Abstract. Background: Serum HER2 testing allows the determination of the real-time HER2 status of breast cancer patients. The aim of this investigation was to study (i) whether changes of serum HER2 status occur during the clinical course of breast cancer and (ii) to evaluate the prognostic significance of serum HER2 status, at the time of first diagnosis of primary breast cancer and at the onset of metastatic disease, for survival after relapse (SAR). Materials and Methods: HER2 serum levels were retrospectively measured in 152 breast cancer patients at the time of first diagnosis of breast cancer and at the onset of metastatic disease. A change of serum HER2 status during clinical course was observed in 43 out of 152 (28%) patients. Serum HER2 status at the time of first diagnosis of breast cancer had no impact on survival after relapse (SAR) (p=0.4). However, the median SAR for serum HER2-positive patients at the onset of metastatic disease was significantly shorter (8 months, 95% CI: 3-12) compared to patients serum HER2-negative at this time (18 months, 95% CI: 14-22) (p<0.01). Conclusion: Serum HER2 status can change during the course of disease. Therefore, the serum HER2 status should be re-evaluated at the time of diagnosis of metastatic disease to optimize treatment decisions.

The HER2 proto-oncogene is located on chromosome 17 and encodes a transmembrane tyrosine kinase growth receptor protein (1,2). Gene amplification and overexpression of the HER2 proto-oncogene occurs in 15% to 20% of breast cancer patients and is associated with poor prognosis, as demonstrated by numerous studies (3-8). Over recent years, the clinical significance of the HER2 status has increased due to its therapeutic implications. HER2-positive metastatic breast cancer patients are eligible for treatment with trastuzumab-based therapies. Trastuzumab (Herceptin®) is a humanized monoclonal antibody which binds with high affinity to the HER2 receptor and inhibits the proliferation of HER2-positive tumor cells (9-11). Several studies indicated that HER2-positive patients are less likely to respond to CMF and tamoxifen but benefit from anthracycline- or taxane-based chemotherapy regimens or aromatase inhibitors (12-15).

The currently accepted method of determining HER2 status is based on tissue testing with either immunohistochemistry for protein overexpression or fluorescence in situ hybridization (FISH) for gene amplification (16). An new alternative to FISH is chromogenic in situ hybridization (17). The HER2 status is usually determined in tissue samples of the primary tumor. However, the HER2 status of the primary tumor does not necessarily reflect the HER2 status in metastatic tissue (18, 19).

An alternative to tissue analysis is a serum test, which is not yet accepted in daily clinical routine for HER2 status determination. This test is based on determination of the extracellular domain of the HER2 receptor protein, which is shed into the blood after proteolytic cleavage from the full-length protein by metalloproteases (20). The circulating serum HER2 protein can be detected by enzyme immunoassay (21-22). Elevated HER2 serum levels are highly correlated with HER2 overexpression and amplification in tumor tissue (23-26). The serum test allows the evaluation of the real-time status of HER2 at the onset of metastatic disease without invasive procedures such as biopsy.

The first aim of this investigation was to study whether changes of serum HER2 status occur during the clinical course of metastatic breast cancer patients, by determining HER2 serum status in breast cancer patients at the time of first diagnosis of primary breast cancer and at the onset of metastatic disease. The prognostic significance of the serum
Table I. Clinical characteristics of the 152 metastatic breast cancer patients.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Serum HER2-positive (primary diagnosis)</th>
<th>Serum HER2-positive (metastatic disease)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td>Total</td>
<td>152</td>
<td>27 (18)</td>
<td>56 (37)</td>
</tr>
<tr>
<td>Menopausal status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>66</td>
<td>9 (14)</td>
<td>27 (41)</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>86</td>
<td>18 (21)</td>
<td>29 (34)</td>
</tr>
<tr>
<td>Estrogen receptor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>76</td>
<td>15 (20)</td>
<td>29 (38)</td>
</tr>
<tr>
<td>Positive</td>
<td>74</td>
<td>11 (15)</td>
<td>25 (34)</td>
</tr>
<tr>
<td>Progesterone receptor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>59</td>
<td>11 (19)</td>
<td>21 (36)</td>
</tr>
<tr>
<td>Positive</td>
<td>91</td>
<td>15 (17)</td>
<td>33 (36)</td>
</tr>
<tr>
<td>Adjuvant treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>10</td>
<td>2 (20)</td>
<td>3 (30)</td>
</tr>
<tr>
<td>Hormonal therapy</td>
<td>106</td>
<td>15 (14)</td>
<td>39 (37)</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>36</td>
<td>10 (28)</td>
<td>14 (39)</td>
</tr>
<tr>
<td>Site of metastases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visceral</td>
<td>82</td>
<td>16 (20)</td>
<td>35 (43)</td>
</tr>
<tr>
<td>Non visceral</td>
<td>69</td>
<td>11 (16)</td>
<td>21 (30)</td>
</tr>
<tr>
<td>DFS</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>≤ 2 years</td>
<td>90</td>
<td>15 (17)</td>
<td>30 (33)</td>
</tr>
<tr>
<td>&gt; 2 years</td>
<td>62</td>
<td>12 (19)</td>
<td>26 (42)</td>
</tr>
</tbody>
</table>

HER2 status at each timepoint was analyzed for survival after relapse. The second part of the study investigated the impact of changes of serum HER2 on prognosis.

Materials and Methods

Patients. Patients for this retrospective analysis were drawn from a prospective study which investigated the clinical utility of the serum marker CA 15-3 to follow-up breast cancer patients during the course of disease by serial CA 15-3 determinations at 3-month intervals (27). To be eligible for this study (i) patients had to be metastasized during follow-up and (ii) blood samples at the time of primary diagnosis and at the onset of metastatic disease had to be available in the sera bank. Moreover, patients had to have a disease-free survival exceeding 6 months to minimize the possibility of including primary breast cancer patients who are considered free of metastases but have already metastasized. The clinical data of the patients are presented in Table I.

For diagnosis of metastatic disease, abdominal ultrasound, chest X-ray and bone scintigraphy had been performed at regular time intervals. The median follow-up was 35 months (range: 7-147 months).

HER2 measurements. Serum samples were stored at -20°C until assayed. Serum HER2 levels were measured by a commercially available sandwich enzyme immunoassay (c-neu ELISA, Dianova, Hamburg, Germany) according to the manufacturer’s instructions without modification. All samples were assayed in duplicate. A cut-off of 1900 ENU/ml was chosen based on a previous study using the same assay (23, 28). All serum samples were analyzed in a blinded fashion. The intra-assay coefficient of variation (CV) was less than 5% and the interassay CV was 10%.

Statistical methods. Serum HER2 was analyzed as a categorical variable (serum HER2-positive versus serum HER2-negative). The Chi-squared test was applied to ascertain statistically significant differences between variables. Disease-free survival (DFS) was the time interval from primary diagnosis to first diagnosis of metastatic disease. Survival after relapse (SAR) was measured from the onset of metastatic disease to the date of death. DFS and SAR were calculated by the methods of Kaplan-Meier. The log rank test was used to compare survival curves. Cox regression model was performed for multivariate analysis. Odds ratios (OR) and their 95% confidence intervals (95% CI) were also determined by the Cox regression model. Statistical analyses were performed using SPSS for Windows (Version 11.5). p-values less than 0.05 were considered statistically significant.

Results

Serum HER2 status at the time of first diagnosis of breast cancer. Twenty-seven out of 152 (18%) metastatic breast cancer patients had elevated serum HER2 levels at the time of first diagnosis of primary breast cancer. Positive serum HER2 status did not correlate with any of the prognostic factors for survival after relapse (SAR) including menopausal status, hormone receptor status, site of metastases or 2-year disease-free survival (Table I). The univariate analysis revealed that serum HER2 status at the time of first diagnosis had no prognostic impact on survival after relapse. The median SAR for serum HER2-positive patients was 13 months (95% CI: 9 - 17 months) compared to 14 months (95% CI: 7 - 22 months) in serum HER2-negative patients (Table II). Figure 1a shows the survival curve after relapse according to the serum HER2 status at the time of first diagnosis of breast cancer.

Serum HER2 status at the time of metastatic disease. At the time of metastatic disease, 56 out of 152 (37%) breast cancer patients were serum HER2-positive. No correlation could be observed between serum HER2 positivity and any of the prognostic factors (Table I). The median survival after relapse for serum HER2-positive patients was 8 months (95% CI: 3 - 12 months), which was significantly shorter compared to 18 months (95% CI: 14 - 22 months) for serum HER2-negative patients (p<0.01) (Table II). To evaluate the independent influence of serum HER2 at the time of metastatic disease on survival after relapse, a multivariate analysis was performed. Factors also included into the analysis were menopausal status, estrogen and progesterone receptor status, disease-free survival and site of metastases. Serum HER2 was the strongest predictor for survival after relapse, followed by progesterone receptor status and disease-free survival. Results of the univariate and multivariate analysis are shown in Table II.
III. Figure 1b demonstrates the survival curve after relapse subdivided by serum HER2 positivity at the onset of metastatic disease.

Change of serum HER2 status during course of disease. The serum HER2 status was available from all patients at the time of first diagnosis of breast cancer and at the time of metastatic disease. Eighty-nine patients remained serum HER2-negative during the clinical course. Twenty patients were HER2 serum-positive only at the time of diagnosis of metastases. A change of serum HER2 status during clinical course of disease could be observed in 43 patients. Seven out of 27 patients, who had elevated HER2 serum levels at the time of primary breast cancer, were serum HER2-negative at the onset of metastatic disease. Conversely, 36 out of 125 initially serum HER2-negative patients were positive for serum HER2 when metastases were diagnosed. The results are summarized in Table II.

To evaluate the prognostic significance of the change in serum HER2 status, the patients were subdivided into four groups according to their serum HER2 status during clinical course (Table II). The first group consisted of patients who were serum HER2-negative at the time of first diagnosis of primary breast cancer and at the time of metastatic disease (serum HER2-negative - negative). The second group included patients who were serum HER2-negative only at the time of first diagnosis (serum HER2-negative - positive). The third group was serum HER2-positive only at the time of metastatic disease (serum HER2-negative - positive), and the fourth group consisted of those patients serum HER2-positive at both timepoints (serum HER2-positive - positive). The survival data are summarized in Table II. No survival differences were observed in serum HER2-negative patients at the time of metastatic disease when subdivided according to their initial serum HER2 status. The median survival after relapse of the serum HER2-negative - negative patients was 17 months (95% CI: 12 - 22 months) compared to 30 months (95% CI: 9 - 50 months) of the serum HER2-positive - negative patients. However, the latter group comprised only 7 patients. Therefore, for further analysis the survival data of both groups were combined.

The initial serum HER2 status also had no influence on survival after relapse in serum HER2-positive patients at the time of metastatic disease. The median SAR for serum HER2-negative - positive patients was 7 months (95% CI: 4 - 10 months) versus 10 months (95% CI: 0 - 21 months) for serum HER2-positive - positive patients. But both subgroups had a significantly shorter survival compared to the serum HER2-negative - negative patients at the time of metastatic disease (p<0.01).

Survival curves of the four subgroups are presented in Figure 2.

Discussion

The eligibility of metastatic breast cancer patients for Herceptin therapy is based on tissue analysis of HER2 overexpression by immunohistochemistry or HER2 gene amplification by FISH. Since in the daily clinical routine, taking a biopsy of the metastasis is not a standard procedure, the HER2 status is usually evaluated on primary tumor tissue. However, the HER2 status may be altered at the time of...
Therefore, a subset of metastatic breast cancer patients may be given an inefficient treatment regimen based on the HER2 status in the primary tumor, while another subset may not receive specific therapy. The determination of HER2 status by the serum test allows the assessment of real-time HER2 status and changes in HER2 status in patients during clinical course. Moreover, studies have also indicated that serial serum HER2 measurements are useful to detect recurrent disease and to monitor therapy response in HER2-overexpressing metastatic breast cancer (23, 28). Recently, Hoopmann et al. (29) reported that metastatic breast cancer patients receiving a trastuzumab therapy with permanently elevated or increasing serum HER2 had a poor clinical outcome compared to those with normal or decreasing serum HER2 during clinical course.

In the first part of the study, the serum HER2 status was investigated in 152 metastatic breast cancer patients at the time of diagnosis of primary breast cancer and at the onset of metastatic disease to evaluate whether alterations of the serum HER2 status occurred. A change of serum HER2 status during the clinical course of disease could be observed in 43 patients (28%). Seven initially HER2-positive patients were negative when metastases were detected. In contrast, 36 patients, who were serum HER2-negative at the time of first diagnosis, were serum HER2-positive at the onset of metastatic disease. These results indicate that alteration of serum HER2 status can occur during the disease course and may represent changes in the pattern of tumor growth, resulting in a changed prognosis.

Most previous studies have analyzed changes of HER2 status by HER2 tissue testing. Gancberg et al. (19) performed immunohistochemistry and FISH analysis on 107 paired primary and metastatic tumors from breast cancer patients. By FISH analysis a discordance could only be observed in 43 patients (28%). Seven initially HER2-positive patients were negative when metastases were detected. In contrast, 36 patients, who were serum HER2-negative at the time of first diagnosis, were serum HER2-positive at the onset of metastatic disease. These results indicate that alteration of serum HER2 status can occur during the disease course and may represent changes in the pattern of tumor growth, resulting in a changed prognosis.
of 20% in HER2 expression and amplification between the primary tumor and metastatic disease. Most of the cases were patients with HER2-negative primary tumors and HER2-positive metastases, although the opposite combination was observed.

Based on tissue testing, a tumor is termed HER2-negative if less than 10% of tumor cells overexpress HER2. It was hypothesized (30,31) that these subpopulations of HER2-positive cells are still sufficient to metastasize due to preferential survival and growth and become the predominant clone in the metastatic lesion, resulting in a HER2-positive metastasis.

Other studies have combined tissue and serum testing to evaluate changes of HER2 status. It could be demonstrated that a subset of patients with HER2-negative tumors at the time of primary diagnosis had elevated serum HER2 levels when recurrence or metastases were diagnosed (24, 33). Discounting the variations in HER2 positivity caused by the different methods, several explanations have been discussed. As mentioned above, in contrast to the primary tumor the metastases may be HER2-positive due to clonal selection and growth and shed HER2 protein in blood. Furthermore, it was hypothesized by Baselga (32) that breast tumors may have different levels of activators which are involved in HER2 cleavage e.g. matrix metalloproteases. These differences may cause variations in serum HER2 concentrations for a given level of tissue HER2 expression. Therefore, serum HER2 levels may also indicate the activity of the HER2 shedding machinery on the HER2 receptor, which might itself correlate with the aggressiveness of disease.

In the second part of the study, the prognostic significance of changes of serum HER2 status for survival after relapse were studied in more detail. Serum HER2 at the time of metastatic disease was an independent prognostic marker for SAR. The median survival after relapse for patients with positive serum HER2 status was significantly shorter compared to those with negative serum HER2. Moreover, in the multivariate analysis HER2 was the strongest independent prognostic factor for SAR, followed by the progesterone receptor status and disease-free survival. Similar results were reported by others, showing shortened survival in serum HER2-positive metastatic patients (29,33-36).

Interestingly, the survival after relapse of HER2-positive metastatic breast cancer patients who were initially serum HER2-negative did not differ significantly from those patients who were initially serum HER2-positive. The same observation was made for survival after relapse in serum HER2-negative patients at the onset of metastatic disease. These results indicate that the serum HER2 status only at the time of metastatic disease is relevant for survival after relapse. The initial serum HER2 status has lost its prognostic significance during clinical course, probably due to the changes in tumor growth behavior of the metastases as discussed above. Similar observations have been reported by Gebauer et al. (37), showing that established prognostic markers in primary breast cancer lose their significance with increasing follow-up.

In conclusion, the (serum) HER2 status can be different at the time of first diagnosis and at the onset of metastatic disease resulting in a changed prognosis for survival after relapse. Therefore, re-evaluation of HER2 status should be performed when metastases or progression of disease occur. The HER2 status can be easily (re-) determined by a serum HER2 test. Prospective studies are now necessary to validate the clinical utility of serum HER2 against tissue-based HER2 evaluation before being accepted into clinical routine.

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References


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