Serum HER2 extracellular domain in metastatic breast cancer patients treated with weekly trastuzumab and paclitaxel: association with HER2 status by immunohistochemistry and fluorescence in situ hybridization and with response rate

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Purpose: We explored the relationship between circulating HER2 extracellular domain (ECD) and tissue HER2 status as determined by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH). We also examined its predictive value in a cohort of metastatic breast cancer patients treated with weekly trastuzumab and paclitaxel.

Methods: Eligible patients had pre- and post-treatment stored serum specimens and were treated on a previously reported phase II trial. Retrospective analysis evaluated: the association between pretreatment serum HER2 ECD and tissue HER2 status by IHC and FISH; and the association between change in serum HER2 ECD after 12 weeks of therapy and response proportion.

Results: Stored serum samples were available for 55/95 (58%) patients. Statistically significant associations were found between HER2 status as assessed by IHC and FISH, and baseline serum HER2 ECD level. Patients whose ECD normalized after 12 weeks of therapy had a higher response proportion compared with patients with persistently high ECD levels (68% versus 15%, P = 0.005). A relative decline of over 55% from baseline HER2 ECD predicted response to therapy.

Conclusion: A statistically significant association was observed between pretreatment serum HER2 ECD and tissue HER2 status as assessed by IHC and FISH. A decrease in serum HER2 ECD level was a significant predictor of response to trastuzumab-based therapy.

Key words: HER2 ECD, trastuzumab, metastatic breast cancer

Introduction

The HER2/neu oncogene, also referred to as c-erbB-2/neu, encodes a protein with a molecular weight of 185,000 Da (p185). The gene product is a transmembrane tyrosine kinase receptor belonging to a family of epidermal growth factor receptors structurally related to the human epidermal growth factor receptor (EGFR) [1, 2]. Amplification of the HER2 proto-oncogene and overexpression of its protein product in breast carcinomas has been linked to a poor prognosis, with more aggressive clinical course and shortened survival [3]. A variety of methods are available to assess tissue HER2 status, but for clinical and research purposes, the most widely applied techniques are immunohistochemistry (IHC), which detects protein overexpression, and fluorescence in situ hybridization (FISH), which detects HER2 gene amplification. Typically, tissue HER2 status by IHC or FISH is performed at the time of initial diagnosis of primary breast cancer or when a metastatic lesion is biopsied. The results may be prognostic and may also predict response to several chemotherapy agents, hormonal treatments and the monoclonal antibody trastuzumab (Herceptin™, Genentech, Inc., So. San Francisco, CA) [3].

The HER2 gene product is composed of a cytoplasmic domain with tyrosine kinase activity, a transmembrane domain and an extracellular domain (ECD). The HER2 ECD may be cleaved and shed from the surface of breast cancer cells and serum HER2 ECD levels can be detected by enzyme-linked immunosorbent assays (ELISA). Serum HER2 ECD testing does not show any significant cross-reactivity with other members of the EGF receptor family [4]. Also, there is no interference between serum HER2 levels and the therapeutic monoclonal antibody, trastuzumab, because the assay
and trastuzumab recognize different and non-overlapping binding sites of the HER2 ECD [4]. The manual and automated ELISA testing methods of serum HER2 are standardized, routine methodologies and are not prone to subjective variable interpretation by a reviewer.

Shed HER2 ECD can be detected in the serum of approximately 15–30% of pre-surgical breast cancer patients at the time of diagnosis, and in the serum of a proportion of patients with metastatic breast cancer [5–7]. In a database of 250 primary breast cancer patients, Molina et al. [7] showed that 28.4% had abnormal serum HER2 ECD levels prior to diagnosis of a recurrence.

These secreted soluble isoforms of the HER2 receptor are currently being examined as biomarkers, and might be useful in breast cancer for detecting early recurrence or metastasis and for predicting response to therapy [8, 9]. High concentrations of HER2 ECD in the serum have been correlated to higher relapse rates, and elevated pretreatment levels of HER2 ECD have been associated with poor clinical responsiveness to hormone therapy and chemotherapy in metastatic breast cancer patients [10–12]. Colomer et al. [12] recently demonstrated that elevated levels of HER2 ECD adversely affected the efficacy of therapy in a cohort of metastatic breast cancer patients treated with biweekly paclitaxel and gemcitabine. On the other hand, HER2 ECD seems to be associated with sensitivity to HER2-targeted agents, such as trastuzumab. Koestler and colleagues [13] reported that response rates to trastuzumab-based treatments were significantly higher in patients with elevated (≥15 ng/ml) ECD levels at baseline, and that early changes in serum ECD predicted both subsequent response and progression-free survival. Finally, serum HER2 ECD levels may accurately predict tumor HER2 status as detected by immunohistochemistry (IHC) [14]. Together, these observations suggest that alterations in serum HER2 could be useful in diagnosing cancer, in monitoring disease recurrence and in predicting therapeutic responsiveness and disease outcome [9].

To explore these associations further, we performed a retrospective analysis of the correlation between circulating HER2 ECD levels and tissue HER2 status, as determined by IHC and FISH, and between pre- and post-treatment ECD levels and response in a cohort of metastatic breast cancer patients, treated with weekly trastuzumab and paclitaxel.

**Materials and methods**

This was a retrospective analysis with serum samples that had been collected previously. Residual serum was collected and stored at −70°C for subsequent testing. No blood specimen was obtained specifically for this study and all specimens were made anonymous after results were obtained and clinical data evaluated. The study included sera from patients with metastatic breast cancer, who were treated on a prospective phase II study of weekly paclitaxel and trastuzumab [15]. Ninety-five patients, with both HER2 overexpressing and HER2 non-overexpressing metastatic breast cancer, were treated in this study, as previously reported [15]. HER2 status had been assessed previously by IHC and FISH on formalin-fixed paraffin-embedded tissue. In the original cohort, HER2 status had been assessed on 80 primary and 15 metastatic tumors. All 55 patients in this report had the primary tumor tested.

In the original study, IHC was performed using four different antibodies, two of which were polyclonal and two of which were monoclonal. For this report, we considered just two of these assays: (i) Dako Hercep-test (rabbit anti-human HER2/neu polyclonal antibody; Dako Corporation, Carpinteria, CA); and (ii) CB11 (mouse anti-human monoclonal antibody; Ventana Medical Systems Inc., Tucson, AZ). These two were selected because they are the most commonly used in clinical practice.

The intensity of the membrane staining was evaluated according to the following criteria set forth by the Dako Hercep-test: score 0, no or up to 10% membrane staining; score 1+, partial and/or faint membrane staining present in more than 10% of tumor cells; score 2+, weak to moderate complete membrane staining present in more than 10% of tumor cells; and score 3+, strong, complete membrane staining present in more than 10% of tumor cells. In this analysis, for Dako Herceptest and CB11, we considered a score of 2 and 3+ as indicative of overexpression.

FISH analysis was performed using the PathVysion HER2 probe kit (Vysis Inc, Downers Grove, IL) according to the manufacturer’s instructions. Signals ≥2 constituted gene amplification.

Baseline serum HER2 ECD status (prior to start of therapy) and after 12 weeks of therapy were determined from stored serum samples with the Immuno-1 immunoassay for HER2 (Bayer Corporation, Tarrytown, NY). The assay is based on two monoclonal antibodies directed against the ECD of the HER2 antigen. This Food and Drug Administration (FDA)-approved automated assay for HER2 in serum was demonstrated to be accurate, precise, resistant to interferences and reliable for longitudinal monitoring [16]. The upper limit of normal was defined as >15 ng/ml, as previously reported [16].

This retrospective analysis was determined to be exempt research by our Institutional Review Board.

**Tumor response criteria on the therapeutic trial**

Radiographic evaluations for tumor response were performed during the trial at weeks 8 and 16, and then every 12 weeks thereafter. Responses were defined as follows: a complete response was the disappearance of all clinical and radiographic signs of tumor for at least 4 weeks; a partial response was a more than 50% reduction in the sum of products of the bi-perpendicular diameters of all measurable lesions with no increase in size of any lesion and no new lesions; a minor response was a 25–49% reduction in the sum of products of the bi-perpendicular diameters of all measurable lesions with no increase in size of any lesion and no new lesions; stable disease was a less than 25% reduction in the sum of products of the bi-perpendicular diameters of all measurable lesions with no increase in size of any lesion and no new lesions; progressive disease was a 25% or greater increase in size of any lesion or the appearance of any new lesion [15].

**Statistical analysis**

Serum HER2 ECD levels were bifurcated at 15 ng/ml. Responders were defined as patients achieving either a partial or complete remission during treatment with paclitaxel and trastuzumab. Fisher’s exact test was used to test for associations between ECD levels and HER2 overexpression and response.

All contingency tables with associated statistics were obtained using SPSS for Windows Release 11.0 (SPSS, Inc., Chicago, IL). Fisher’s exact tests were used to determine the significance of the association in all 2 × 2 tables. The Fisher’s test was chosen over the McNemar test because of the time differential between IHC tests and serum results. Binomial confidence intervals were developed in Microsoft Excel.
Results

Stored serum samples were available for 55/95 (58%) patients enrolled in the therapeutic trial. Of these, FISH data were available in 44. Patients’ characteristics are described in Table 1. These 55 patients’ characteristics did not differ significantly from the initial study cohort [15].

The therapeutic trial allowed enrollment of patients with metastatic breast carcinoma, which could be either HER2 positive or negative.

Pretreatment serum ECD

The median pretreatment serum ECD level for the 55 patients considered in this analysis was 23.4 ng/ml (range 8.4–1512). Of all 55 patients, 38 (69%) had pretreatment ECD levels of at least 15 ng/ml; the median ECD level for these was 29.85 ng/ml (range 15.6–1512).

Association between IHC and pretreatment serum HER2 ECD

Associations between tissue HER2 status as determined by Dako Herceptest and CB11, and serum HER2 ECD pretreatment levels were explored. Tables 1 and 2 show the bivariate distributions of each IHC value and pretreatment levels of serum HER2/neu. The Fisher’s exact $P$ indicates an association between elevated serum HER2 levels and each IHC. Concordance was estimated for each IHC test.

ECD levels were concordant with tissue HER2 overexpression by Dako Herceptest: 85% of patients whose disease showed 2+ overexpression by Dako, also had elevated ECD in the serum prior to start of therapy ($P = 0.022$). However, among patients whose disease tested 0/1+ by Dako, 55% had elevated ECD levels (Table 2).

A similar concordance was observed with the monoclonal antibody CB11 (Table 3): 95% of patients with CB11 staining intensity of 2/3+ had elevated levels of ECD at baseline ($P = 0.002$).

Association between FISH and pretreatment serum HER2 ECD

Concordance was estimated for pretreatment serum HER2 ECD and tissue HER2 status as assessed by FISH (Table 4). Among patients whose disease showed gene amplification by FISH, 83% also had elevated ECD levels ($P = 0.03$). On the other hand, among those patients whose disease did not show gene amplification, 50% had elevated baseline HER2 ECD levels.
Association between serum HER2 ECD and response to therapy

To correlate HER2 ECD levels with response proportion to treatment, HER2 ECD was determined at baseline and after 12 weeks of therapy with paclitaxel and trastuzumab. Of all 55 patients, 38 (69%) had baseline ECD levels of at least 15 ng/ml. The overall response rate among these patients was 50%, compared with 47% in patients with low baseline HER2 ECD levels ($P = 1$). We therefore found no correlation between baseline ECD level and likelihood of response to therapy (Table 5).

An alternative analytic strategy utilizes the relative change in HER2 ECD with treatment. The change in serum HER2 ECD level after therapy was bifurcated at 55%. The value of 55% was determined using a ROC curve with response as the outcome. The value of 55% was the first cut-off point to produce an odds ratio that was significantly different from 1.0. At the cut-off point the true positive fraction was 0.593 and the true negative fraction was 0.679.

Table 7 shows the association between response and therapy for all 55 patients, using a 55% relative decrease in serum ECD over 55% at 12 weeks significantly predicted a response to therapy.

Discussion

The clinical relevance of HER2 status is related to its potential role as both a prognostic indicator and as a predictive factor for patients affected by breast carcinoma. Amplification of the HER2 proto-oncogene and overexpression of its protein product in breast carcinomas has been linked to a poor prognosis, and a differential response to a variety of systemic treatments [3, 17]. With the availability of the monoclonal antibody trastuzumab as therapy for metastatic breast cancer, there is an increased need to evaluate accurately the HER2 status of breast cancers, to identify those patients who might benefit from this treatment.

Clinically, HER2 status is most often determined in tissue from primary or metastatic breast cancer by immunohistochemical assays that evaluate the protein expression or by FISH assays that determine the oncogene amplification. Results of these tests are generally in agreement, although discrepancies are possible due to inherent variability in
The serum extracellular domain of HER2 is shed into the circulation and can be elevated in the serum of women with metastatic breast cancer [19]. HER2 ECD might provide a novel and useful tool for the management of patients with breast cancer, as it might illuminate prognosis, treatment selection and might predict clinical response. In fact, serum HER2 ECD has been approved by the FDA for the follow-up and monitoring of patients undergoing treatment for metastatic breast cancer on various therapies [20, 21].

There is evidence that HER2 ECD might be clinically useful in breast cancer for detecting early recurrence or metastasis and for predicting response to both hormonal therapy and chemotherapy [7, 10, 12]. Lipton et al. [10] previously reported their experience in this setting: 719 patients with estrogen-receptor-positive metastatic breast cancer, receiving therapy with an aromatase-inhibitor, underwent serum HER2 ECD testing at baseline. The response rate, median duration of treatment response and median survival, were all inferior among those patients with elevated serum HER2 levels. Similarly, Colomer et al. [12] recently demonstrated that elevated levels of HER2 ECD adversely affected the efficacy of chemotherapy with biweekly paclitaxel and gemcitabine in a cohort of metastatic breast cancer patients.

Several investigators have presented data regarding serum HER2 ECD testing as a tool for monitoring patients undergoing treatment, and reported that HER2 ECD seems in fact to be associated with sensitivity to HER2-targeted agents, such as trastuzumab. Esteva et al. [9] published a phase II study in which 30 women with metastatic breast cancer received therapy with docetaxel and trastuzumab. The efficacy of therapy was correlated with serum HER2 ECD levels, which were determined at baseline, after completion of two cycles and four cycles, and after three cycles thereafter. Variation in HER2 ECD concentrations correlated significantly with response to treatment: serum HER2 ECD decreased in 87% of the responding patients, indicating that changes in serum biomarker correlate well with the clinical course of disease. Fountzila et al. [22] also described similar findings. Koestler and colleagues [13] recently reported a pilot study: serial levels of serum ECD were obtained from 55 patients with HER2 overexpressing metastatic breast cancer, undergoing therapy with trastuzumab-based combinations. Response rates to trastuzumab-based treatments were significantly higher in patients with elevated (>15 ng/ml) ECD levels at baseline. In addition, in patients responding to treatment, ECD levels decreased significantly as early as from day 8 of therapy onwards. Multiple logistics regression analyses identified kinetics of ECD levels as the only factor that allowed for accurate prediction response likelihood. Burstein and colleagues [23] reported their experience: among 54 patients with metastatic breast carcinoma receiving first-line therapy with vinorelbine and trastuzumab, a lack of decline in HER2 ECD during cycle 1 predicted tumor progression. However, in this database, neither the baseline level of HER2 ECD, nor a decrease in HER2 ECD with therapy predicted clinical response after one cycle of treatment. Perez et al. [11] published a randomized phase II study: among 33 patients undergoing chemotherapy for metastatic breast cancer with either docetaxel followed by doxorubicin and cyclophosphamide (AC), or vice versa, serum HER2 concentrations tended to decrease over the course of treatment. The Cancer and Leukemia Group B (CALGB) trial 98–40 and its companion trial (CALGB 159806) will prospectively evaluate the value of HER2 ECD in patients with metastatic breast carcinoma undergoing treatment with paclitaxel and trastuzumab [24].

In our retrospective analysis, we compared tissue HER2 status as detected by IHC and FISH with HER2 ECD levels drawn at baseline, before starting treatment with paclitaxel and trastuzumab. Although we were analyzing a subset of treated patients, we found a statistically significant correlation among the different tests: patients whose breast cancer showed tissue overexpression or gene amplification of HER2, also tended to have elevated serum HER2 ECD levels, as can be noted in Tables 2, 3 and 4. Our finding suggests that the determination of serum HER2 ECD might aid in the assessment of the HER2 status, in addition to the conventional assays performed on the tumor specimen. However, elevated HER2 ECD levels were also observed among patients whose disease did not show protein overexpression or gene amplification. This could perhaps be explained by the fact that the concentration of HER2 ECD might be a function of tumoral HER2 receptor density, rate of ECD cleavage, and most importantly tumor burden [10].

In our analysis, we did not observe a correlation between baseline ECD levels and likelihood of response to therapy, as shown in Table 5. This is in contrast to what was previously reported by other authors, and could be partially related to differences in patient populations [9, 13]. In our trial, we enrolled patients with both HER2-negative and HER2-positive disease, whilst other authors only included patients with HER2 overexpressing metastatic breast carcinoma [9, 13]. In addition, the median baseline ECD serum level in our population was 23.4 ng/ml; Koestler et al. [13] and Esteva et al. [9] reported higher median baseline ECD levels of 45.1 and 41.9 ng/ml, respectively.

Notably, among the subset of patients with an elevated HER2 ECD level at baseline, we found that response proportion among those patients whose HER2 ECD normalized after 12 weeks of therapy, was significantly higher than among those patients who had persistently elevated levels of ECD: 68% versus 15%, P = 0.005 (Table 6, Figure 1). Even more interesting, when considering a relative drop over 55% from pretreatment to post-treatment ECD values, a very strong correlation was detected. This indicates that a relative drop in serum HER ECD over 55% at 12 weeks in our database significantly predicted a response to therapy (P = 0.015) (Table 7). If failure to detect at least a 55% drop in serum HER2 ECD were to be prospectively validated as a predictor of unresponsiveness to trastuzumab, it would have profound clinical relevance.
Clinically, negative tumor assays for HER2 do not exclude elevated circulating HER2 ECD, raising the possibility that the latter test could identify additional patients who might benefit from treatment with trastuzumab. In our database, 34% of patients (19/55) had an elevated baseline ECD level, and HER2-negative disease by IHC/FISH. Response proportion among this subgroup was 37% (7/19). Because of the small sample size, and because these patients were also receiving therapy with paclitaxel, we do not think any assumption can be made regarding the potential role of HER2 ECD as a biomarker to select patients with tissue HER2-negative disease, for trastuzumab-based therapy.

The clinical utility of serum HER2 as a prognostic indicator has not yet been fully established and needs to be evaluated prospectively in primary breast cancer patients with early stage disease. On the other hand, there is increasing evidence in the literature of the predictive role of HER2 ECD, for patients with HER2 overexpressing metastatic breast carcinoma, treated with trastuzumab-based combinations. In our database, we found that a drop in serum HER ECD levels at 12 weeks significantly predicted response to therapy with paclitaxel and trastuzumab in a cohort of patients with metastatic breast carcinoma. Our findings are consistent with other reports [9, 13] and support the conclusion that HER2 ECD may constitute a useful method of monitoring patients receiving trastuzumab-based therapy. If validated, such a strategy could improve the therapeutic index for this drug.

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References