HER2/neu Gene Determination in Women Screened for Breast Carcinoma: How Screening Programs Reduce the Skyrocketing Cost of Targeted Therapy

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Abstract. *Few data on Human Epidermal Growth Factor Receptor 2 (HER2)-positive breast carcinomas have been reported for screen-detected breast carcinoma. Assessing the impact of a targeted intervention with anti-HER2 inhibitors on costs is required in order to plan for better strategies in screening programs. A total of 54,472 women were screened and 323 cases were found to be invasive cancer. We performed immunophenotypical-fluorescent in situ hybridization (FISH) analysis. Among 153 evaluable breast carcinomas, tumours displayed a 3+ scoring status 3+ in 16 (10%), 2+ in 12 (8%), 1+ in 29 (19%) and 0 in 96 (63%) of cases, respectively. All 3+ HER2+ cases and 2/12 2+ (17%) cases exhibited HER2/neu gene amplification, the remaining cases did not. In contrast to the higher incidence reported at the population level, 20-30% HER2-positive cases for metastatic carcinomas, and only 11% of the screen-detected breast carcinomas displayed HER2/neu gene amplification. Breast cancer detection by screening programs hijacks the skyrocketing cost of the use of targeted therapy in HER2-positive carcinoma.*

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Overexpression of the human epidermal growth factor receptor-2 (HER-2) protein or *HER2/neu* gene amplification (HER2 positivity) are associated with rapid tumour growth, increased risk of recurrence after surgery, poor response to conventional chemotherapy and shortened survival in patients affected by breast carcinomas (1, 2). Clinical studies have demonstrated a statistically significant reduction of breast cancer relapse and improved overall survival by adding trastuzumab to therapy for one year after adjuvant chemotherapy in patients with HER2-positive breast cancer (3, 4). Lapatinib is a HER2 tyrosine kinase inhibitor that has clinical activity in *HER2/neu*-amplified breast cancer. Both trastuzumab and lapatinib have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of HER2–positive breast cancer and are clearly associated with improved clinical outcomes in metastatic (5) and, for trastuzumab, early node-positive and node-negative HER2-positive invasive breast cancer (4).

HER2-positive breast carcinoma has been reported in up to 30% of cases of metastatic breast cancer (6), this trend does not seem to be reproduced in a series of studies on early breast carcinomas (7-10).

Few data are available on the prevalence of HER2-positive breast carcinomas at the screen-detection level (11). This information may have a potential impact on the health care cost-effectiveness. Regarding the costs involved in the conventional management of patients affected by breast cancer, HER2-targeted therapies add a significant drug-related cost, so accurate information on the incidence of HER2-positive breast cancer is required to estimate the resources needed in clinics.

Materials and Methods

Code of ethics. Our Institution's Review Board (Nucleo Ricerca e Innovazione) approved the study proposal (CODEETH9721SD).

Patient samples. Between July 1, 1999 and June 30, 2004, 54,472 women were screened. Of the 418 carcinomas detected, 95 were *in situ*, and 323 were invasive. The results and performance indicators of the Breast Cancer Screening Program in Verona have been reported previously (12, 13).

Tissue samples. A total of 153 formalin-fixed and paraffin-embedded tissue blocks were available for the study.

Tissue microarrays. Tissue microarrays were constructed using a tissue arraying instrument (Beecher Instruments, Hackensack, NJ, USA). Tissue cylinders with a diameter of 0.6 mm were punched out from each donor paraffin block in targeted areas corresponding to previously demarcated neoplastic areas on the parallel slide. These tissue cores were then deposited into a recipient, 'master' paraffin block. The punched-out cores were placed 1 mm apart on the x-axis and 1.5 mm apart on the y-axis. Two microarray blocks respectively contained three neoplastic punches per each case: 456 cores were finally evaluated.

Sections 5-μm-thick were cut from the master block, stained with haematoxylin and eosin, and reviewed to ensure the presence of cores with morphological features of breast cancer for each neoplastic case. Morphological features of each core were confirmed by reviewing the corresponding whole tissue sections stained with haematoxylin and eosin. Sections were then used for *in situ* hybridization.

Immunohistochemical analysis. According to the recommendations from the manufacturer of the HercepTest kit (Dako, Milan, Italy), tissue microarray sections mounted on slides and stored at room temperature were stained within four to six weeks from sectioning to maintain antigenicity.

The guidelines for American Society of Clinical Oncology/College of American Pathology (ASCO/CAP) scoring were as follows: 3+ strong, complete membrane staining in more than 30% of the malignant cells; 2+, weak to moderate complete membrane staining in more than 10% of the malignant cells; 0/1+, no or fever than 10% of cells staining, respectively. The guidelines for FDA scoring were also used: differently from the ASCO/CAP criteria, a case was scored 3+ when showing strong, complete membranous staining in more than 10% of the malignant cells.

The final score was assessed by combining score findings of all three neoplastic cores. Heterogeneity was evaluated when comparing findings from each neoplastic cores. We matched findings from tissue arrays with those from whole sections.

Oestrogen (ER rabbit-antibody, SP1, 1:50; Neomarker) and progesterone (PgR 636, 1:150; Dako) receptors and Ki67 (MM1, 1:50; Novocastra) were also assessed and scored as low, medium or high expression levels based on percentage of positive neoplastic nuclei.

Fluorescence in situ hybridization analysis. FISH was performed by using the *PathVysion* HER2/neu DNA probe kit from Vysis Inc./Abbott (Olympus S.p.a). The technical procedure has been previously described (14). Between 60-90 neoplastic nuclei per core were counted.

Table I. *Human epidermal growth factor receptors-2 (HER-2) immunomolecular profile of 153 out of 323 screen-detected infiltrative breast carcinomas from 54,472 women.*

HER2 immunoscore	No. cases (%)	Her-2/neu gene amplification by FISH	Eligibile for therapy
0	96 (63%)	0 (63%)	N
1+	29 (19%)	0 (19%)	N
2+	12 (8%)	2 (1%)	Y
3+	16 (10%)	16 (10%)	Y
	153	18 (11%)	

N: No; Y: yes.

Specimens were determined to exhibit amplification if the ratio of Her-2/neu signals to chromosome 17 centromere signals was higher than 2.2 according to ASCO/CAP recommended criteria (2). A case was scored as equivocal when the signal ratio ranged from 1.8 to 2.2. FDA cut-offs were also used (amplification if the ratio of *HER2/neu* signals to chromosome 17 centromere signals was higher than 2).

Polysomy of chromosome 17 (without HER2/neu gene amplification) was defined as a centromeric chromosome 17 spot count of 3.0 or more in at least 80% of tumour cells (15). Moreover, among *HER2/neu*-amplified cases we distinguished those with high grade amplification (ratio ≥4.0) from those with a low-grade amplification (ratio from >2.2 to 4) (16). Each core was scored for gene amplification and a minimum of 60 nuclei were counted. The final score was assessed combining the score findings on all three neoplastic cores.

Heterogeneity was evaluated when comparing findings from each neoplastic cores (17). We matched findings from tissue arrays with those from whole sections.

Digital image analysis. FISH slides were also digitalized and stored by D-Sight/Fluo instrument (Menarini-Visia Imaging, Florence, Italy).

Results

Tissue samples. There were 118 ductal (56 cases G1, 40 G2 and 12 G3), 23 lobular (20 G1, two G2 and one G3), 9 mixed ductal-lobular infiltrative breast carcinomas (one G1, eight G2), 1 tubular, 1 colloid and 1 papillary carcinoma. A total of 135 breast carcinomas were staged as pT1, 10 pT2, 5 pT3 and three pT4b. Overall, 129 cases (84%) and 109 cases (71%), respectively, were positive for ER and PgR. Ki67 staining was positive (>15% of neoplastic cells) in 13 cases (9%). The mean age was 58 years (range 50-70 years).

HER2 immunohistochemical and fluorescence in situ hybridization findings. The immunohistochemical and fluorescence *in situ* hybridization findings are presented in Table I and shown in Figures 1, 2 and 3.

By using the FDA and ASCO/CAP cut-offs, no significant differences were noted. Overall, 10% and 11% of 153

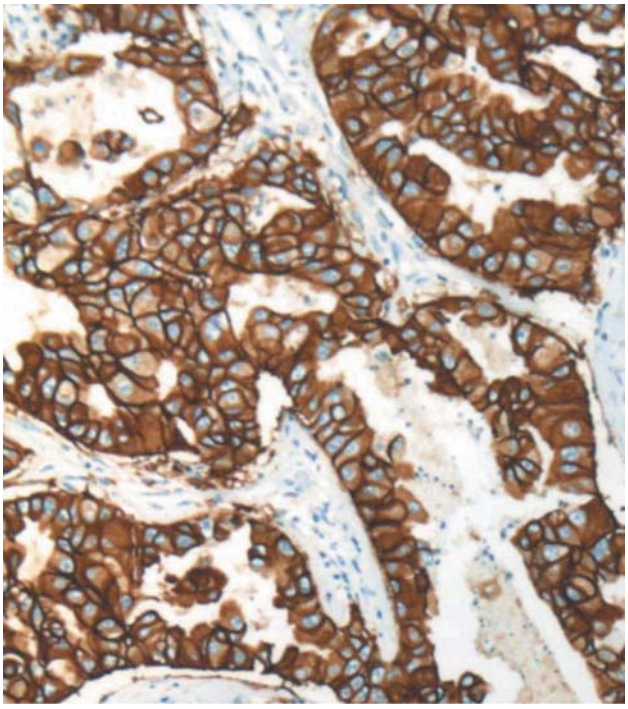

Figure 1. *Strongly positive (3+) immunoexpression for HER2 in infiltrative ductal breast carcinoma (×20).*

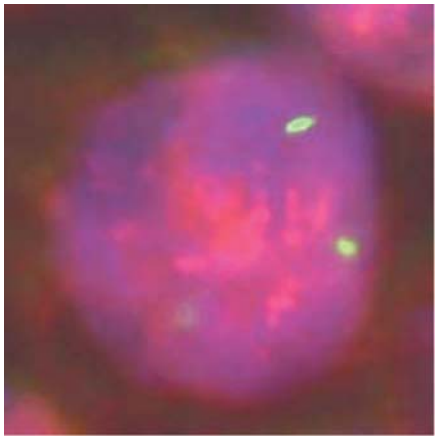

Figure 2. *High-grade HER2/neu gene amplification in infiltrative ductal breast carcinoma by fluorescence in situ hybridization analysis (oil immersion ×100).*

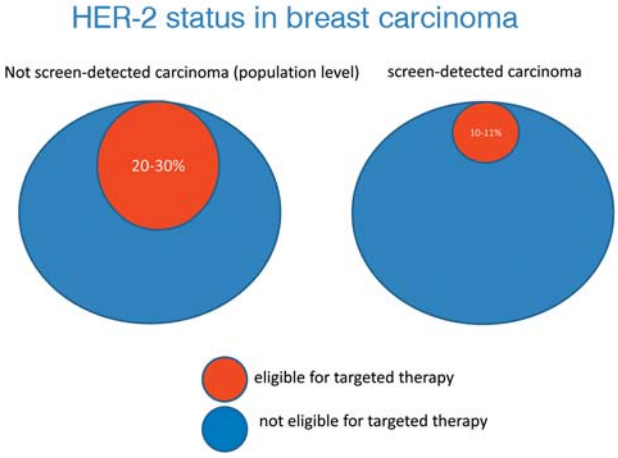

Figure 3. *Decreasing rate of Her-2-positive expression in breast carcinomas detected at population versus screening levels.*

screen-detected breast carcinomas assayed displayed strong 3+ HER2 immunoexprssion and *HER2/neu* gene amplification, respectively.

Immunophenotypically, carcinomas in the majority of cases scored 0 (96/153; 63% of cases). All cases with 3+ and 2+ immunoscore represented ductal infiltrative breast carcinoma.

All 16 cases with 3+ HER2 score and two out of twelve scoring 2+ exhibited high-grade *HER2/neu* gene amplification (mean ratio: 3.2; range from 2.8 to 4.3) by fluorescence *in situ* hybridization (overall, 12% of our cohort). The remaining cases did not exhibit *HER2/neu* gene amplification (mean ratio: 1.5, range from 1.2 to 1.9). Eight cases were staged as pT1c, seven pT1b, two pT1a and one pT2 among 18 cases with *HER2/neu* amplification.

Polysomy of chromosome 17 (without *HER2/neu* gene amplification) was observed in 3 of the 12 (25%) 2+ cases and in 2 out of 29 (7%) 1+ cases. Polysomy of chromosome 17 was also present in 3/96 (3%) cases with 0 immunoscore.

Differences (heterogeneity) between different cores were recognized in 12/153 (8%) cases for fluorescent *in situ* hybridization analysis, when comparing arrays to whole tissue; all cases were scored equivocally according to ASCO/CAP cut-offs.

Concordance between the immunohistochemical and genotypical findings in selecting patients for therapy (matching negative immunostaining with absence of *HER2/neu* gene amplification and positive immunostaining with *HER2/neu*-amplified cases) was high (concordance 0.80).

Discussion

We conclude that: i) only 11% of screen-detected breast carcinomas displayed *HER2/neu* gene amplification; ii) this represents a small proportion of more advanced disease-bearing patients selected for trastuzumab targeted therapy (up to 30% cases usually detected out of screening programs); iii) no significant differences were noted between the FDA and

ASCO/CAP cut-offs; iv) the cost-effectiveness of HER2 testing and trastuzumab therapy in a patient with screen-detected breast cancer is more affordable than that detected at a population level; v) breast cancer screening could be used to help reduce the cost of use of trastuzumab targeted therapy in HER2-positive breast carcinoma. This economic evaluation has to be considered when evaluating the value, cost-effectiveness, strategies and final decisions on screening.

The HER2 immunophenotypical and *HER2/neu* genotypical status is required for the appropriate selection of post-surgical targeted therapies (2). Compared to original incidence of HER2-positive breast carcinomas detected at a population level (not detected at screening level) and in a metastatic setting (25-30% of cases), a lower incidence of HER-2 positivity has been observed in different cohorts of early breast cancer (8, 18). The lower incidence has also been justified in different ways, *i.e.* the restricted ASCO/CAP 2007 criteria used in a certain period of time, different level of internal and external quality assessment, and different kits of probes used. In the present study, we detected a low rate of breast carcinomas with 3+ HER2 immunoexpression (10%) and *HER2/neu* gene amplification (11%) in a cohort of screen-detected breast carcinomas. These findings significantly differ from the subset (ranging from 20 to 30% HER2-positive breast carcinoma) observed at an unselected population level (1, 6, 16). Our findings highlight how screening programs can help to reduce the cost of targeted therapy based on the fact that only a minor subset of screen-detected breast carcinomas exhibit HER-2-positive expression. It is hypothesised that intensified screening for breast cancer may detect a larger proportion of slowly growing HER2-negative tumours with a longer lead-time than for other tumour types. HER2-positive tumours are under-represented in the screen-detected patient group (18), thus screening is likely to partly explain the different incidence of prognostic and predictive biological markers (19) and molecular subtype distribution of screen-detected breast cancer differs from that of carcinomas found outside of screening and accounts in part for the better outcome of screen-detected cancers. Screen-detected carcinomas are more likely to be smaller in size and well- differentiated (pT1a), with less involvement of regional lymph nodes and the survival benefit has been attributed to the stage shift. Notably, several studies have shown that screen detection remains an independent prognostic factor after adjusting for disease stage (19).

Our group recently evaluated the HER2 status in a series of consecutive ductal breast carcinomas by using the FDA and ASCO/CAP scoring systems (8). We showed that the FDA and ASCO/CAP schemes for HER2 evaluation selected patients differently for trastuzumab therapy and that the ASCO/CAP scheme has a great concordance co-efficient between strong 3+ immunohistochemical cases and cases with high-grade, granular *HER-2/neu* amplification. Similarly, in the series reported in our study, we recorded a good concordance between the subset of breast carcinoma characterized as 3+ by immmunohistochemical analysis and with gene amplification by fluorescence *in situ* hybridization analysis. At a population level, discrepancy between breast carcinoma with 3+ immunoscore and gene amplification may be observed, although rarely after ASCO/CAP criteria corrections. Moreover, we previously evaluated the intratumoural heterogeneity of ductal breast carcinomas with HER2/neu amplification scored by ASCO/CAP criteria, and 3+ immunoexpression and showed that the routine assessment of *HER2/neu* amplification on whole-tissue sections is not significantly confounded by intra-tumoural heterogeneity in breast cancer with high-grade amplification (14). In the screen-detected breast carcinomas reported here, heterogeneity of *HER2/neu* status is an infrequent event. The use of multicore tissue microarrays may be an efficient approach in the study of heterogeneity, and it has also been proposed for routine HER2 assessment (20).

Affordability of personalised healthcare is limited by the high costs of currently available targeted therapy drugs. There is a worldwide need for detecting breast cancer at its earliest stages and access to screening and diagnostic medical equipment need to be increased in order to reduce the overall cost of breast cancer management. Bleyer *et al.* recently reported that despite substantial increases in the number of cases of early-stage breast cancer detected, screening mammography has only marginally reduced the rate at which women present with advanced cancer and although they were not certain which women were affected, the imbalance suggested that there was substantial overdiagnosis, accounting for nearly a third of all newly-diagnosed breast carcinomas, and that screening is having, at best, only a small effect on the rate of death from breast cancer (21). Adjuvant trastuzumab increases life expectancy by 1.54 (1.18 discounted) quality-adjusted life-years (QALYs) (22). The cost-effectiveness of HER2 testing and the addition of one year adjuvant trastuzumab after adjuvant chemotherapy from a societal perspective have been evaluated in different settings (22-25). Liberato *et al.* reported that at a cost of 675€ and $767 per weekly dose in the Italian and US settings, respectively, trastuzumab achieves its clinical benefit at a cost of 14,861€ (95% confidence interval, 3,917€ to 44,028€) and $18,970 (95% CI=$6,014 to $45,621) per QALY saved (26). The incremental cost effectiveness was higher than 50,000€/QALY (or $60,000 /QALY) for time perspectives shorter than 7.8 years and for patients older than 76 years, or with a 10-year risk of relapse lower than 15%. Their results confirmed the cost effectiveness when simulating a Herceptin Adjuvant Trial (HERA)-like scenario at a multiway sensitivity analysis and concluded that from the long-term perspective, adjuvant trastuzumab is a cost-effective therapy for patients with HER2-positive, high-risk, early breast cancer (23, 25). Again, Hedden *et al.* evaluated the cost-effectiveness of the

currently recommended 12-month adjuvant protocol of trastuzumab using a Markov modeling approach and real-world cost data (27). In the base case analysis, treatment with a 12-month adjuvant trastuzumab regimen resulted in a gain of 1.38 QUALYs or 1.17 LYG at a cost of $18,133 per patient. Thus, the cost per QALY gained for the base case is $13,095 and that per LYG was $15,492 (27). They concluded that over the long term, treatment of *HER-2/neu* mutation-positive breast cancer with a 12-month protocol of trastuzumab in the adjuvant setting is predicted to be cost-effective in the Canadian context. Similar findings were obtained in different cohort of patients (28-32).

Dendukuri *et al*. showed that the strategy with the best cost-effectiveness ratio for HER2 testing is to screen all breast cancer patients by immunohistochemistry and to confirm 2+ and 3+ scores with fluorescence *in situ* hybridization (33). At the technical level, in addition to improving specificity, this approach could lead to major cost savings in treatment with trastuzumab. In a screen-detected setting of breast carcinoma, the decreasing incidence of HER2-positive breast carcinoma again acquires less restriction in performing the double (immunohisotchemical and fluorescence *in situ* hybridization analyses) approach for 2+ and 3+ tumours. Again, the spectrum of saving money per one year's worth of trastuzumab therapy significantly decreases from 20-30% to 10-11% for patients with HER2-positive breast carcinoma (675€ and $767 per weekly dose in the Italian and US setting, respectively, per patient), when comparing the population *versus* the screen-detected cohorts of patients, respectively. Moreover, at the screening level, most carcinomas detected are usually of stage pT1 (around 80%) and this indicator is the determinant for the efficacy of the end-point of screening; in contrast at the population level patients affected by pT1 carcinomas are more infrequent. These aforementioned indicators and their cross-sectional representation of more pT1 cancers and fewer HER2-positive breast carcinomas, demonstrate the potential of screening detection of HER2-positive breast cancer for the dramatical reduction of the otherwise high costs of targeted therapy.

In conclusion, the evaluation of HER2 expression in a series of screen-detected breast carcinomas helped to identify a relatively small subset of patients with HER2-positive carcinoma suitable for trastuzumab therapy. This revealed a greater affordability and cost-effectiveness for the use of targeted therapies, even in light of the current high cost of such therapy.

Assessing the impact of a targeted intervention on costs and health outcomes requires explicit consideration of the method of targeting. In this study, we report on the low incidence of HER2-positive breast carcinoma detected at the screening level and highlight how the screening programs can help to reduce the cost of anti-HER2 targeted therapy with trastuzumab or anti-HER2 inhibitors.

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