Comparison of HER-2 overexpression in primary breast cancer and metastatic sites and its effect on biological targeting therapy of metastatic disease

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HER-2 overexpression, a predictive marker of tumour aggressiveness and responsiveness to therapy, occurs in 20–30% of breast cancer. Although breast cancer is a heterogeneous disease, HER-2 measurement is carried out in primary tumour. This study aims to evaluate HER-2 overexpression in primary and metastases and its effect on treatment decisions. Biopsies from primary breast cancer and corresponding metastases from 58 patients were studied. HER-2 overexpression was evaluated immunohistochemically in all primary and metastatic sites. Positive overexpression in primary and/or metastases was confirmed by fluorescence in situ hybridisation (FISH). Discordance in HER-2 overexpression between primary and metastatic sites was 14% (eight of 58 patients). Concordance was found in 50 (86%) of patients (95% CI: 77–95). In one patient (2%), HER-2 was negative in metastasis but positive in primary. In seven (12%) patients, HER-2 was positive in metastases and negative in primary (95% CI: 3.7–20), and three of them responded to trastuzumab. Gene amplification by FISH was found in all cases with HER-2 positive (+2 and +3) by immunohistochemistry. Our data suggest that a possible discordance of HER-2 overexpression between primary and metastases should be considered when making treatment decisions in patients with primary HER-2-negative tumours.

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The choice of therapy for treatment of breast cancer is based on tumour stage, histopathologic features, hormone receptor status and biological markers (Hamilton and Piccart, 2000). The most promising biological marker in terms of predicative value for breast cancer treatment is HER-2 (Thor et al, 1998). The HER-2 oncprotein is a transmembrane receptor, belonging to the epidermal growth factor receptor family, with tyrosine kinase activity, resulting in intracellular signalling and activation of genes involved in cell growth, which is associated with shortened survival, enhanced aggressiveness and other poor prognostic factors (Slamon et al, 1987; Tsuda et al, 1989). Most of the literature reports a correlation between HER-2 overexpression and increased sensitivity to anthracycline-based chemotherapy in comparison with CMF and tamoxifen (Elledge et al, 1998; Paik et al, 1998). Most importantly, HER-2 overexpression identifies the subset of patients who can benefit from trastuzumab, recombinant humanised anti-HER-2 monoclonal antibody (Genentech Inc., South San Francisco, CA, USA), in advanced and metastatic breast cancer (MBC) (Hortobagyi, 2001; Lohrisch and Piccart, 2001). Monotherapy with trastuzumab has yielded response rates of 35% when given as first-line therapy and 18% in patients previously treated with chemotherapy (Cobleigh, 1999; Vogel et al, 2001). Compared with chemotherapy alone, combination of chemotherapy and trastuzumab improves response rates and overall survival for women with HER-2-positive MBC (Lohrisch and Piccart, 2001). Breast cancer is a heterogeneous tumour with high individual variability as far as response to treatment is concerned (Harris et al, 1997).

Generally, assessment of HER-2 is performed in the primary tumour even if the metastases appear several years later. As biopsy of metastases is not routine, it is difficult to test a large number of cases. Clinical metastases may grow from micrometastases resistant to adjuvant therapy and may represent one particularly aggressive clone from among many clones of primary breast cancer (Harris et al, 1997). It is still unknown whether HER-2 expression differs in metastases compared to primary breast cancer. Few data have been published regarding this issue (Cardoso et al, 2001).

The objective of the present study is to determine the expression of HER-2 in the primary breast cancer and its metastases and to update our previous results by studying additional patients (Zidan et al, 2002). All tumours and their metastases were evaluated for HER-2 using immunohistochemistry (IHC) (Hercept Test). The fluorescence in situ hybridisation (FISH) test was utilised for HER-2-overexpressing cases. In addition, we examined the utility of evaluating HER-2 overexpression in metastases on the decision to treat these patients with trastuzumab. A comprehensive review of the literature was also performed.

MATERIALS AND METHODS

Between 1990 and 2002, 209 patients with MBC were treated in our department. Patients were enrolled in this study if tumour samples
from both primary and corresponding metastases were available and suitable for IHC analysis. Patients with metastatic ipsilateral axillary lymph nodes at the primary operation were excluded.

Formalin-fixed paraffin-embedded samples of primary breast cancer and metastases were collected from 58 patients. For each case, the primary tumour and the corresponding metastases were sampled for HER-2 staining at the same time by the same two experienced pathologists. Immunohistochemical staining for HER-2 was performed by using CB11 monoclonal antibody with antigen retrieval. Expression was scored using the scoring system outlined in the DAKO Hercept Test as 0, 1+, 2+ and 3+ according to the standardised criteria (Slamon et al, 1989). Gene amplification by FISH was tested in all cases where the primary tumour or metastases were positive (+ 2 or + 3) by IHC.

Statistical analysis

Rates and positivity were compared using McNamara tests for paired data. Confidence intervals for concordance/discordance rates were calculated. The Kendall's tau-b coefficient was used to assess concordance for HER-2 status. Although the difference between HER-2 overexpression in the primary tumour and the metastases was marginally significant (P = 0.07), a discordance was observed (Kendall’s tau-b coefficient = 0.69, 95% CI = 0.53 – 0.80) due to more cases of HER-2 overexpression in the metastases. Two-tailed P-values of 0.05 or less was considered to have a statistical significance.

RESULTS

All 58 cases with available biopsies from the primary tumour and metastases were included in this study. Median age at diagnosis of the primary breast cancer was 56 years (range: 29 – 82 years). One patient was male. Metastases were found in bone of 68% of patients (n = 39), in skin and soft tissue in the surgical scar region of 35% (n = 20), in 36% (n = 21) in liver, in lungs of 33% (n = 19) of patients and in the pleura of 19% (n = 11) of patients. In 35% (n = 20) of patients, there was one metastatic site, in 41% (n = 24) of patients two sites were found and in 24% (n = 14) three metastases were found (Table 1). Invasive ductal carcinoma was diagnosed in 88% of patients. Oestrogen and progesterone receptors (ER, PR) were positive in 60 and 53% of the primary tumours, respectively. All patients underwent surgery at diagnosis: 60% (n = 35) of patients had lumpectomy and 40% (n = 23) had mastectomy. In all, 29 (50%) patients were treated with tamoxifen as adjuvant treatment with or without chemotherapy.

Metastases were diagnosed between 1 and 12 years after primary breast cancer surgery (median: 4.5 years). HER-2 was positive by IHC in 24% (n = 14) of the primary breast cancer tissues (two with 2+ and 12 with 3+) and negative in the remaining 76% (n = 44). In 35% (n = 20) of patients, HER-2 was positive in metastases (two with 2+ and 18 with 3+). Of the total 58 patients, 86% (n = 50) revealed a concordance between the primary tumour and its metastases (95% CI: 77 – 95). In 2% of patients (n = 1), HER-2 was positive in the primary tumour and negative in the metastatic site. In 12% (n = 7) of patients, HER-2 was negative in the primary tumour and positive in the corresponding metastases (95% CI: 3, 7 – 20) (P = 0.07) (Table 2). The single patient with HER-2 positive in primary carcinoma of breast and negative in skin and bone metastases was a 48-year-old woman in whom metastases were diagnosed 2 years postmastectomy. Trastuzumab and vinorelbine were given as second-line therapy for 3 months then stopped because of disease progression.

Four patients were treated with trastuzumab due to HER-2 evaluation in the metastases as follows:

Case 1: A 32-year-old male patient had metastases in skin, bone and lung 3 years after mastectomy. HER-2 was negative in the primary tumour but positive in skin and bone metastases. After failure with doxorubicin and paclitaxel, he received trastuzumab as a single agent and achieved partial response for 9 months.

Case 2: A 58-year-old woman with primary HER-2 negative but positive in metastases in liver (after 6 years) received trastuzumab monotherapy as third-line therapy after chemotherapy failure for 6 months with minimal response and no side effects.

Case 3: A 61-year-old woman had a local recurrence in skin and soft tissue of chest wall 5 years after mastectomy. Oestrogen receptor and PR were positive. She received tamoxifen as an adjuvant treatment for 5 years. At the end of the 5th year, she was diagnosed with multiple local recurrences in skin and soft tissue. HER-2 was negative in the primary and positive (+ 2) in the recurrence. Gene amplification by FISH was negative in the primary and positive in the recurrence. ER and PR were found to be positive in the recurrence as well. After chemotherapy failure (two lines), the patient received trastuzumab with vinorelbine weekly, achieving complete response after 5 months. For the past 13 months, this patient has received only trastuzumab and still in complete remission with high quality of life.

Case 4: A 52-year-old woman with lung metastases with HER-2 negative in primary and positive in metastases did not respond to trastuzumab therapy. Three deceased patients who had not received trastuzumab were studied retrospectively.

DISCUSSION

The most promising predictive factor for breast cancer today is HER-2. Breast tumours with HER-2 overexpression are resistant to hormonal therapy and alkylating agents, but more sensitive to anthracycline-based chemotherapy (Elledge et al, 1998; Ross and

| Table 1 Patient characteristics |
|---------------------------------|
| **Number of patients %**        |
| Total number of patients        | 58 | 100 |
| Gender                          |    |
| Female                         | 57 | 98  |
| Male                           | 1  | 2   |
| Age (years) Median              | 56 |
| Range                          | 29 – 82 |
| Receptor status, primary tumour |    |
| ER positive                     | 35 | 60  |
| PR positive                     | 31 | 53  |
| Primary surgery                 |    |
| Lumpectomy                      | 35 | 60  |
| Mastectomy                      | 23 | 40  |
| Histology                      |    |
| Invasive ductal carcinoma       | 51 | 88  |
| Invasive lobular carcinoma      | 7  | 12  |
| Number of metastatic sites      |    |
| 1                              | 20 | 35  |
| 2                              | 24 | 41  |
| 3                              | 14 | 24  |
| Prior therapy                   |    |
| Adjuvant chemotherapy           | 29 | 50  |
| Adjuvant hormonal therapy       | 10 | 18  |
| Adjuvant chemo+hormonal therapy| 19 | 32  |

ER = oestrogen receptor; PR = progesterone receptor.
Fletcher, 1998; Hamilton and Piccart, 2000). Studies that have addressed HER-2 as a predictive factor for hormonal therapy have demonstrated lower response rates or reduced survival in hormonally treated patients whose tumours overexpressed HER-2 (level 2+ or 3+), reaching statistical significance in most studies (Nicholson et al., 1993; Leitzel et al., 1995; Elledge et al., 1998).

HER-2 overexpression is gaining acceptance as an indicator that defines breast cancer patients who should preferentially receive anthracycline-based or CMF adjuvant or therapeutic treatment (Paik et al., 1998; Thor et al., 1998; Hamilton and Piccart, 2000; Andrulis et al., 1998). HER-2 overexpression is a prerequisite for treating patients with MBC with trastuzumab, and may be considered for adjuvant treatment in the future (Ross and Fletcher, 1998; Baselga, 2001; Eiermann, 2001). Generally, evaluation of HER-2 overexpression for the treatment of MBC is carried out in the primary tumour. Overexpression is shown in 20–77% of human breast cancers (Slichenmyer and Thor, 1998). HER-2 overexpression is gaining acceptance as a predictor of response to hormonal therapy (Table 3). These studies show discordance rates of between 0 and 26%. Niehans et al. (1993) retrospectively evaluated HER-2 overexpression in primary breast cancer and different metastatic sites in autopsy tumour samples. The uncontrolled fixation time of autopsy samples jeopardises the IHC data that can be obtained from samples.

Masood and Bui (2000) evaluated HER-2 overexpression in 56 patients, but only 11 cases of metastases were from distant sites. At the 2000 San Antonio Breast Cancer Meeting, Edgerton et al. (2000) presented preliminary results of a study comparing HER-2 status in 193 patients, only 93 of which were distant metastases. Only seven of the 21 metastatic samples evaluated by Shimizu et al. (2000) were from distant sites. Tanner et al. (2001) reported on 46 primary breast cancers and their metastases. Only 12 of the 46 cases were metastases from distant sites. Gancberg et al. (2002) utilised both IHC and FISH techniques retrospectively in 107 patients between 1991 and 1999. Only 68 out of the 107 cases were available for FISH test and five (7%) of them were discordant. In our previous study, 40 patients with primary and metastases were evaluated by IHC test. Discordance was found in 18% of cases (Zidan et al., 2002), and in 14% when 58 patients were evaluated using both IHC and FISH (Zidan et al., 2004).

The present study suggests a 14% discordance of HER-2 overexpression between primary tumour and the metastases ($P=0.07$ McNamara). HER-2 was positive in the primary and negative in the metastatic site in one patient (2%) and negative in the primary and positive in metastases in seven patients (12%). In these seven patients, findings affected the selection of therapy, particularly by trastuzumab, in four who otherwise would not have been selected for trastuzumab treatment.

Table 2

| Case # | HER-2 (IHC) in primary | HER-2 (FISH) in primary | Metastatic site | HER-2 (IHC) in metastases | HER-2 (FISH) in metastases |
|--------|------------------------|------------------------|----------------|---------------------------|---------------------------|
| 1      | +3         | Positive               | Skin, soft tissue | +3 | Positive |
| 2      | +3         | Positive               | Skin, bone       | +3 | Positive |
| 3      | +3         | Positive               | Local recurrence in breast | +3 | Positive |
| 4      | +3         | Positive               | Pleura           | +3 | Positive |
| 5      | +3         | Positive               | Liver            | +3 | Positive |
| 6      | +3         | Positive               | Liver            | +3 | Positive |
| 7      | +2         | Positive               | Lung             | +3 | Positive |
| 8      | +3         | Positive               | Skin             | +3 | Positive |
| 9      | +3         | Positive               | Bone             | +2 | Positive |
| 10     | +3         | Positive               | Lung             | +3 | Positive |
| 11     | +2         | Positive               | Bone             | +3 | Positive |
| 12     | +3         | Positive               | Skin             | +3 | Positive |
| 13     | +3         | Positive               | Liver            | +3 | Positive |
| 14     | +3         | Positive               | Skin and bone    | Negative | Negative |
| 15     | Negative   | Negative               | Skin, soft tissue | +3 | Positive |
| 16     | Negative   | Negative               | Soft tissue, bone | +3 | Positive |
| 17     | Negative   | Negative               | Skin, bone, lung | +3 | Positive |
| 18     | Negative   | Negative               | Liver            | +3 | Positive |
| 19     | Negative   | Negative               | Skin, soft tissue | +2 | Positive |
| 20     | Negative   | Negative               | Pleura           | +3 | Positive |
| 21     | Negative   | Negative               | Lung             | +3 | Positive |

IHC = immunohistochemistry; FISH = fluorescence in situ hybridisation.
Table 3  Studies comparing HER-2 overexpression in primary breast cancer and distant metastases

| Investigator | # of pts. | HER-2 overexpression: evaluation method | Discordance (%) in HER-2 between primary and metastasis |
|--------------|-----------|----------------------------------------|-----------------------------------------------------|
| Neihans      | 30        | IHC                                    | 3                                                   |
| Masood       | 56        | IHC                                    | 2                                                   |
| Edgerton     | 193       | IHC and FISH                           | 25                                                  |
| Shimizu      | 21        | IHC                                    | 0                                                   |
| Tanner       | 46        | IHC and CISH                           | 0                                                   |
| Ganberg      | 107       | IHC and FISH                           | 7                                                   |
| Zidan        | 40        | IHC                                    | 18                                                  |
| Zidan        | 58        | IHC and FISH                           | 14                                                  |
| Dowsett      | 39        | IHC                                    | 8                                                   |
| Lipton       | 240       | Serum ECD                              | 26                                                  |
| Luftner      | 80        | IHC                                    | 18                                                  |

IHC = immunohistochemistry; FISH = fluorescence in situ hybridisation; CISH = chromogenic in situ hybridisation; ECD = extracellular domain.

four studies showed a discordance rate of 0–3%. These four studies were actually based on small numbers of distant metastases and include mostly locoregional relapses.

Immunohistochemistry staining has certain limitations, this cannot, however, explain the discordance observed between primary and metastases in the expression of HER-2 in the present study. The same IHC method was used for all patients and evaluation was carried out by the same pathologist. Primary and metastatic lesions from the same patients were immunostained in the same staining run, thus the probability of error is minimised.

The best method of measuring HER-2 is a point of controversy. A consensus conference was held in August 1999 in an attempt to resolve some of these issues (Author unlisted, 1999). Data presented at that meeting indicate that the concordance between IHC and FISH methods is in the range of 80–90%, comparing 3+ overexpression. As the median time between primary tumour and metastases in our study is 4.5 years, it is difficult to compare HER-2 overexpression by using frozen tissue samples from both primary and metastases. This technical limitation may affect the results of IHC test for HER-2 expression in some cases. FISH technique was used for testing and confirming all cases with HER-2 overexpression by IHC (+2 and +3). All cases in our study showed amplification by FISH. The first limitation of FISH is the much higher cost when compared to IHC test. The second limitation is that, although IHC can be carried out in nearly every pathology laboratory, FISH can be performed only in special laboratories with specialised equipment.

The present study is one of the first to address the issue of heterogeneity between breast cancer primaries and metastatic sites. The rationale behind the present study is the fact that breast cancer is a biologically and genetically heterogeneous tumour that contains multiple different clones (Teixeira et al, 1995). An early stem line clone may migrate to the metastatic site and grow independently from its counterpart in the primary tumour, resulting in complete heterogeneity between primary and metastatic sites in the same patient. Heterogeneity between primary and metastatic sites may also result from the genetic instability of cancer cells. Breast cancer cells that survive adjuvant treatment may undergo genetic changes resulting in either a loss or gain of expression of some biological markers (Kuukasjarvi et al, 1997). Molecular changes, including upregulation of HER-2, were observed in breast cancer relapsing after adjuvant hormone therapy (Slighemmyer and Fry, 2001; Tanner et al, 2001). In our study, four of seven patients with HER-2 positive in metastases and negative in the primary initially received adjuvant treatment with tamoxifen before developing metastases. Two became ER negative in metastases. Additionally, some small clones in the primary tumour overexpress HER-2, but are negligible in the tumour mass and may not be detected in the staining of the larger primary breast cancer. Metastases developing from such clones are HER-2 positive (Teixeira et al, 1995).

In all, 12% of patients in the current study were found to be eligible for treatment with trastuzumab, according to results of HER-2 overexpression in metastatic sites. To our knowledge, ours is the first study to address the possibility of treating patients with metastases of breast cancer with trastuzumab based on the discordance in HER-2 overexpression between primary tumour and metastases.

Three other techniques appear easy and applicable in evaluating HER-2 overexpression in MBC. One promising method is the testing of extracellular domain of HER-2 in serum of breast cancer patients (Burstein et al, 2003). Another technique is the evaluation of HER-2 amplification by chromogenic in situ hybridisation. This technique is easy, inexpensive and can be carried out in most hospitals (Dandachi et al, 2004). HER-2 amplification detected by FISH in fine-needle aspiration (FNA) from metastases is also a promising technology. Bozzetti et al (2002) evaluate HER-2 amplification by FISH on 66 breast cancer FNAs. Paired results by FISH cytology and FISH histology showed a concordance of 91%. They concluded that HER-2 gene amplification can be reliably estimated by FISH on breast cancer FNAs. This makes the evaluation of HER-2 from metastases easy and implementable.

To summarise, the present study demonstrates a relatively high discordance rate (14%) in HER-2 overexpression between primary and metastases of the same breast cancer, emphasising the existence of biological differences between primary and metastases. We suggest taking HER-2 evaluation into consideration in metastatic sites when HER-2 is negative in the primary tumour and the patient can benefit from treatment with trastuzumab. Additional studies with larger numbers of patients are needed to verify our results.

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