Prognostic Significance of Serum HER2 and CA 15-3 at the Time of Diagnosis of Metastatic Breast Cancer

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Abstract. Background: Serum HER2 testing allows the determination of real-time HER2 status during clinical course. The aim of this investigation was: (1) to study the prognostic significance of serum HER2 at the time of first diagnosis of metastatic breast cancer and (2) to evaluate its relationship to CA15-3 which is a surrogate marker for tumor load. Materials and Methods: Serum samples of 120 breast cancer patients were assayed for HER2 and CA15-3 at the onset of metastatic disease. Results: Forty-seven out of 120 (39%) metastatic breast cancer patients had elevated serum HER2 levels. The positivity rate of CA15-3 was 51%. The median survival after relapse (SAR) for HER2-positive patients was shorter (10 months, 95%-CI: 6-14 months) compared to the SAR of HER2-negative patients (19 months, 95%-CI:15-23 months) (p<0.01). The median survival of patients with increased CA 15-3 was 13 months (95%-CI: 9-17 months) compared to 18 months (95%-CI: 15-21 months) for patients with normal CA 15-3 concentrations (p<0.05). In the multivariate analysis serum HER2 was an independent prognostic marker for SAR even when adjusted for tumor load measured by CA15-3 levels. Conclusion: Serum HER2 is a strong independent prognostic factor for survival after relapse in metastatic breast cancer patients with normal CA 15-3 concentrations (p<0.05). Overexpression and amplification of HER2 - protooncogene in breast cancer has turned out to be an important prognostic marker with therapeutic implications. The HER2 protooncogene is located on chromosome 17q21 and encodes a transmembrane tyrosine kinase growth receptor (1,2). Gene amplification and overexpression of HER2 protooncogene occurs in approximately 15% to 30% of breast carcinomas (3-5). Primary and metastatic patients who overexpress HER2 or show HER2 amplification are in general eligible for treatment with the anti-HER-2/neu targeted monoclonal antibody, trastuzumab.

The HER2 status is determined by tissue analysis in clinical routine. The overexpression is analyzed by immunohistochemical staining of tumor tissue whereas the gene amplification is evaluated by fluorescence in situ hybridization (FISH) analysis. An additional method to determine the HER2 status is based on a serum test which measures the extracellular domain of the receptor protein. The external domain is shed into the blood circulation and can therefore be detected in the serum of breast cancer patients (6,7). Serum HER2 is correlated to tumor load and HER2 overexpression in tumor tissue (8-11). Several studies indicated that elevated serum HER2 predicts a worse clinical outcome (9-14) and poor response to hormonal treatment and chemotherapy (14-17). Moreover, the current published literature suggests that serial serum HER2 determinations may be useful to detect metastatic disease and monitor metastatic breast cancer patients during clinical course of disease (9,11-13,18-20).

The aim of this study was to evaluate the clinical utility of HER2 in metastatic breast cancer patients alone and in combination with CA 15-3. CA 15-3 is a well-established surrogate marker for tumor load in breast cancer. The prognostic significance of CA 15-3 at the time of metastatic disease is mainly related to tumor burden (21). Serial CA 15-3 measurements are used to detect early recurrence and monitor metastatic breast cancer patients during follow-up (21-25). In the first part of the investigation the positivity rates of CA 15-3 and HER2 at the time of detection of metastatic disease were analyzed and correlated to prognostic factors. To assess whether serum HER2 is a prognostic marker when adjusted for tumor burden measured by CA 15-3 levels, the influence of CA 15-3 and serum HER2 for survival time after relapse – alone and in combination – was studied.

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Key Words: Serum HER2, CA 15-3, breast cancer, prognosis, survival, metastasis.
Materials and Methods

Patients. Metastatic breast cancer patients for this analysis were drawn from a previous prospective study which evaluated the ability of CA 15-3 to detect metastatic disease in breast cancer patients during follow-up by serial CA 15-3 determinations (22). Serum samples at the time of detection of metastases of 120 patients were available. Clinical characteristics of these patients are shown in Table I. For diagnosis of metastases, abdominal sonography, chest X-ray and bone scintigraphy were performed at regular intervals. The median follow-up of patients was 19 months (range: 1-118 months).

HER2 measurements. All serum samples had been taken at the time of first diagnosis of metastatic disease and stored at -20°C in a sera bank until assayed. The sera were thawed and HER2 was measured by a commercially available sandwich enzyme immunoassay (Dianova, Hamburg, Germany) according to the manufacturer’s instructions. All samples were assayed in duplicate. The intraassay coefficient of variation (CV) was 4% and the interassay CV was 10%. 1,900 HNU/ml (HNU: human neu unit) was chosen as the cut-off level from a previous study using the same assay (7). CA 15-3 levels were measured routinely using a commercially available enzyme immunoassay (Enzymun-Test CA 15-3, Roche Diagnostics, Germany). The cut-off was 40 U/ml as previously reported (22). All samples were analyzed in a blinded fashion.

Statistical methods. Serum HER2 and CA 15-3 were analyzed as continuous and dichotomous variables (e.g. "HER2-positive" versus "HER2-negative"). A Chi-squared test was used to evaluate the correlation between variables. Disease-free survival (DFS) was calculated from the date of diagnosis of the primary breast cancer until detection of metastases. Survival after relapse (SAR) was defined as the time interval between the first diagnosis of metastases until death. Survival curves were generated by the methods of Kaplan-Meier and compared by log-rank test. A Cox regression model was performed in the univariate analysis to screen for prognostic factors and in multivariate analysis to determine the independent prognostic significance for survival after relapse. Factors included in the Cox regression model were menopausal status, estrogen and progesterone receptor status, disease-free survival, localization of the metastases, CA 15-3 as well as HER2. Odds ratios (OR) and their 95% confidence intervals (CI) were also determined by the Cox regression model. Statistical analyses were performed using SPSS for Windows (Version 11.0). p-values less than 0.05 were considered statistically significant.

Results

HER2 and CA 15-3 serum levels at the time of first diagnosis. The HER2 serum levels of the 120 breast cancer patients ranged from 410 U/ml to 25,000 U/ml with a median of 1,207 U/ml. Using 1,900 U/ml as cut-off level, 47 out of 120 (39%) patients had HER2 concentrations above the cut-off level. Elevated CA 15-3 concentrations were detected in 61 out of 120 patients (51%). Both markers were increased in 27 out of 120 patients (23%) (Table II). Combining HER2 and CA 15-3, the sensitivity rate increased to 68%. Neither CA 15-3 nor HER2 serum levels correlated with menopausal status, site of
metastases, disease-free survival (DFS) or estrogen and progesterone receptor status (Table I). CA 15-3 and HER2 showed a weak correlation when analyzed as continuous variables ($r=0.19, p<0.04$), but not as a categorized variables (elevated versus non elevated) (Table I).

**Univariate analysis for HER 2 and CA 15-3.** The univariate analysis revealed that elevated HER2 and CA 15-3 serum levels were associated with a worse prognosis. The median survival after relapse (SAR) for HER2-positive patients was 10 months (95%-CI: 6-14 months) and therefore shorter compared to the SAR of HER2-negative patients which was 19 months (95%-CI:15-23 months) ($p<0.01$). The survival differences were less for patients with elevated CA 15-3 ($p<0.05$). The median survival of patients with increased CA 15-3 was 13 months (95%-CI: 9-17 months) compared to 18 months (95%-CI: 15-21 months) for patients who had normal CA 15-3 concentrations. Figure 1 shows the survival curves after relapse according to HER2 serum status (a) and CA 15-3 positivity (b).

Other factors significant for survival in the univariate analysis were estrogen receptor status, progesterone receptor status, site of metastases and disease-free survival. Menopausal status did not influence the clinical outcome (Table III).

**Multivariate analysis for HER2 and CA 15-3.** To evaluate the independent prognostic factor of HER2 and the interrelationship of the other prognostic factors, a multivariate analysis was performed. The factors included in the analysis and their categories are shown in Table III. HER2 was the strongest independent prognostic factor for SAR followed by progesterone receptor status, CA 15-3 and disease-free survival. Estrogen receptor status and site of metastases lost their prognostic significance when the other factors were taken into account. The results of the Cox regression analysis are shown in Table III.

**CA 15-3 and HER 2 as a combined marker.** Finally, the prognostic significance of the combination of CA15-3 and HER2 was studied. Patients were subdivided into four groups according to their CA 15-3 and HER2 levels. Both markers were elevated in 23% of patients. Seventeen percent of the patients had only increased HER2 levels and 28% of the patients presented only elevated CA 15-3. None of the markers were increased in 32% of the patients. Data are shown in Table II. Elevation of both CA 15-3 and HER2 serum levels appeared not to be related to any of the prognostic markers (Table I). Survival curves for all four groups are summarized in Figure 2. Patients with elevated concentrations for both markers had a significantly shorter survival than those in which none or only one marker was elevated ($p<0.01$). The survival data are summarized in Table II. For the multivariate analysis patients were subdivided into two groups (HER2 / CA 15-3 elevated versus one or no marker elevated). "HER2 and CA 15-3 elevated" turned out to be the strongest independent factor followed by progesterone receptor.

**Discussion**

The role of serum HER2 in clinical routine is still under controversial discussion. The currently accepted method to determine HER2 status in breast cancer patients is FISH or immunohistochemistry of the (primary) tumor. When patients develop a metastatic disease and no metastatic tissue is available for re-evaluation of HER2 status, treatment decisions are based on the results of the primary tumor. However, the HER-2 status may change during clinical course resulting in an uncertain response to anti-HER2 therapy (20,26,27). A complementary method to determine HER2 status is based on a serum test that measures the extracellular domain of the HER2 oncoprotein. Serum HER2 testing can easily be repeated and allows the determination of the real time status of HER2 during clinical course. The aim of this study was to evaluate the prognostic significance of serum HER2 in metastatic breast cancer at the time of first diagnosis and its relationship to CA15-3, an established surrogate marker for monitoring breast cancer patients.

The positivity rate of serum HER2 in metastatic breast cancer was 39%. Similar results ranging from 30% to 59% were reported by others (8,11,13,15,16,18,19,26,28,29). The percentage of serum HER2-positive patients observed in advanced or metastatic breast cancer patients is higher than in primary breast cancer patients where positivity rates range between 3% to 14% based on the literature (8,9,11,14,18,19,26,29). These differences in positivity rates support the hypothesis that a genetic change of the tumor can occur during clinical course. Another possible explanation is that the
Table III. Univariate and multivariate analysis of survival after relapse.

<table>
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<tr>
<th>Variable</th>
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<th>p-value</th>
<th>Multivariate</th>
<th>p-value</th>
<th>Multivariate²</th>
<th>p-value</th>
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<td>1.6 (1.0-2.5)</td>
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<td>2.3 (1.5-3.5)</td>
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<tr>
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<td>&lt;0.01</td>
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<td>3.3 (2.0-5.4)</td>
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¹not significant, ²HER2 and CA 15-3 in combination
percentage of HER2 shedding cells in the primary tumor is initially too small to produce detectable HER2 levels. During disease progression the HER2 shedding cells may become the dominant cell clone due to a growth advantage which leads to increasing serum HER2 levels. Therefore, the HER2 status should be re-evaluated at the onset of metastatic disease or when new metastatic lesions occur. If no tumor tissue is available, determination of serum HER2 may be an alternative to obtain real-time status of HER2. Otherwise fewer patients would be eligible to receive HER2-targeted therapies.

In our study serum HER2 was a strong prognostic marker for SAR. Patients with elevated HER2 serum levels had a significantly shorter SAR compared to others. Moreover, HER2 was the strongest independent prognostic factor for SAR followed by progesterone receptor status, CA 15-3 and DFS in the multivariate analysis. These results were supported by others reporting shortened survival in serum HER2-positive metastatic patients (13,15,16,26,28,30). These data may justify future studies with anti-HER2-directed drugs in patients with positive HER2 serum status.

CA 15-3 is an established tumor marker to follow-up breast cancer patients. The positivity rate of CA 15-3 was 51%. Combining HER2 and CA 15-3 serum status, the detection rate increased up to 68%. Therefore 17% patients would have been missed by the determination of CA 15-3 alone. Similar results were confirmed by other studies (9,10,18,20,26,28) and suggest that the determination of serum HER2 together with CA 15-3 may improve the sensitivity for the early diagnosis of metastatic disease compared to the determination of one marker alone. In addition, the combination of serum HER2 and CA 15-3 will be useful to identify a group of patients with an aggressive tumor and high tumor load as supported by the survival analysis, since patients with both elevated CA 15-3 and HER2 had a significantly shorter survival after relapse compared to those with increased serum HER2 or CA 15-3 alone.

Tumor mass which is reflected by CA 15-3 levels is a prognostic marker in metastatic disease and associated with shortened survival (25). It has been suggested that, besides the surrogate marker CA15-3, serum HER2 is mostly an indicator for tumor mass (11) since a high correlation between CA 15-3 and HER2 could be observed (26). However, in our study CA 15-3 and HER2 showed only a weak correlation when analyzed as continuous variables. When patients were subdivided into categories (elevated versus non elevated) no relationship between these two markers could be observed. These results may indicate that serum HER2 not only reflects tumor load (of HER2 positive cells) but also aggressiveness of disease. This hypothesis was supported by the results of the multivariate analysis. When serum HER2 was adjusted for tumor load measured by CA15-3 level, serum HER2 was still an independent prognostic factor. Similar results were obtained by Ali et al. (28) who performed CA 15-3 and HER2 measurements in metastatic patients receiving second-line hormonal therapy. In concordance with our results only a weak correlation of HER2 and CA 15-3 could be observed. The multivariate analysis revealed that HER2 neu remained an independent prognostic factor when adjusted for tumor load by CA15-3 levels.

In conclusion, our results indicate that serum HER2 is a strong independent prognostic factor for survival after relapse, even when CA 15-3 as a marker for tumor load is taken into account. Therefore, the prognostic significance of serum HER2 may reflect the biological behavior of the tumor in metastatic disease. Moreover, the combination of increased serum HER2 and CA15-3 identifies a subset of patients with worse prognosis compared with patients having only one marker elevated. Therefore, that subset of patients should be considered for inclusion into clinical trials with anti-HER2-directed drugs.

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References


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