Predicting Tissue HER2 Status Using Serum HER2 Levels in Patients with Metastatic Breast Cancer

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**Background:** Immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) are reliable ways to identify amplification of the HER-2/neu (HER2, symbol ERBB2) gene, but each technique requires a high-quality tissue sample, which may not be available. We investigated whether serum concentrations of the HER2 extracellular domain (ECD) can be used as an alternative to tissue HER2 status in metastatic breast cancer, and we defined an optimal decision level of serum HER2 for prediction of tissue HER2 status.

**Methods:** We determined HER2 expression in 195 metastatic breast cancer patients by IHC and performed FISH analysis on tumors for which IHC staining was graded as 2+. We measured serum HER2 by immunoadassay and used receiver operating characteristics (ROC) curve analysis to determine optimal serum HER2 ECD concentrations for differentiation between positive and negative HER2 status.

**Results:** IHC results were 0/1+ for 30 (15%) of the patients, 2+ for 89 (46%), and 3+ for 76 (39%). FISH revealed HER2 amplification in 19 (21%) of the IHC 2+ tumors. Mean (SE) serum HER2 ECD was 22.2 (5.1) μg/L in the tissue HER2-negative group, significantly lower than the concentration of 363 (96) μg/L in the tissue HER2-positive group (P <0.0001). ROC curve analysis showed 95% specificity and 62% sensitivity for tissue HER2 positivity at 37 μg/L of serum HER2.

**Conclusion:** To use serum HER2 concentration as an alternative to direct determination of tissue HER2 status, we suggest 37 μg/L as a cutoff for predicting positive tissue HER2 with 95% specificity. Sensitivity, however, is low.

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The HER-2/neu (HER2) protooncogene (symbol ERBB2) encodes a transmembrane glycoprotein with tyrosine kinase activity that mediates proliferation and differentiation in normal epithelial cells (1). As a result of gene amplification, ~20%–30% of breast cancers overexpress HER2 protein, and such overexpression is associated with poor prognosis (2–5). Metastatic breast cancers that test positive for HER2 amplification and/or overexpression are candidates for anti-HER2 trastuzumab (Herceptin) therapy, so it is essential to evaluate HER2 status accurately (6, 7).

Tissue HER2 status is generally determined by immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH) (8). IHC is the method of choice, with 0/1+ signifying HER2 negative status and 3+ signifying HER2 positive status. FISH is performed when IHC results are indeterminate (2+), and cancers that show HER2 amplification are classified as HER2 positive (9). Because IHC and FISH require a high-quality tissue sample, which is not always available, and because FISH is a costly procedure—more than 10 times more expensive than IHC in Korea—we sought an alternative method for evaluating HER2 status. We based our method on the finding that the HER2 extracellular domain (ECD) may be cleaved and shed from the surface of breast cancer cells and released into the circulation (10, 11). This leads to increased serum HER2 ECD concentrations in ~30%–40% of patients with metastatic breast cancer and serves as a marker for prognosis and resistance to hormone and alkylating therapy (12–14). Serum HER2 ECD concentration correlates with tissue HER2 status (by IHC or FISH) (15), and the possibility of using it as an alternative to tissue analysis has recently been examined (16). In this study, we investigated that possibility further in view of the discordant positive rates between tissue HER2 and serum HER2 in patients with metastatic breast cancer.
metastatic breast cancer, and we suggest a method that optimizes predictive power of serum HER2 ECD concentration.

**Materials and Methods**

**PATIENTS AND SAMPLES**

This retrospective study included 195 patients with metastatic breast cancer who were treated at the National Cancer Center, Korea, from February 2002 to May 2005. During this period, we diagnosed 320 patients with metastatic breast cancer; however, we excluded 125 patients because we could not obtain their serum samples from the time when their diagnoses were made. Clinicopathologic features such as age, disease site, number of metastases, and estrogen and progesterone receptor status were evaluated. We typically do not perform tissue biopsy for metastatic breast cancer that has advanced from primary breast cancer, so prior tissue samples were obtained from 114 patients. We performed tissue IHC and FISH analysis using tissue samples from the time the patient was diagnosed with primary breast cancer (stage I, II, III) in 114 patients and using tissue samples from the time metastasis was diagnosed in 81 patients. Thirty-four of 81 patients had metastatic breast cancer at the initial diagnosis (stage IV disease), and in 47 of 81 patients, original primary breast tissue could not be retrieved. The tissue specimens were fixed in formaldehyde and embedded in paraffin by standard methodology. All serum samples were obtained when the patients were diagnosed with metastatic breast cancer and before chemotherapy or Herceptin therapy.

**IMMUNOHISTOCHEMISTRY**

Paraffin-embedded tissue was cut into 4-μm sections and mounted on glass slides. The sections were deparaffinized in xylene and rehydrated through alcohols to distilled water. Sections were incubated for 15 min in 10 mmol/L citrate buffer, pH 6.0, at 98 °C. Polyclonal rabbit antihuman c-erbB-2 oncoprotein (Code A0485; Dako Corp.) was applied at 1:100 dilutions. The slides were stained in an ES automatic stainer (Ventana Medical Systems Inc.), and the degree of staining was scored by a pathologist, according to the method recommended for the Dako HercepTest.

**FISH**

Paraffin-embedded tissue was cut into 4-μm sections, mounted onto positively charged slides, baked at 56 °C overnight, and deparaffinized. FISH analysis was performed using a PathVision HER2 DNA Probe Kit (Vysis) according to the manufacturer’s recommendations. Briefly, the tissues were pretreated and subjected to protease digestion. A hybridization solution containing directly labeled probes of SpectrumGreen for the chromosome 17 centromere (CEP) and SpectrumOrange for the HER2 gene locus were applied, and the denaturation and hybridization procedures were performed in a HYBrite system (Vysis). The slides were washed in 2 × SSC buffer (0.15 mol/L NaCl and 0.015 mol/L sodium citrate; pH 7.2) at 72 °C for 2 min and counterstained with diaminopyrrolylindole. Two observers assessed each with a Nikon Eclipse E600 fluorescence microscope equipped with narrow bandpass filters. They scored at least 60 cells per specimen for HER2 (ERBB2) and CEP signals. We set a HER2:CEP ratio >2.0 as evidence of HER2 (ERBB2) amplification.

**SERUM HER2 ECD ASSAY**

Blood was drawn into serum separator tubes and centrifuged at 2000 × g for 10 min at room temperature. Serum samples were stored at −70 °C until used. To measure the serum HER2 ECD concentration, we used a 2-site chemiluminescence sandwich immunoassay (ADVIA Centaur System, Bayer Diagnostics) with a detection limit of 0.5 μg/L; the interassay CV in our laboratory was 5.9%. Following the manufacturer’s recommendation, we used 15 μg/L as the serum HER2 ECD cutoff to discriminate normal concentrations from patients with breast cancer (17). Serum HER2 ECD measurement was carried out in a blinded manner without knowledge about the results of IHC and FISH. When clinically indicated, we measured carcinoembryonic antigen (CEA) using a cutoff value of 5 μg/L (n = 65) (ARCHITECT i2000, Abbott Diagnostics) and CA15.3 using a cutoff value of 38 units/mL (n = 126) (ADVIA Centaur System).

**STATISTICAL ANALYSIS**

We used SAS version 8 software for statistical analysis. To examine the relationship between HER2 status and other clinicopathologic variables, we used the χ² test and logistic regression analysis. To measure concordance of IHC, FISH, and serum HER2 results, we changed the serum HER2 value to a dichotomous variable using the normal cutoff level and estimated the Spearman correlation. We used the t-test to compare the serum HER2 level with tissue HER2 status in each group. We performed receiver operating characteristic (ROC) curve analysis to evaluate the discriminating ability of serum HER2 and to arrive at a serum HER2 cutoff measure for predicting tissue HER2 positivity. Also, we separately analyzed ROC curve according to tissue origins (tissue at the site of primary diagnosis vs tissue at the site of metastasis). We analyzed CA15.3 and CEA ROC curve.

**Results**

Table 1 shows the clinicopathologic characteristics and tissue HER2 status of all 195 cases. The median age of the patients was 49 years (range, 24–85). IHC results were 0/1+ for 30 (15%) of the patients, 2+ for 89 (46%), and 3+ for 76 (39%). FISH revealed HER2 amplification in 19 (21%) of the IHC 2+ tumors. Tissue HER2 positivity was significantly (P = 0.001) associated with negative estrogen receptors, negative progesterone receptors, liver metastases, and brain metastases.

A flow diagram for the study is shown in Fig. 1.

Serum HER2 was >15 μg/L in 119 (61%) of the
patients, including 35 from the tissue HER2-negative group (n = 100) and 84 from the tissue HER2 positive group (n = 95).

The serum HER2 results were correlated with the IHC (\(P < 0.01\)) and FISH (\(P < 0.01\)) results and showed significant (\(P < 0.01\)) correlation with progesterone receptor negativity and liver metastases.

Mean (SE) serum HER2 ECD in the tissue HER2-negative group was 22.2 (5.1) \(\mu\)g/L, significantly lower than the concentration of 363 (96) \(\mu\)g/L in the tissue HER2-positive group (\(P < 0.0001\)) (Fig. 2.). Mean (SE) CEA [13.9 (6.3) vs 40.3 (14.1)] and CA15.3 [120 (22) vs 231 (48)] concentrations did not differ significantly (\(P > 0.05\)) between the two groups.

ROC curve analysis showed 62% specificity and 88% sensitivity for tissue HER2 positivity at 15 \(\mu\)g/L serum HER2. A 90% specificity and 65% sensitivity were seen at 30 \(\mu\)g/L HER2/L (AUC, 0.87; 95% confidence interval (CI), 0.81–0.92) (Fig. 3.). At 37 \(\mu\)g/L serum HER2, specificity was 95% and sensitivity 62%. Using 30 \(\mu\)g/L as the decision level, 71 (36.4%) of the patients showed increased serum HER2; using 37 \(\mu\)g/L, 64 (32.8%) of the patients showed increased serum HER2.

When we analyzed only data from patients in whom the primary site of cancer was studied (n = 114), a cutoff of 33 \(\mu\)g/L was associated with 95% specificity and 66% sensitivity (AUC, 0.87; 95% CI, 0.80–0.94). The corresponding cutoff (that is, for 95% specificity) derived from the data on metastatic tissue was slightly higher than that for primary tissue data, but the distributions of serum HER2 in the negative (primary tissue vs metastatic tissue) and positive tissue HER2 group showed no significant difference by t-test (\(P = 0.56\) in tissue HER2 negative group, and \(P = 0.63\) in tissue HER2 positive group).

Because the serum HER2 results showed significant (\(P < 0.01\)) correlation with liver metastasis, we analyzed ROC curve for the patients with liver metastasis vs without liver metastasis. The cutoff for 95% specificity in patients with liver metastasis was 133 \(\mu\)g/L, at which the sensitivity was 51% and the AUC 0.84 (95% CI 0.75–0.92); the corresponding cutoff for patients without liver metastasis was 32 \(\mu\)g/L which had a 55% sensitivity and AUC of 0.89 (95% CI 0.82–0.96).

For CEA and CA15.3, the optimal cutoffs were 38.0 \(\mu\)g/L (AUC, 0.70; 95% CI, 0.57–0.83; \(P = 0.006\)), and 342 kU/L (AUC, 0.59; 95% CI, 0.49–0.69; \(P = 0.09\)), respectively.

**Discussion**

This study, like previous ones (15, 16), demonstrated a good correlation between serum HER2 ECD concentration and IHC and FISH results in metastatic breast cancer. Here, in addition, we determined that 37 \(\mu\)g/L serum HER2 ECD showed 95% specificity and 62% sensitivity in predicting tissue HER2, and we suggest that that value be used as the cutoff because we focused on low false-positive rate for tissue positivity.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients, n (%)</th>
<th>Tissue HER2 negative, n (%)</th>
<th>Tissue HER2 positive, n (%)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen receptor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>90 (46.2)</td>
<td>28 (31.1)</td>
<td>62 (68.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Positive</td>
<td>105 (53.8)</td>
<td>72 (68.6)</td>
<td>33 (31.4)</td>
<td></td>
</tr>
<tr>
<td>Progesterone receptor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>117 (60.0)</td>
<td>46 (39.3)</td>
<td>71 (60.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Positive</td>
<td>75 (38.5)</td>
<td>53 (70.7)</td>
<td>22 (29.3)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>3 (1.5)</td>
<td>1 (33.3)</td>
<td>2 (66.7)</td>
<td></td>
</tr>
<tr>
<td>Serum HER2 ECD</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Not increased</td>
<td>74 (37.9)</td>
<td>63 (85.1)</td>
<td>11 (14.9)</td>
<td></td>
</tr>
<tr>
<td>Increased</td>
<td>121 (62.1)</td>
<td>37 (30.6)</td>
<td>84 (69.4)</td>
<td></td>
</tr>
<tr>
<td>Sites of metastasis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>119 (61.0)</td>
<td>56 (47.1)</td>
<td>63 (52.9)</td>
<td>0.141</td>
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<tr>
<td>Liver</td>
<td>93 (47.7)</td>
<td>36 (38.7)</td>
<td>57 (61.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>Lung</td>
<td>90 (46.2)</td>
<td>48 (53.3)</td>
<td>42 (46.7)</td>
<td>0.596</td>
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<tr>
<td>Pleura</td>
<td>46 (23.6)</td>
<td>24 (52.2)</td>
<td>22 (47.8)</td>
<td>0.890</td>
</tr>
<tr>
<td>Skin</td>
<td>27 (13.8)</td>
<td>12 (44.4)</td>
<td>15 (55.6)</td>
<td>0.445</td>
</tr>
<tr>
<td>Brain</td>
<td>22 (11.3)</td>
<td>3 (13.6)</td>
<td>19 (86.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>No. of disease sites</td>
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<td></td>
<td></td>
<td>0.025</td>
</tr>
<tr>
<td>1</td>
<td>54 (27.7)</td>
<td>34</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>57 (29.2)</td>
<td>32</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>≥3</td>
<td>84 (43.1)</td>
<td>34</td>
<td>50</td>
<td></td>
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</table>

Tissue HER2 positivity was significantly associated with negative estrogen receptors, negative progesterone receptors, liver metastases, and brain metastases.
A previous study determined 16 \( \mu g/L \) as the cutoff point (16), but the specificity at 16 \( \mu g/L \) in our study was only 72%. The discrepancy might result from differences in the assays, but if we consider the different positive rates—30%–40% when the serum HER2 ECD cutoff level was 15 \( \mu g/L \) and 20%–30% in tissue HER2 studies in metastatic breast cancer—the decision cutoff level for tissue HER2 positivity would be higher than the general cutoff point to diagnose for breast cancer. Thus, we chose a serum HER2 ECD level with higher specificity to achieve a more stringent clinical application of trastuzumab therapy, especially where no tissue HER2 data was available.

Some cases with negative tissue HER2 in our study showed high serum HER2 ECD concentrations (up to 500.4 \( \mu g/L \)), which might be the result of transformation of tissue HER2 status at disease recurrence, as has been reported (19, 20).

To obtain optimal cutoff according clinical variables, we considered tissue origin and liver metastasis and analyzed ROC curves separately. The cutoff determined from data of metastatic tissue was slightly higher than that of primary tissue data, but we observed no significant difference of serum HER2 between each of the negative (primary tissue vs. metastatic tissue) and positive tissue HER2 groups. The cutoff values of serum HER2 according to the presence or absence of liver metastasis showed differences as wide as 32 \( \mu g/L \) vs 133 \( \mu g/L \); thus, we

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**Fig. 1. Flow diagram of patients in study.**

Tissue HER2 (ERBB2) status was determined by IHC and FISH. IHC 0/1+ was classified tissue HER2-negative status, and 3+ was classified HER2-positive status. FISH was performed when IHC results were 2+ (indeterminate), and cancers that showed HER2 amplification were classified as tissue HER2-positive.
might considered a higher cutoff level of serum HER2 when assessing HER2 status in patients with liver metastasis.

When we compared serum HER2 with CA15.3 and CEA, 2 other common tumor markers in metastatic breast cancer, serum HER2 showed a higher discrimination power, although CA15.3 and CEA were evaluated in a smaller number of patients.

Serum HER2 ECD concentration was useful not only for assessing prognosis, predicting the response to trastuzumab, and reflecting tumor responses reported in previous studies (7, 14, 21, 22), but also, at the higher cutoff plane, for reflecting tissue HER2 status with enhanced specificity.

In conclusion, determination of serum HER2 ECD concentration in metastatic breast cancer may provide a useful index of tissue HER2 status, especially in light of its relatively easy and inexpensive methodology and the simplicity of sample collection.

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References


