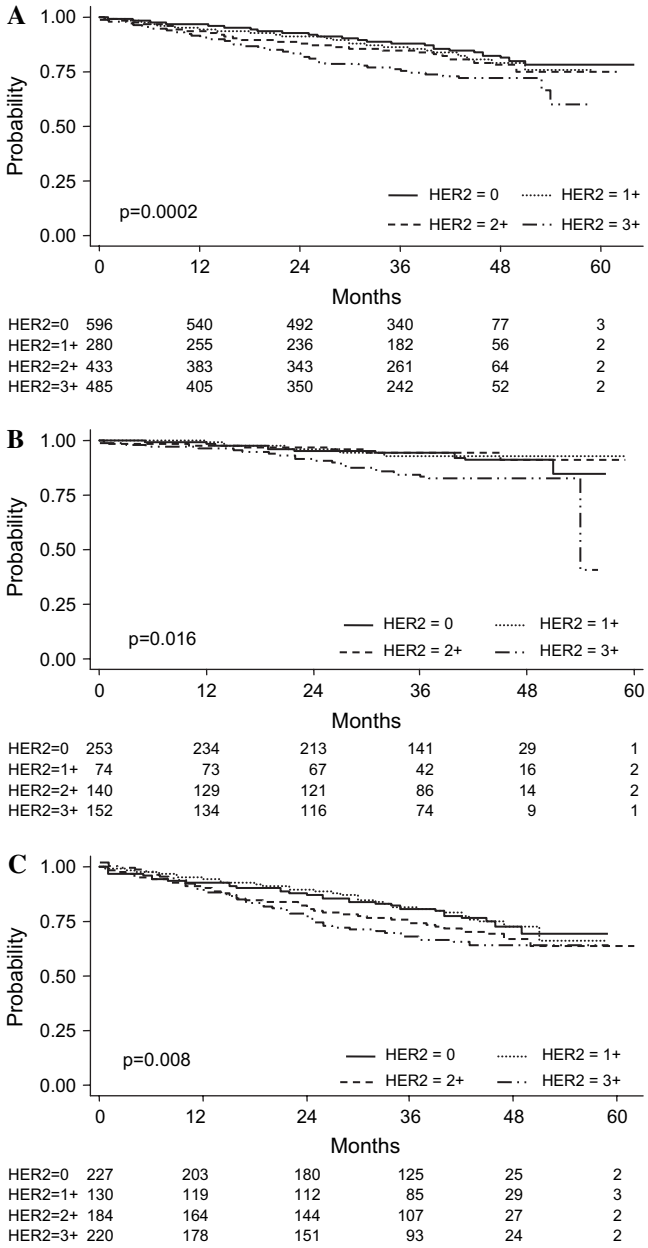


**Figure 1.** Disease-free survival according to HER2 score of entire series (A) and lymph node-negative (B) or -positive (C) patients.

each variable strata (Supplementary Figure S1, available online), was found associated with prognosis. Similar analyses considering only the HER2 3+ subgroup (Table 3) identified tumor size and nodal status as parameters dictating prognosis, but, interestingly, worse prognosis for T2 tumors was not observed. Estrogen receptor expression was found to be an independent prognostic factor. When the HER2 2+ subgroup was analyzed concerning tumor size, only T4 was associated with worse prognosis (Table 3).

Analysis of relapse frequency according to surgical invasiveness (Figure 2A) revealed a higher relapse rate in patients who underwent invasive surgery (mastectomy with or without reconstruction) than in quadrantectomized patients as expected considering the more advanced stage of disease in the former. Among mastectomized patients, relapse frequency for HER2 2+ and 1+ cases was somewhat similar to that of the HER2 3+ cases, while among cases who underwent conservative surgery, relapse frequencies among HER2 2+, 1+, and 0 subgroups were essentially the same (Figure 2A).

Relapse rates among the HER2 subgroups showed a different visual trend depending on type of therapy (HT, chemotherapy, or both) (Figure 2B). Note that the trend of relapse rate in HER2 3+ patients treated with HT only was comparable to that of the entire patient series (Figure 2B). When type of chemotherapy was considered (Figure 2C), regimens including taxanes reversed the visual trend of relapse rate, with fewer relapses in HER2 2+ and 3+ than in the HER2 1+ and 0 cases; treatment with anthracyclines alone decreased the relapse rate in the HER2 3+ subgroup. The relapse rate in the four subgroups treated with CMF alone appeared to be similar to that of the entire series (Figure 2C).

**Table 2.** Multivariate analysis[a]

| Parameter | Hazard ratio | $P > \|z\|$ | 95% confidence interval |
|---|---|---|---|
| HER2 = 1+ | 1.132002 | 0.516 | 0.778761–1.645470 |
| HER2 = 2+ | 1.193688 | 0.283 | 0.864057–1.649069 |
| HER2 = 3+ | 1.401578 | **0.033** | 1.027065–1.912656 |
| Age >50 | 1.144098 | 0.279 | 0.896550–1.459996 |
| Age missing | 3.66656 | **0.001** | 1.721824–7.807804 |
| Height >160 | 0.6741441 | **0.028** | 0.474368–0.958055 |
| Height missing | 0.8040753 | 0.140 | 0.601759–1.074412 |
| Blood group B | 0.9164832 | 0.812 | 0.447486–1.877021 |
| Blood group AB | 1.392653 | 0.487 | 0.547683–3.541253 |
| Blood group 0 | 1.058672 | 0.778 | 0.711710–1.574780 |
| Blood group missing | 1.481669 | **0.045** | 1.008984–2.175797 |
| Vascular invasion no | 0.534094 | **0.000** | 0.396553–0.719340 |
| Vascular invasion missing | 0.8926212 | 0.486 | 0.648406–1.228818 |
| Grading II | 1.034842 | 0.906 | 0.584765–1.831329 |
| Grading III | 1.327571 | 0.344 | 0.738331–2.387069 |
| Grading missing | 1.206596 | 0.601 | 0.597002–2.438642 |
| ER positive | 0.9548555 | 0.785 | 0.685384–1.330274 |
| ER missing | 1.232484 | 0.793 | 0.258053–5.886464 |
| PGR positive | 0.7034678 | **0.026** | 0.515769–0.959473 |
| PGR missing | 0.5470443 | 0.437 | 0.119405–2.506243 |
| MIB-1 positive | 1.505257 | **0.046** | 1.007151–2.249711 |
| MIB-1 missing | 1.352388 | 0.167 | 0.881128–2.075697 |
| T2 | 1.423145 | **0.012** | 1.080606–1.874264 |
| T3 | 3.421668 | **0.000** | 2.190447–5.344940 |
| T4 | 2.738686 | **0.000** | 1.876386–3.997260 |
| T missing | 2.203058 | **0.019** | 1.137508–4.266751 |
| N+ | 2.29032 | **0.000** | 1.654984–3.169555 |
| N missing | 1.775668 | **0.005** | 1.184885–2.661015 |

Log likelihood = −2095.7425; prob > $\chi^2$ = 0.0000.
[a]No. of subjects = 1794; No. of failures = 310; time at risk = 60608.
ER, estrogen receptor; PGR, progesterone receptor. Significant *P* values are given in bold.

## discussion

This is the first large and recent patient series to allow analysis of the biology and prognosis of breast carcinomas classified according to the HercepTest-like score of HER2 expression

**Table 3.** Multivariate analysis of HER2 3+[a] and HER2 2+[b] patients data

| Parameter | HER2 3+ | | | HER2 2+ | | |
|---|---|---|---|---|---|---|
| | Hazard ratio | $P > \|z\|$ | 95% confidence interval | Hazard ratio | $P > \|z\|$ | 95% confidence interval |
| Age >50 | 1.304837 | 0.211 | 0.859782–1.980270 | 1.185974 | 0.521 | 0.704296–1.997081 |
| Age missing | 2.944244 | 0.304 | 0.375953–23.057590 | 5.292267 | **0.046** | 1.033023–27.112760 |
| Height >160 | 0.984496 | 0.958 | 0.548645–1.766594 | 0.451991 | **0.017** | 0.235021–0.8692673 |
| Height missing | 0.918885 | 0.747 | 0.549214–1.537378 | 0.351161 | **0.001** | 0.193429–0.6375174 |
| Blood group B | 0.570228 | 0.464 | 0.126857–2.563192 | 1.409425 | 0.601 | 0.390014–5.093350 |
| Blood group AB | 1.416838 | 0.741 | 0.179790–11.165430 | 1.410015 | 0.580 | 0.417582–4.761081 |
| Blood group 0 | 1.084340 | 0.817 | 0.546283–2.152351 | 1.320024 | 0.487 | 0.602819–2.890523 |
| Blood group miss | 1.368879 | 0.337 | 0.720957–2.599085 | 3.128732 | **0.014** | 1.264201–7.743203 |
| Vascular invasion no | 0.689147 | 0.150 | 0.415208–1.143822 | 0.636456 | 0.161 | 0.338221–1.197667 |
| Vascular invasion miss | 1.097194 | 0.756 | 0.611849–1.967537 | 0.713891 | 0.355 | 0.349555–1.457968 |
| Grading II | 0.570180 | 0.403 | 0.152610–2.130293 | 1.283642 | 0.694 | 0.370685–4.445118 |
| Grading III | 0.800130 | 0.744 | 0.209738–3.052401 | 1.969249 | 0.306 | 0.538344–7.203459 |
| Grading missing | 0.541447 | 0.398 | 0.130572–2.245235 | 1.994960 | 0.342 | 0.480618–8.280715 |
| ER positive | 0.521561 | **0.027** | 0.293068–0.928199 | 1.418014 | 0.339 | 0.693357–2.900042 |
| ER missing | $1.22 \times 10^{8}$ | – | – | 0.483598 | 0.349 | 0.105848–2.209470 |
| PGR positive | 0.888615 | 0.682 | 0.505539–1.561968 | 0.456553 | **0.010** | 0.250483–0.8321531 |
| PGR missing | $4.86 \times 10^{-9}$ | **0.000** | $2.11 \times 10^{-9}$ to $1.12 \times 10^{-8}$ | – | – | – |
| MIB-1 positive | 0.905467 | 0.843 | 0.339767–2.413039 | 1.261965 | 0.591 | 0.539537–2.951706 |
| MIB-1 missing | 0.770163 | 0.598 | 0.291743–2.033126 | 1.293852 | 0.597 | 0.497951–3.361885 |
| T2 | 1.319874 | 0.274 | 0.802329–2.171262 | 1.052448 | 0.869 | 0.574155–1.929176 |
| T3 | 6.303701 | **0.000** | 2.873910–13.826690 | 1.844534 | 0.242 | 0.661502–5.143308 |
| T4 | 2.675114 | **0.006** | 1.330930–5.376859 | 4.407831 | **0.000** | 2.098214–9.259767 |
| T missing | 3.671057 | **0.003** | 1.537969–8.762634 | 3.241387 | 0.126 | 0.717816–14.636880 |
| N+ | 2.370482 | **0.002** | 1.376601–4.081927 | 3.683241 | **0.002** | 1.631889–8.313224 |
| N missing | 1.127768 | 0.750 | 0.538816–2.360474 | 2.989023 | **0.024** | 1.158065–7.714814 |

[a]No. of subjects = 485; No. of failures = 108; time at risk = 4995; log likelihood = −589.29834; prob > $\chi^2$ = 0.0000.
[b]No. of subjects = 433; No. of failures = 75; time at risk = 14762; log likelihood = −388.8725; prob > $\chi^2$ = 0.0000.
ER, estrogen receptor; PGR, progesterone receptor. Significant *P* values are given in bold.

levels. The significant association of HER2 3+ tumors with the majority of the pathobiologic parameters distinguishes this HER2 subgroup from the other three categories. The characteristics of HER2 3+ tumors found in this study largely resembled those previously described in tumors generally classified as HER2 positive [1, 2] and probably reflect the amplification of HER2 as a driven event that conditions other pathologic and biologic features, i.e. high vascular invasion and high proliferation rates. In our study, HER2 2+ tumors almost showed intermediate values of parameters between the negative and the 3+ subgroups, suggesting that oncoprotein expression level in HER2-positive carcinomas correlated with aggressiveness. This assumption is also supported by characteristics of HER2 1+ tumors which were in between those of HER2 0 and 2+ tumors.

Follow-up of the patients clearly indicated worsened prognosis with increased HER2 expression, even if considerable improvement compared with results in other previous retrospective studies was observed [10]. The prognostic improvement observed in our study may reflect more appropriate therapies for these tumors. Thus, multivariate analysis in the present study indicated borderline prognostic significance of HER2.

Follow-up according to the four HER2 categories indicated that not only HER2 3+ cases but also patients with HER2 2+ tumors fared significantly worse than HER2-negative (0 and 1+) patients, especially in the first years from surgery. However, when 2+ were considered according to nodal status, this was no longer prognostic in node-negative patients. The contrasting data reported so far concerning the prognostic significance of HER2 in node-negative patients may well rest in the cut-off used for HER2 positivity, i.e. studies in which 2+ were included as positive were likely to find no prognostic value for HER2 positivity, whereas analyses in which positivity was restricted to 3+ cases were expected to find that HER2 was associated with poor prognosis, even in node-negative patients. Accordingly, HER2 was no longer prognostic in node-negative patients when we considered 2+ and 3+ tumors together.

The multivariate analysis of prognostic factors in the total series indicated that tumor size had the most significant impact on prognosis followed by nodal status, estrogen receptor expression, vascular invasion, and proliferation measured as MIB1 positivity. Note that when multivariate analysis was restricted to the HER2 3+ subgroup, T2 tumor size was not associated with relapses, suggesting that even patients with tumors <2 cm should receive adjuvant treatment.

The impact of type of surgery on relapse of HER2 2+ and 1+ tumors might rest in the stimulation of micrometastatic cells by growth factors released during the surgical maneuver [11]. Tumors expressing intermediate levels of HER2, most of which are likely to be without HER2 amplification, could be more responsive to exogenous growth factors since growth deregulation is still factor dependent.

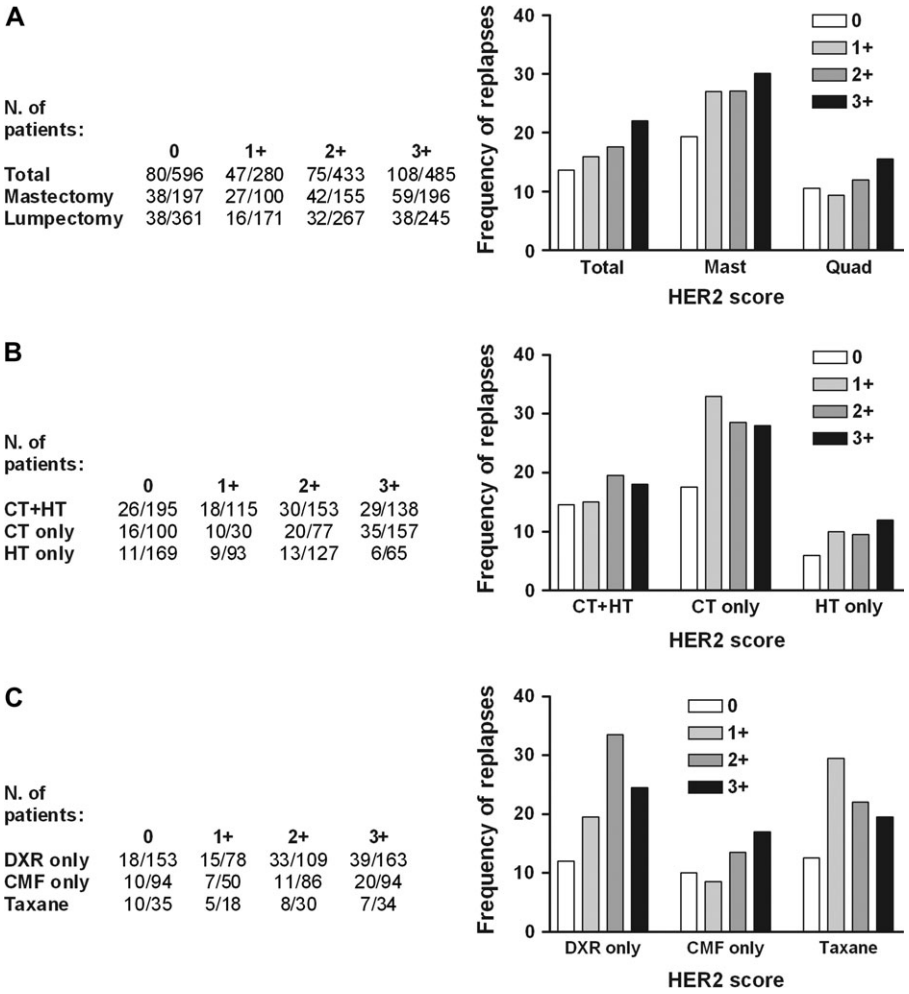


**Figure 2.** Frequency of relapse according to HER2 score in patients who underwent different surgery (A), adjuvant therapy (B), or chemotherapy (C). (Mast, mastectomy; Quad, quadrantectomy; CT, chemotherapy; HT, hormone therapy; DXR, doxorubicin; CMF, cyclophosphamide, methotrexate and fluorouracil).

With respect to the impact of different therapy regimens, we found no difference in prognosis between HER2 3+ patients treated with HT and those who received no HT, arguing against a previously reported particular HT resistance of HER2-positive tumors expressing hormone receptor [12]. Patients treated with chemotherapy alone, most of whom were hormone receptor negative, displayed an improved prognosis according to HER2 expression level. Note that the majority of estrogen receptor-positive patients were treated with CMF plus HT, whereas the receptor-negative patients, considering the poor prognosis, received more aggressive chemotherapy including anthracyclines and/or taxanes. The inversion of relapse rate in patients treated with taxanes suggests the high sensitivity of 2+ and 3+ tumors to this treatment, as previously shown in different studies [13–15]. The better prognosis of HER2 3+ patients in the American herceptin trials [16], all treated with taxanes, compared with the same type of patients included in the Hera European trial [17], in which only 20% of the patients received taxanes, further supports the HER2 sensitivity to this drug. Similarly, although to a lesser extent, doxorubicin appears to be active on 3+ tumors since relapse rates were found to decrease in patients treated with this drug. Such high sensitivity to doxorubicin is not surprising since HER2 is frequently coamplified with topoisomerase II, a known target of anthracyclines [18, 19]. By contrast, the worsening prognosis associated with HER2 level was maintained in CMF-treated patients, indicating no differences in response according to HER2 status.

Data from this retrospective study, indicating the present prognostic significance of HER2 according to expression level of this receptor, point to the need to consider HER2 2+, with or without lymph node infiltration, and HER2 3+ tumors as distinct diseases with different outcomes and specific features that can guide the optimal choice of therapy.

## funding

Associazione Italiana per la Ricerca sul Cancro; Roche Spa.

## acknowledgement

We thank Mrs L. Mameli for excellent technical assistance in manuscript preparation.

## references

1. Ferrero-Pous M, Hacene K, Bouchet C et al. Relationship between c-erbB-2 and other tumor characteristics in breast cancer prognosis. Clin Cancer Res 2000; 6: 4745–4754.
2. Toikkanen S, Helin H, Isola J Joensuu H. Prognostic significance of HER-2 oncoprotein expression in breast cancer: a 30-year follow-up. J Clin Oncol 1992; 10: 1044–1048.
3. Gasparini G, Gullick WJ, Maluta S et al. c-*erb* B-3 and c-*erb* B-2 protein expression in node-negative breast carcinoma—an immunocytochemical study. Eur J Cancer [A] 1994; 30A: 16–22.
4. Esteva FJ, Hortobagyi GN, Sahin AA et al. Expression of erbB/HER receptors, heregulin and P38 in primary breast cancer using quantitative immunohistochemistry. Pathol Oncol Res 2001; 7: 171–177.
5. Press MF, Bernstein L, Thomas PA et al. HER-2/*neu* gene amplification characterized by fluorescence in situ hybridization: poor prognosis in node-negative breast carcinomas. J Clin Oncol 1997; 15: 2894–2904.
6. Ross JS, Fletcher JA. The HER-2/neu oncogene in breast cancer: prognostic factor, predictive factor, and target for therapy. Oncologist 1998; 3: 237–252.
7. Volpi A, De Paola F, Nanni O et al. Prognostic significance of biologic markers in node-negative breast cancer patients: a prospective study. Breast Cancer Res Treat 2000; 63: 181–192.
8. Ménard S, Tomasic G, Casalini P et al. Lymphoid infiltration as a prognostic variable for early-onset breast carcinomas. Clin Cancer Res 1997; 3: 817–819.
9. Van de Vijver MJ, He YD, Van'T Veer LJ et al. A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 2002; 347: 1999–2009.
10. Rilke F, Colnaghi MI, Cascinelli N et al. Prognostic significance of HER-2/*neu* expression in breast cancer and its relationship to other prognostic factors. Int J Cancer 1991; 49: 44–49.
11. Tagliabue E, Agresti R, Carcangiu ML et al. Role of HER2 in wound-induced breast carcinoma proliferation. Lancet 2003; 362: 527–533.
12. Berry DA, Muss HB, Thor AD et al. HER-2/neu and p53 expression versus tamoxifen resistance in estrogen receptor-positive, node-positive breast cancer. J Clin Oncol 2000; 18: 3471–3479.
13. Konecny GE, Thomssen C, Luck HJ et al. Her-2/neu gene amplification and response to paclitaxel in patients with metastatic breast cancer. J Natl Cancer Inst 2004; 96: 1141–1151.
14. Guarneri V, Broglio K, Kau SW et al. Prognostic value of pathologic complete response after primary chemotherapy in relation to hormone receptor status and other factors. J Clin Oncol 2006; 24: 1037–1044.
15. Yamauchi H, Stearns V Hayes DF. When is a tumor marker ready for prime time? A case study of c-erbB-2 as a predictive factor in breast cancer. J Clin Oncol 2001; 19: 2334–2356.
16. Romond EH, Perez EA, Bryant J et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med 2005; 353: 1673–1684.
17. Piccart-Gebhart MJ, Procter M, Leyland-Jones B et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med 2005; 353: 1659–1672.
18. Pritchard KI, Messersmith H, Elavathil L et al. HER-2 and topoisomerase II as predictors of response to chemotherapy. J Clin Oncol 2008; 26: 736–744.
19. Mano MS, Rosa DD, De AE et al. The 17q12-q21 amplicon: Her2 and topoisomerase-IIalpha and their importance to the biology of solid tumours. Cancer Treat Rev 2007; 33: 64–77.