The Predictive Value of EGFR and HER-2/neu in Tumor Tissue and Serum for Response to Anthracycline-Based Neoadjuvant Chemotherapy of Breast Cancer

Walter Schippinger, MD,1 Nadia Dandachi, PhD,1 Peter Regitnig, MD,2 Günter Hofmann, MD,1 Marija Balic, MD,1 Rainer Neumann, PhD,3 Hellmut Samonigg, MD,1 and Thomas Bauernhofer, MD1

Key Words: Breast cancer; Epidermal growth factor receptor; EGFR; HER-2/neu; Predictive parameters

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Abstract

We investigated the predictive value of HER-2/neu and epidermal growth factor receptor (EGFR) in tumor tissue and prechemotherapy serum for histopathologic response in 108 patients with breast cancer undergoing neoadjuvant anthracycline-based chemotherapy. Response to chemotherapy, assessed by histopathologic classification of regression (grade 0 [no therapy effect] to 4 [no residual tumor]), correlated significantly with prechemotherapy serum HER-2/neu levels. Median prechemotherapy serum HER-2/neu levels were significantly higher in patients with regression grades 1 through 4 compared with those in patients with regression grade 0 (9.6 vs 8.55 ng/mL; P = .011; 95% confidence interval [CI], .009-.014). Median pretreatment serum HER-2/neu levels of patients with complete pathologic response (pCR) were significantly higher than in patients with moderate or no treatment response (10.95 vs 9.1 ng/mL; P = .041; 95% CI, .036-.046). Receiver operating characteristic curve analysis revealed a serum HER-2/neu value of more than 10.3 ng/mL to predict a pCR with 80% sensitivity and 69.4% specificity. There was no significant correlation of response with HER-2/neu and EGFR scores in tumor tissue or with serum EGFR levels.

Results demonstrate prechemotherapy serum HER-2/neu to be a significant predictor of response to neoadjuvant anthracycline-based chemotherapy for breast cancer.

The epidermal growth factor receptor (EGFR), a 170-kd membrane-bound tyrosine kinase receptor, and HER-2/neu are members of the HER growth factor receptor family. The HER-2/neu proto-oncogene encodes a 185-kd transmembrane glycoprotein that contains an extracellular ligand-binding domain and intracellular tyrosine kinase activity.1 The extracellular domain (ECD) of the HER-2/neu protein is shed from the receptor by actively regulated proteolytic cleavage and can be detected in serum as a protein of approximately 105 kD.2-4 HER-2/neu gene amplification or protein overexpression is observed in 15% to 20% of breast carcinomas.5,6 HER-2/neu gene amplification and overexpression of the receptor protein are associated with poor prognosis.7-10 Data from retrospective analyses examining the benefit from adjuvant CMF chemotherapy suggested reduced disease-free survival for patients with HER-2/neu–overexpressing breast cancer.11 Also, several studies on endocrine treatment with tamoxifen revealed an impaired therapy effect in patients with HER-2/neu overexpression in breast carcinoma tissue or with elevated serum levels of HER-2/neu ECD.12-14 Concerning the efficacy of anthracycline-based adjuvant chemotherapy, studies have demonstrated an increased benefit for patients with HER-2/neu–overexpressing breast cancer.15-17

EGFR is expressed in 30% to 60% of breast carcinomas.18-20 Expression of EGFR in breast cancer tissue has been shown to be frequently associated with high tumor grade, elevated growth fraction, and an inverse relationship with estrogen receptor status.18,20-23 Several analyses examining the prognostic significance of EGFR expression in breast cancer suggest a possibly poor prognosis for patients with EGFR+ tumors.19,24 There exists little information about a possible influence of EGFR expression on response to chemotherapy.24
Systemic antitumor therapy before surgery (neoadjuvant therapy) for breast cancer aims to decrease the size of the primary tumor, leading to a higher chance for breast conservation, and to decrease the risk of disease relapse and death. The neoadjuvant treatment setting is an ideal model to examine the influence of possibly predictive parameters because tumor tissue is removed after preoperative therapy and histopathologic response can be assessed without the limitations of radiologic imaging methods.

The purpose of this study was to determine whether assessment of EGFR and HER-2/neu in tumor tissue and measurement of the levels of EGFR ECD (serum EGFR) and HER-2/neu ECD (serum HER-2/neu) in pretreatment serum samples can be useful in predicting response to neoadjuvant anthracycline-based chemotherapy. To assess response to treatment, we used a histopathologic scoring system defining the degree of the cytotoxic effect of neoadjuvant chemotherapy.

Materials and Methods

Samples

From March 1995 to December 2003, 150 consecutive patients were treated at the Division of Oncology, Department of Internal Medicine, Medical University of Graz, Graz, Austria, with neoadjuvant chemotherapy for invasive primary breast cancer. All patients required preoperative tumor size reduction to facilitate breast-conserving surgical removal of the tumor. Before initiation of neoadjuvant chemotherapy, tumor tissue was obtained by core needle biopsy or surgical biopsy for routine histopathologic examination. A serum sample was obtained directly before initiation of neoadjuvant chemotherapy and was stored in the serum bank of the Division of Oncology, Medical University of Graz, at –80°C.

From 108 patients, prechemotherapy serum samples and postchemotherapy paraffin-embedded tumor tissue samples were available for use in this retrospective study. From 90 of these patients, paraffin-embedded prechemotherapy biopsy samples also were available.

All patients received at least 3 cycles of an anthracycline-based neoadjuvant chemotherapy regimen. Patient characteristics are summarized in Table 1.

Written informed consent was obtained from all patients to use the stored material for scientific purposes.

Chemotherapy

All patients whose samples were used in this study were treated with epirubicin in a dose of 60 to 90 mg/m². In 55 patients, epirubicin was administered in combination with docetaxel, 75 mg/m², or paclitaxel, 200 mg/m², intravenously every 3 weeks. The other 35 patients were treated with epirubicin and cyclophosphamide, 600 mg/m², every 3 weeks. A median of 4 (range, 3-6) neoadjuvant chemotherapy cycles were administered.

Determination of Histopathologic Response

After neoadjuvant chemotherapy, all surgical specimens were routinely evaluated macroscopically, and several tissue samples of each specimen were embedded in paraffin. Special attention was given to areas showing vital tumor, tumor necrosis, or scar-like fibrosis. All specimens were reevaluated by 1 pathologist (P.R.) independent of immunohistochemical evaluation. To evaluate the histopathologic response, all samples were classified into regression grades according to Sinn et al., which are comparable to a regression grading classification developed by Chevallier et al. The Sinn regression grade 3 was further subclassified into 3a, isolated invasive tumor cells only, and 3b, residual invasive tumor less than 5 mm in greatest dimension. A detailed description of the Sinn regression grading is shown in Table 2.

Determination of EGFR Expression in Tumor Tissue

The EGFR expression in tumor tissue was determined by immunohistochemical analysis. Specimens of core needle biopsies and surgically removed tumor biopsies were fixed in 7% neutral buffered formalin and embedded in paraffin. From the tissue blocks, 5-µm sections were cut and mounted

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Patient and Tumor Characteristics in 108 Cases of Breast Cancer*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
<td>Result</td>
</tr>
<tr>
<td>Age (y)</td>
<td>Median 51,5 27-71</td>
</tr>
<tr>
<td>Menopausal status</td>
<td>Premenopausal 47 (43.5) Postmenopausal 61 (56.5)</td>
</tr>
<tr>
<td>Histologic diagnosis</td>
<td>Invasive ductal (NOS) 79 (73.1) Invasive lobular 16 (14.8) Mixed ductal/lobular and others 13 (12.0)</td>
</tr>
<tr>
<td>Prechemotherapy tumor size</td>
<td>CT1 5 (4.6) CT2 52 (48.1) CT3 42 (38.9)</td>
</tr>
<tr>
<td>Histopathologic grading</td>
<td>G1 6 (5.6) G2 42 (38.9) G3 46 (42.6)</td>
</tr>
<tr>
<td>ER and PR status</td>
<td>ER+ and PR+ 54 (50.0) ER+ or PR+ 20 (18.5) ER- and PR- 34 (31.5)</td>
</tr>
</tbody>
</table>

ER, estrogen receptor; NOS, not otherwise specified; PR, progesterone receptor.

* Data are given as number (percentage) unless otherwise indicated.
formed at least 3 days after the reaction was finished. Slides run with each assay. The analyses of the FISH tests were performed according to the manufacturer’s instructions.

Assessment of HER-2/neu Expression in Tumor Tissue

Sections from the formalin-fixed, paraffin-embedded tumor tissue were analyzed immunohistochemically by using the HercepTest (DAKO). Membrane immunoreactivity and membrane staining patterns were evaluated and scored using the 0 to 3+ scoring system according to the protocol of the manufacturer and as approved by the US Food and Drug Administration.

Statistical Analysis

Correlations of serum HER-2/neu levels, serum EGFR levels, and clinical and pathologic parameters were calculated by using the Mann-Whitney U test for continuous variables and with the \( \chi^2 \) test for categorical data. Logistic regression was used for multivariate analysis of binary therapy response variables. Odds ratios were calculated and presented with their 95% confidence intervals (95% CIs). A receiver operating characteristic (ROC) curve analysis was performed to define the optimal cutoff value in terms of sensitivity and specificity for serum HER-2/neu as a predictive factor for response to chemotherapy.

Statistical significance was set as a \( P \) value less than .05. All reported \( P \) values are 2-sided. For statistical analyses, SPSS software (version 12.0 for Windows, SPSS, Chicago, IL) was used.
Results

Histopathologic Response to Neoadjuvant Chemotherapy

Response to neoadjuvant chemotherapy as defined by regression grades 0 (no therapy effect) to 4 (no residual tumor) was found in the following frequencies: 0, 34 (31.5%) of tumors; 1, 27 (25.0%); 2a, 15 (13.9%); 2b, 22 (20.4%); 3, 2 (1.9%); and 4, 8 (7.4%).

Histopathologic Response to Chemotherapy and Correlation With Clinical, Histopathologic, and Serum Parameters

Histopathologic response to neoadjuvant chemotherapy correlated significantly with age, menopausal status, type of chemotherapy, and prechemotherapy serum HER-2/neu levels.

There was no statistically significant correlation between histopathologic response and tissue HER-2/neu status categories with scores 0, 1+, and 2+ without gene amplification and with scores 3+ and 2+ with gene amplification; tissue EGFR status categories with score 0 and with scores 1+, 2+, and 3+; histologic tumor type; tumor size; grade; estrogen and progesterone receptor status; and serum EGFR level. No statistically significant correlation was found between prechemotherapy serum HER-2/neu level and type of chemotherapy, age, or menopausal status.

Correlations of histopathologic and clinical parameters with response to neoadjuvant chemotherapy are shown in Table 3.

Correlation of Pretreatment Serum HER-2/neu Level With Response to Chemotherapy

In patients with complete pathologic response of invasive tumor (pCR; regression grades 3 and 4), the median prechemotherapy serum HER-2/neu value was 10.95 ng/mL (range, 7.8-22.1 ng/mL); in patients with moderate response (regression grades 1 and 2), it was 9.35 ng/mL (range, 6.1-26.5 ng/mL); and in patients with no signs of histopathologic response (regression grade 0), it was 8.55 ng/mL (range, 3.9-17.2 ng/mL). The median prechemotherapy serum HER-2/neu level was significantly higher in patients responding to chemotherapy (regression grades 1 to 4) than in patients with no histopathologic signs of response (9.6 vs 8.55 ng/mL; \( P = .011; 95\% \text{ CI, .009-.014} \)).

Table 3
Response to Chemotherapy by Clinical, Histopathologic, and Serum Parameters in 108 Cases of Breast Cancer*

<table>
<thead>
<tr>
<th>Response</th>
<th>Regression Grade 0</th>
<th>Regression Grades 1-4</th>
<th>( P^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td>(.010)</td>
</tr>
<tr>
<td>Menopausal status</td>
<td></td>
<td></td>
<td>(.006)</td>
</tr>
<tr>
<td>Premenopausal (n = 47)</td>
<td>8 (17)</td>
<td>39 (83)</td>
<td></td>
</tr>
<tr>
<td>Postmenopausal (n = 61)</td>
<td>26 (43)</td>
<td>35 (57)</td>
<td></td>
</tr>
<tr>
<td>Histologic diagnosis</td>
<td></td>
<td></td>
<td>(.094)</td>
</tr>
<tr>
<td>Invasive ductal (n = 79)</td>
<td>29 (37)</td>
<td>50 (63)</td>
<td></td>
</tr>
<tr>
<td>Invasive lobular (n = 16)</td>
<td>4 (25)</td>
<td>12 (75)</td>
<td></td>
</tr>
<tr>
<td>Mixed ductal/lobular and others (n = 13)</td>
<td>1 (8)</td>
<td>12 (92)</td>
<td></td>
</tr>
<tr>
<td>Prechemotherapy tumor size</td>
<td></td>
<td></td>
<td>(.338)</td>
</tr>
<tr>
<td>cT1/2 (n = 57)</td>
<td>14 (25)</td>
<td>43 (75)</td>
<td></td>
</tr>
<tr>
<td>cT3 (n = 42)</td>
<td>14 (33)</td>
<td>28 (67)</td>
<td></td>
</tr>
<tr>
<td>Grading</td>
<td></td>
<td></td>
<td>(.055)</td>
</tr>
<tr>
<td>G1/2 (n = 48)</td>
<td>22 (46)</td>
<td>26 (54)</td>
<td></td>
</tr>
<tr>
<td>G3 (n = 46)</td>
<td>12 (26)</td>
<td>34 (74)</td>
<td></td>
</tr>
<tr>
<td>ER and PR status</td>
<td></td>
<td></td>
<td>(.196)</td>
</tr>
<tr>
<td>ER+ and PR+ (n = 54)</td>
<td>21 (39)</td>
<td>33 (61)</td>
<td></td>
</tr>
<tr>
<td>ER+ or PR+ (n = 20)</td>
<td>6 (30)</td>
<td>14 (70)</td>
<td></td>
</tr>
<tr>
<td>ER− and PR− (n = 34)</td>
<td>7 (21)</td>
<td>27 (79)</td>
<td></td>
</tr>
<tr>
<td>HER-2/neu status</td>
<td></td>
<td></td>
<td>(.733)</td>
</tr>
<tr>
<td>0, 1+, 2+ without gene amplification (n = 72)</td>
<td>23 (32)</td>
<td>49 (68)</td>
<td></td>
</tr>
<tr>
<td>2+ with gene amplification, 3+ (n = 18)</td>
<td>5 (28)</td>
<td>13 (72)</td>
<td></td>
</tr>
<tr>
<td>EGFR score</td>
<td></td>
<td></td>
<td>(.362)</td>
</tr>
<tr>
<td>0 (n = 72)</td>
<td>24 (33)</td>
<td>48 (67)</td>
<td></td>
</tr>
<tr>
<td>1+, 2+, 3+ (n = 18)</td>
<td>4 (22)</td>
<td>14 (78)</td>
<td></td>
</tr>
<tr>
<td>Serum HER-2/neu</td>
<td></td>
<td></td>
<td>(.011)</td>
</tr>
<tr>
<td>Serum EGFR</td>
<td></td>
<td></td>
<td>(.117)</td>
</tr>
<tr>
<td>Type of chemotherapy</td>
<td></td>
<td></td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Anthracycline with taxane (n = 55)</td>
<td>5 (9)</td>
<td>50 (91)</td>
<td></td>
</tr>
<tr>
<td>Anthracyline without taxane (n = 53)</td>
<td>29 (55)</td>
<td>24 (45)</td>
<td></td>
</tr>
</tbody>
</table>

EGFR, epidermal growth factor receptor; ER, estrogen receptor; PR, progesterone receptor.

* Data are given as number (percentage).

† Univariate analysis. Significant values are shown in bold type.
In addition, the median pretreatment serum HER-2/neu level in patients with pCR was significantly higher than in patients with moderate or no treatment response (10.95 vs 9.1 ng/mL; \( P = .041; 95\% \ CI , .036-.046 \)). There was no statistically significant difference between the median prechemotherapy serum HER-2/neu value for patients with pCR and patients with a moderate response (\( P = .091 \)).

Box plots of prechemotherapy serum HER-2/neu levels compared with regression grades are shown in "Figure 1".

Sensitivity and Specificity of Serum HER-2/neu as Predictive Factor for Response to Chemotherapy

To evaluate a cutoff value for the serum HER-2/neu level to distinguish responders (regression grades 1 to 4) from non-responders (regression grade 0) to neoadjuvant chemotherapy, an ROC curve analysis was performed that showed a serum HER-2/neu value of 8.7 ng/mL to distinguish responders from nonresponders with 68.9% sensitivity and 58.8% specificity.

In addition, ROC curve analysis revealed a cutoff serum HER-2/neu value of 10.3 ng/mL that distinguished patients with pCR from patients with moderate or no response with 80% sensitivity (95% CI, 44.4%-96.9%) and 69.4% specificity (95% CI, 59.3%-78.3%). The ROC curve for serum HER-2/neu as a predictive factor for pCR is shown in "Figure 2".

Pretreatment Serum EGFR Level and Response to Chemotherapy

The median prechemotherapy serum EGFR value for patients with pCR was 67 ng/mL (range, 55-80.4 ng/mL); in patients with moderate response, it was 61.5 ng/mL (range, 40.9-83.2 ng/mL); and in patients without response, it was 60.1 ng/mL (range, 43.8-72.4 ng/mL). There was no statistically significant correlation between median prechemotherapy serum EGFR level and histopathologic response to chemotherapy.

Expression of HER-2/neu and EGFR in Primary Tumor Biopsy Specimens

Of the 90 tumors examined, immunohistochemical HER-2/neu score 0 was found in 34 tumors (38%), score 1+ in 31 (34%), score 2+ in 11 (12%), and score 3+ in 14 (16%). Tumors with an immunohistochemical HER-2/neu score of 2+ were tested additionally for HER-2/neu gene amplification by FISH. HER-2/neu gene amplification was detected in 4 (36%) of 11 tumors with an immunohistochemical HER-2/neu score of 2+, and 7 (64%) of 11 tumors with an immunohistochemical HER-2/neu score of 2+ showed no gene amplification.

EGFR expression was found immunohistochemically in 18 tumors, as follows: 1+, 9 (50%); 2+, 6 (33%); and 3+, in 3 (17%). Seventy-two (80%) tumors showed no EGFR expression (score 0). Of 72 tumors without immunohistochemically detectable EGFR expression, 13 (18%) showed an HER-2/neu score of 3+, and 59 (82%) showed an HER-2/neu score of 0,
1+, or 2+. Within the 18 breast carcinomas with an EGFR score of 1+, 2+, or 3+, 17 (94%) were characterized with an HER-2/neu score of 0, 1+, or 2+, and only 1 tumor (6%) showed coexpression with an HER-2/neu score of 3+.

Discussion

The goal of predictive parameters for response to neoadjuvant chemotherapy is to select patients who most likely will benefit from preoperative antitumor treatment to avoid ineffective treatment with unnecessary toxic effects and to offer patients with a poor chance of response alternative treatment modalities.

The results of this study showed a statistically significant correlation between histopathologic response to neoadjuvant anthracycline-based chemotherapy and levels of pretreatment serum HER-2/neu, indicating that a higher pretreatment serum HER-2/neu level predicts increased histopathologic regression of tumor tissue. In addition, results of the present study showed a statistically significant correlation of the pretreatment serum HER-2/neu level with pCR as the response to neoadjuvant chemotherapy. No significant correlation was found for serum EGFR level and response to anthracycline-based chemotherapy.

Previous studies examining potentially predictive parameters for chemotherapy focused on clinical and radiologic responses. Clinical response assessment is influenced by several tissue factors such as edema, necrosis, and desmoplastic stromal reaction, which cannot be exactly differentiated from vital tumor tissue by palpation only. Further problems with clinical response assessment are the tendency of subjective overestimation of tumor size and variations in tumor measurement, especially with various investigators within a study.

Radiologic assessment of tumor size by mammography and ultrasound correlates better with histologically measured tumor dimensions compared with clinical tumor assessment. However, pathologic complete remission of tumor tissue has been shown to be the only relevant parameter improving survival of patients after neoadjuvant chemotherapy. Therefore, in this study, histopathologic response to preoperative chemotherapy was chosen as the end point to evaluate the predictive value of EGFR and HER-2/neu in serum and tumor tissue. pCR was defined in the present study as regression grade 3 or 4 according to the response scoring system of Sinn et al., indicating disappearance of all invasive carcinoma independent of residual carcinoma in situ. This definition of pCR was based on the results reported from previous studies showing the same favorable prognosis for patients with residual carcinoma in situ and patients without invasive or in situ carcinoma following neoadjuvant chemotherapy for breast cancer.

Results of previous studies of the predictive value of HER-2/neu tissue status for adjuvant chemotherapy suggest that HER-2/neu overexpression predicts anthracycline sensitivity. In the neoadjuvant treatment setting, a recently reported study showed a nonsignificant trend toward poorer clinical and radiologic response to anthracycline-based preoperative chemotherapy in HER-2/neu–overexpressing tumors. However, the study showed no difference in pCR rates between HER-2/neu–overexpressing carcinomas and tumors without HER-2/neu overexpression. In our study, serum HER-2/neu was shown to correlate with response to neoadjuvant anthracycline-based chemotherapy, whereas the HER-2/neu status of tumor tissue did not correlate with response to treatment. A possible explanation for this discrepancy can be that elevated serum levels of HER-2/neu ECD are not surrogates for HER-2/neu overexpression in early, nonmetastasized stages of breast cancer. In a recently published study investigating serum HER-2/neu concentrations in patients with primary breast cancer, serum HER-2/neu concentrations ranged from 5.0 to 17.5 μg/L; however, no correlation was found with HER-2/neu status as assessed by immunohistochemical analysis or FISH. In addition, results of our study reported previously, investigating the prognostic significance of serum HER-2/neu in metastatic breast cancer, demonstrated that elevated serum HER-2/neu values are not restricted to patients with HER-2/neu–overexpressing primary tumors.

In previous studies examining the predictive value of serum HER-2/neu for anthracycline-based chemotherapy in metastatic breast cancer, divergent results were reported. Colomer et al. reported that in 58 patients with metastatic breast cancer treated with doxorubicin and paclitaxel, circulating HER-2/neu ECD levels correlated inversely with radiologic response to therapy. In another study including 103 patients treated with epirubicin and cyclophosphamide or paclitaxel for metastatic breast cancer, no significant correlation between radiologic response to chemotherapy and serum HER-2/neu levels was observed for the entire study population. We demonstrated a significant correlation of serum HER-2/neu levels and histopathologic response of breast cancer to anthracycline-based neoadjuvant chemotherapy. There are few data about the predictive significance of EGFR expression in metastatic breast cancer, but up to now, no reported data describe the impact of EGFR expression on response to neoadjuvant anthracyclines. To the best of our knowledge, the present study is the first to examine the predictive significance of HER-2/neu and EGFR levels in the serum of patients with early, nonmetastasized stages of breast cancer receiving neoadjuvant anthracycline-based chemotherapy.

The findings of this study demonstrate a statistically significant correlation of pretreatment serum HER-2/neu and
response to neoadjuvant anthracycline-based chemotherapy in breast cancer. In our study, a pretreatment serum HER-2/neu value of more than 10.3 ng/mL predicted a pCR with 80% sensitivity and 69.4% specificity. Serum EGFR, as well as HER-2/neu and EGFR scores in tumor tissue, were found to have no predictive value in this setting. Results of this study add important information on the possible use of the serum marker HER-2/neu as a predictive marker for tumor response in patients with breast cancer undergoing neoadjuvant chemotherapy. Further studies are necessary to evaluate the definitive clinical usefulness of serum HER-2/neu as a predictive marker for neoadjuvant anthracycline-based chemotherapy in breast cancer.

From the 1Department of Internal Medicine, Division of Oncology and 2Department of Pathology, Medical University of Graz, Graz, Austria; and 3Bayer Vital, Leverkusen, Germany. Supported by the Austrian Cancer Aid Styria (project No. EF 05/2004), Graz, Austria.

Address reprint requests to Dr Schippinger:
Universitätsklinik für Innere Medizin Graz, Klinische Abteilung für Onkologie, Auenbruggerplatz 15, A-8036 Graz, Austria.

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