Evaluation of serum HER2 extracellular domain in early breast cancer patients: correlation with clinicopathological parameters and survival


1Medical Oncology Division, Azienda Ospedaliera of Perugia, Perugia; 2Department of Oncology, Istituto di Ricerche Farmacologiche ‘Mario Negri’, Milan; 3Biochemistry and Clinical Chemistry Laboratory, Azienda Ospedaliera of Perugia, Perugia; 4Institute of Pathology; 5‘Breast Unit’, Department of Surgical, University of Perugia, Perugia, Italy

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Background: We explored the correlation between serum human epidermal growth factor receptor-2 (HER2) extracellular domain (ECD) and tissue HER2 status, their relationship with clinicopathological parameters and their impact on disease-free survival (DFS) and overall survival in early breast cancer patients.

Patients and methods: This prospective trial included patients with stage I–III breast cancer. Serum HER2 ECD levels were measured by two enzyme-linked immunosorbent assays before surgical treatment. Tissue HER2 status was analyzed by immunohistochemistry (IHC) in all tumors; FISH assay was utilized in HER2 2+ tumors by IHC.

Results: From May 2000 to July 2005, 256 consecutive stage I–III breast cancer patients were included in this study. High serum HER2 ECD levels (‡15 ng/ml) were reported in 23 patients (9.0%) and HER2-positive status in tumor tissue was observed in 42 patients (16.4%) with a concordance of 87.1%. High HER2 ECD levels were significantly associated with high histological grade (P = 0.003), stage III (P = 0.008), lymph node involvement (P = 0.035) and negativity of both estrogen (P = 0.016) and progesterone (P = 0.007) receptors. At multivariate analysis, high serum HER2 ECD levels were a significant independent prognostic factor of worse DFS (P = 0.009).

Conclusions: A statistically significant association was observed between high serum HER2 ECD levels and worse DFS in early breast cancer patients.

Key words: early breast cancer, HER2 ECD, prognosis

Introduction

The human epidermal growth factor receptor-2 (HER2) proto-oncogene, also known as HER2/neu and c-erbB-2, encodes a growth factor receptor that has been found to play an important role in breast cancer [1].

In up to 30% of breast cancer patients, the HER2 gene is amplified and its associated receptor protein is overexpressed on the tumor cell surface, thus playing an important role in the malignant transformation and clinical aggressiveness of breast cancer.

HER2 overexpression is an independent predictor of shorter overall survival (OS) and disease-free survival (DFS) in invasive primary breast cancer [2].

Retrospective evaluations of randomized clinical trials have revealed a correlation between HER2 overexpression and resistance to tamoxifen [3–5] while an interaction between HER2 overexpression and enhanced response to doxorubicin-containing regimens has been reported [6–7].

A variety of methods are available to assess tissue HER2 status: immunohistochemistry (IHC), which detects protein overexpression and FISH, which detects HER2 gene amplification are the most widely applied techniques. Tissue HER2 status is very important because HER2 protein provides a target for therapeutic approaches. Trastuzumab, a mAb (Herceptin™; Genentech Inc., South San Francisco, CA) alone or in combination with chemotherapy is known to be an effective treatment for HER2-positive breast cancer (IHC 3+ or FISH amplified) in metastatic, neo-adjuvant and adjuvant settings [8].

HER2 receptor 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 (ELISAs) without any significant
cross-reactivity with other members of the HER receptor family [9]. The manual and automated ELISA testing methods of serum HER2 ECD are standardized methodologies and are not prone to subjective variable interpretation by a reviewer. Shed HER2 ECD has been detected in the serum from 20% to 40% of patients with metastatic disease [10–11] and high serum concentrations of HER2 ECD have been associated with HER2 overexpression [12], increased tumor burden [13], worse survival [14] and resistance to endocrine therapy and chemotherapy [15–17]. On the other hand, HER2 ECD seems to be associated with sensitivity to HER2-targeted agents, such as trastuzumab. A decrease in serum HER2 ECD levels in metastatic breast cancer patients treated with trastuzumab was predictive of response [18]. In early breast cancer, shed HER2 ECD has been detected up to 20% of patients [19], but the clinical significance of circulating HER2 ECD is uncertain and the relationship of baseline HER2 ECD levels with tissue HER2 overexpression in primary tumors has been little studied.

Therefore, we carried out a prospective study in operable breast cancer patients to evaluate the correlation between HER2 ECD serum levels and HER2 tumor status measured by IHC and FISH, their relationship with clinical–pathological parameters and their impact on DFS and OS.

patients and methods

This prospective trial was conducted in patients with a histological diagnosis of stage I–III breast cancer treated with conservative surgery or mastectomy. Tumor staging followed the tumor–node–metastasis (TNM)–American Joint Committee on Cancer classification [20] and the pTNM was obtained after classical pathological examination. Patients with metastatic disease and with other previous tumors were excluded from this study.

Estrogen receptors (ERs), progesterone receptors (PgRs) and HER2 status were assessed at the time of surgery on formalin-fixed paraffin-embedded tissue blocks of the primary tumor in the Pathology Department of the University of Perugia.

Recorded clinical and pathological features for each patient include age, menopausal status, histology, grade, Ki67, ER and PgR status, stage, surgical treatment and medical adjuvant therapy. Follow-up, including clinical examination (every 3 months for the first 2 years, every 6 months for the next 3 years and yearly thereafter) and annual Rx-mammography, was carried out in all patients.

Recurrence was defined as the first documented evidence of new disease manifestation in the locoregional area, in the contralateral breast, in distant sites, or in a combination of these. The study was reviewed and approved by the institution’s Ethics Committee and informed consent was obtained by all patients.

serum HER2 ECD assays

Serum samples were prospectively collected from breast cancer patients before surgery.

Five milliliters of peripheral blood were collected in a sterile test tube (without anticoagulants) and centrifuged at 3000 g for 10 min at room temperature. Serum was stocked in 0.5-ml aliquots in cryovials and stored at −80°C until the time of HER2 ECD automated and manual analyses. Serum HER2 ECD levels were measured using both HER2 assays to compare the two ELISA methods. With the automated method, baseline serum HER2 ECD levels were determined with the ADVIA Centaur HER2/neu assay (Bayer Corporation, Tarrytown, NY), on the basis of two mAbs directed against the ECD of the HER2 antigen, using direct chemiluminescent technology.

The measured chemiluminescence is directly proportional to the quantity of HER2/neu antigen in the sample. Quality control was ensured by assaying the two levels of control sera supplied with the kit in each series. Mean ± standard deviation (SD) and coefficient of variation (CV) for the controls were 15.7 ± 0.75 ng/ml (CV 4.5%) and 112.9 ± 4.99 ng/ml (CV 4.4%), respectively. This automated assay for HER2 ECD was demonstrated to be accurate, precise, resistant to interferences and reliable for longitudinal monitoring [21]; the upper limit of normal was defined as 15 ng/ml [21].

With the manual method, the HER2 ECD levels were measured using a sandwich enzyme immunoassay (Human Neu Oncoprotein ELISA; Oncogene Science/Bayer Diagnostics, Cambridge, MA), following the manufacturer’s recommended protocol. The concentration of HER2 ECD in the samples was determined by interpolation of sample absorbance from the standard curve. The minimum detectable was 0.3 ng/ml and intra- and inter-assay coefficients of variation were <10%. The upper limit of normal was defined as 15 ng/ml, as previously reported [18].

tissue HER2 analyses by IHC and FISH

IHC staining of specimens was carried out on formalin-fixed paraffin-embedded breast cancer tissues using the mAb C81 which targets the intracellular domain of HER2/neu protein (BioGenex, San Ramon, CA). According to the HercepTest™ criteria, immunoreaction was scored as: 3+ if >10% of tumor cells showed strong and complete membrane staining, 2+ if membrane positivity was moderate and complete in >10% cells, 1+ if membrane positivity was weak and incomplete in >10% cells and 0 if membrane staining was absent or present in <10% cells. Tumors scored as 3+ were considered HER2 positive while tumors scored as 0/1+ were designated as HER2 negative. In 2+ tumors evaluated by IHC, FISH analysis was carried out, using the Abbott-Vysis Path Vysion™ HER2 DNA Probe Kit (Abbott Laboratories, Abbott Park, IL), following the manufacturer’s recommended protocol. The results were reported as the ratio between the average copy number of the HER2/neu gene and that of the chromosome 17 centromere, analyzing 60 neoplastic nuclei. Specimens with a signal ratio of <2.0 were considered as nonamplified and 2.0 or greater as amplified.

statistical analysis

DFS was defined as the time from surgery to first appearance of disease or death for any cause. Patients known to be alive and without disease at the time of analysis were censored at their last follow-up date. OS was defined as the time from surgery to death for any cause. Survival curves were estimated using the Kaplan–Meier method.

Cox proportional hazards model, as implemented in the PHREG program in SAS (SAS Institute Inc., Cary, NC) were used to evaluate the effect of serum HER2 ECD and HER2 tissue and clinicopathological variables on OS and DFS. A proportional hazards model was used for univariate and multivariate analyses. Variables that were found to be associated with OS and DFS in the univariate analysis (statistical significance was set at P < 0.05) were considered for the multivariate analysis. Results are expressed as hazard ratios (HRs) with 95% confidence intervals (CIs).

The χ² test was used to assess the association among clinical–pathological features and the expression of HER2 tissue or the levels of HER2 ECD.

Our analyses were carried out utilizing the results obtained from automated ELISA assay and treating the levels of HER2 ECD as a dichotomous variable (HER2 high ≥ 15 ng/ml versus low < 15 ng/ml).

All P values were derived from significance two-sided tests. Statistical analysis was carried out using SAS (Statistical Analysis System, SAS Institute Inc., version 9.1) software.
results

patient characteristics

From May 2000 to July 2005, a total of 256 consecutive patients with radically resected primary breast cancer, referred to the Breast Unit Surgical Department of the University of Perugia, Italy, were recruited. Histological diagnosis was made at the Institute of Pathology, University of Perugia.

The main clinicopathological characteristics of the patients in our series are summarized in Table 1. Eastern Cooperative Oncology Group performance status was zero to one in 99.2% of patients. Ductal infiltrating carcinoma histology was reported in 216 patients (84.4%).

All patients underwent surgery; conservative surgery was carried out in 214 patients (83.6%) and mastectomy in 42 patients (16.4%). Radiotherapy was delivered to 198 patients.

Adjuvant chemotherapy was administered to 191 patients (74.6%); 90 patients (47.1%) received combination chemotherapy with cyclophosphamide, methotrexate and fluorouracil, 89 patients (46.6%) anthracycline-based therapy, 2 patients (1.1%) taxane-based therapy and 10 patients (5.2%) anthracycline- and taxane-based regimens. Endocrine therapy was administered to 181 of 256 patients (70%) (Table 1).

baseline serum HER2 ECD and tissue HER2 status

With a cut-off value of 15 ng/ml, 23 of the 256 patients (9.0%) had HER2 ECD levels ≥15 ng/ml (high levels) and 233 patients (91.0%) had HER2 ECD levels <15 ng/ml (low levels).

Tissue HER2 status was positive (3+ by IHC and 2+ by IHC with FISH1 amplification) in 42 patients (16.4%) and negative in 214 patients (83.6%).

correlation between automated and manual methods to assay serum HER2 ECD levels

The correlation between the ADVIA Centaur and Oncogene ELISA methods was evaluated in all 256 breast cancer patients. The mean value of HER2 ECD measured by automated ADVIA Centaur was 10.8 ng/ml (SD 3.3 ng/ml, median 9.75 ng/ml, range 4.0–26.5 ng/ml). Pearson’s correlation test, applied to determine the linear regression relationship was found between HER2 ECD levels and the other clinicopathological variables

Table 1. Tumor and patient characteristics (n = 256)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. of patients</th>
<th>%</th>
</tr>
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<tbody>
<tr>
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<td>(25.9–84.9)</td>
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<td></td>
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<tr>
<td>Postmenopausal</td>
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<tr>
<td>Stage</td>
<td></td>
<td></td>
</tr>
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<td>(53.9)</td>
</tr>
<tr>
<td>II</td>
<td>79</td>
<td>(30.9)</td>
</tr>
<tr>
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<td>(15.2)</td>
</tr>
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<td></td>
</tr>
<tr>
<td>≤2</td>
<td>180</td>
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<tr>
<td>&gt;2</td>
<td>61</td>
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</tr>
<tr>
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<tr>
<td>Positive</td>
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<tr>
<td>ER positive</td>
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<td>(66.0)</td>
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<td>(46.9)</td>
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<tr>
<td>PgR positive</td>
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<td>(52.7)</td>
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<tr>
<td>Unknown</td>
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<td>(0.4)</td>
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<td>Ki67 status (cut-off &gt; 13%)</td>
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<td>Ki67 positive</td>
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<td>(46.5)</td>
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<td>(0.8)</td>
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<td>Adjuvant CT</td>
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</tr>
<tr>
<td>Endocrine therapy</td>
<td>181</td>
<td>(70.0)</td>
</tr>
<tr>
<td>Only endocrine therapy</td>
<td>54/181</td>
<td>(29.8)</td>
</tr>
<tr>
<td>Endocrine therapy after CT</td>
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<td>(70.1)</td>
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<td>≥15 ng/ml</td>
<td>23</td>
<td>(9.0)</td>
</tr>
<tr>
<td>&lt;15 ng/ml</td>
<td>233</td>
<td>(91.0)</td>
</tr>
<tr>
<td>Tissue HER2 (IHC/FISH)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
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<td>(16.4)</td>
</tr>
<tr>
<td>Negative</td>
<td>214</td>
<td>(83.6)</td>
</tr>
</tbody>
</table>

*HIC 3+, IHC 2+ and FISH amplified. ER, estrogen receptor; PgR, progesterone receptor; CT, chemotherapy; HER2, human epidermal growth factor receptor-2; ECD, extracellular domain; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization.

association between tissue HER2 status (IHC/FISH) and baseline serum HER2 ECD levels

The bivariate distributions of patients with tissue HER2 status and baseline serum HER2 ECD levels are shown in Table 2. We observed high serum HER2 ECD levels in 38.1% (16 of 42) of patients with tissue HER2-positive status versus only 3.3% (7 of 214) of patients with tissue HER2-negative status. The χ² statistic indicates an association between high HER2 ECD levels and tissue HER2 status (P < 0.0001). HER2 ECD levels were moderately concordant with tissue HER2 status (87.1%, K statistic = 0.42, 95% CI 0.26–0.58).

relationship between baseline serum HER2 ECD levels and clinicopathological variables

High HER2 ECD levels were significantly associated with stage III (P = 0.008), lymph node involvement (P = 0.035), high histological grade (P = 0.003), negativity of ER (P = 0.016) and negativity of PgR (P = 0.007) (Table 3). No statistical relationship was found between HER2 ECD levels and the other
variables, such as age, menopausal status, histological tumor type and tumor size.

relationship between HER2 tissue status and clinicopathological variables

Statistically significant associations were found among tissue HER2-positive status, high histological grade \((P < 0.0001)\) and negativity of ER \((P < 0.0001)\) and PgR \((P = 0.015)\) (Table 4). No statistically significant relationship was found between HER2 tissue status and the other variables (menopausal status, histological tumor type and tumor size, stage and lymph node involvement).

survival analysis

At a median follow-up of 3.14 years \((2.85–3.38\) years\), 24 patients \((9.4\%)\) had a recurrence. Locoregional recurrence was observed in 8 patients \((33.4\%)\) and metastatic disease in 16 patients \((66.6\%)\); dominant site was visceral in 12 of 16 patients \((75\%)\). Two patients died without recurrence of breast cancer; so far 26 events occurred in our series for the analysis of DFS.

We observed 9 events in 23 patients \((39.1\%)\) with high HER2 ECD levels and only 17 events in 233 patients \((7.3\%)\) with low HER2 ECD levels. Dominant site of relapse was visceral in 6 of 9 patients with high HER2 ECD levels \((66.6\%)\) versus 6 of 15 patients with low HER2 ECD levels \((40\%)\). DFS was significantly shorter in patients with high HER2 ECD levels at diagnosis when compared with patients with low HER2 ECD levels \((HR = 5.76, 95\% CI 2.50–13.27, P < 0.0001)\) (Figure 2). DFS was also worse in patients with tissue HER2-positive status in comparison to those with tissue HER2 negative, but this difference was not statistically significant \((HR = 2.14, 95\% CI 0.91–5.04, P = 0.082)\) (Figure 3).

To date, 12 patients died and no difference in OS has been observed either according to serum HER2 ECD levels \((HR = 2.40, 95\% CI 0.51–11.35, P = 0.269)\) or to HER2 tissue expression \((HR = 2.18, 95\% CI 0.59–8.09, P = 0.245)\).

To assess the effect of each variable on DFS and OS, a Cox model was carried out first using univariate analysis. High HER2 ECD levels \((P < 0.0001)\), stage III \((P < 0.0001)\), lymph node positivity \((P = 0.001)\) and negative PgR status \((P = 0.026)\) were significantly associated with worse DFS (Table 5). Stage III status \((HR = 4.53, 95\% CI 1.83–11.23, P = 0.001)\) and lymph node positivity \((HR = 6.43, 95\% CI 1.37–30.10, P = 0.018)\) were significantly associated with OS (data not shown).
A multivariate Cox regression model for DFS and OS was built using the variables that were found significant at the univariate analysis. High serum HER2 ECD levels (HR = 3.25, 95% CI 1.34–7.88, \( P = 0.009 \)) and positive lymph nodes (HR = 2.90, 95% CI 1.01–8.29, \( P = 0.048 \)) were significantly associated with worse DFS (Table 5). Stage (III versus I and II) was an independent prognostic factor for OS (HR = 4.41, 95% CI 1.76–11.06, \( P = 0.002 \) (data not shown).

We also explored the impact of systemic adjuvant therapy on DFS and OS, but no association was observed. Adjuvant trastuzumab was not administered to any patient. After recurrence, seven of nine patients with tissue HER2-positive disease received trastuzumab-based therapy.

**Discussion**

The role of serum HER2 ECD in patients with early breast cancer as prognostic factor is not well defined. The percentage of elevated serum levels of HER2 ECD are extremely variable in early breast cancer patients at the time of diagnosis. In our study, high HER2 ECD levels were observed in 9.0% of early breast cancer patients, similar to the results reported by other authors [22–24].

To measure the levels of HER2 ECD, we used both the automated and manual ELISA methods which are standardized, sensitive, reproducible and appropriate for routine analysis. Both assays utilize the same two antibodies (NB-3 and TA-1) that are directed against different epitopes of the HER2 ECD and both use a value of 15 ng/ml as cut-off. We showed that there was a strong correlation between these two ELISA methods, as reported by others [25, 26]. Both methods have been cleared by the Food and Drug Administration for use in

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### Table 4. Relationship between tissue HER2 status and clinicopathological variables

<table>
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<tr>
<th>Characteristics</th>
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<th>HER2 positive</th>
<th>( P ) value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Menopausal status</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Premenopausal</td>
<td>80</td>
<td>(84.2)</td>
<td>15</td>
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<td>Postmenopausal</td>
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<td>(83.2)</td>
<td>27</td>
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<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>I</td>
<td>11</td>
<td>(83.3)</td>
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<tr>
<td>II</td>
<td>68</td>
<td>(86.1)</td>
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</tr>
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<td>(79.5)</td>
<td>8</td>
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<td>Tumor size, cm</td>
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<td>( \leq 2 )</td>
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<td>(86.7)</td>
<td>24</td>
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<td>&gt;2</td>
<td>49</td>
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<td>Lymph nodes status</td>
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<tr>
<td>Negative</td>
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<td>Grade</td>
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</tr>
<tr>
<td>G1</td>
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<td>G2</td>
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<td>(88.4)</td>
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<tr>
<td>PgR positive</td>
<td>12</td>
<td>(88.9)</td>
<td>15</td>
</tr>
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</table>

HER2, human epidermal growth factor receptor-2; ER, estrogen receptor; PgR, progesterone receptor.

*\( P \) value <0.05, statistically significant.
the management and monitoring of women with metastatic breast cancer besides the automated Immuno-1 HER2 test.

High HER2 EDC levels were associated with shorter DFS in our series of early breast cancer patients and according to Cox regression analysis, patients with elevated HER2 ECD showed a relative risk of 3.25 for reduced DFS. Elevated HER2 ECD levels were also correlated with the risk of visceral recurrence (66.6% versus 40%), in accordance with observations already reported, analyzing tissue HER2 overexpression in early breast cancer patients [27].

Few trials have evaluated the prognostic role of serum HER2 ECD in early breast cancer. The results of our prospective study are in agreement with two retrospective studies [28, 24] that showed a shorter DFS in patients with elevated serum HER2 ECD levels. The adverse prognostic value of elevated HER2 ECD levels in early breast cancer could reflect the presence of micrometastases or high HER2 cleavage and shedding with the production of truncated cell-associated fragments that contain the signaling kinase domain activated in absence of the ECD [29]. Therefore, these tumors with a deregulated growth-promoting pathway could have a more aggressive behavior.

Moreover, in our study, elevated levels of HER2 ECD were associated with several factors related to tumor aggressiveness, such as stage III, lymph node involvement, poor histological differentiation, ER negativity and PgR negativity (Table 3).

The correlation of tissue HER2 status (detected by IHC and/or FISH) with serum HER2 ECD levels was also analyzed. High serum HER2 ECD levels were observed in 38.1% (16 of 42) of patients with tissue HER2 positivity and in only 3.3% (7 of 214) of the patients with tissue HER2 negativity, with a moderate concordance (87.1%) as reported by others [23]. The targeted molecular domains and protein function are different: the IHC method recognizes intracytoplasmic domains of the entire HER2 receptor; the serum HER2 assay measures the ECD of the HER2 receptor by using two mAbs recognizing two independent epitopes [30].

![Figure 3. Kaplan–Meier estimates for disease-free survival according to tissue human epidermal growth factor receptor-2 (HER2) expression](image)

| Table 5. Univariate and multivariate analyses for variables considered for DFS (Cox proportional hazards regression model) |
|---|---|---|
| **DFS** | **Hazard ratio** | **95% confidence interval** | **P value** |
| **Univariate analysis** | | | |
| Stage (III versus I and II) | 5.02 | 2.32–10.90 | <0.0001* |
| Lymph nodes status (positive versus negative) | 4.66 | 1.96–11.10 | 0.001* |
| PgR (cut-off ≥ 10%) (negative versus positive) | 0.39 | 0.17–0.89 | 0.026* |
| Serum HER2 ECD (high versus low) | 6.46 | 2.75–15.17 | <0.0001* |
| HER2 tissue (positive versus negative) | 2.14 | 0.91–5.04 | 0.082* |
| **Multivariate analysis** | | | |
| Serum HER2 ECD (high versus low) | 3.25 | 1.34–7.88 | 0.009* |
| Lymph nodes status (positive versus negative) | 2.9 | 1.01–8.29 | 0.048* |

*HER2 ECD: high (≥215 ng/ml) versus low (<15 ng/ml).

*Positive tissue HER2 status: IHC 3+, IHC 2+ and FISH amplified.

DFS, disease-free survival; PgR, progesterone receptor; HER2, human epidermal growth factor receptor-2; ECD, extracellular domain; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry.

*P value <0.05, statistically significant.
In conclusion, the ELISA method for measuring the circulating HER2 ECD is a useful tool to obtain the real-time status of HER2 and both manual and automated methods may be used. Our findings indicate that the determination of serum HER2 ECD might aid to assess the HER2 status, in addition to the conventional assays carried out on tumor specimens.

Due to the correlation between HER2 ECD levels and HER2 expression in tumoral tissue, patients could be considered for trastuzumab therapy only if tissue is not available, on the basis of high HER2 ECD levels.

Elevated HER2 ECD levels may also represent a subgroup of HER2-positive tumors with a higher level of HER2 cleavage and shedding. In vitro studies have shown that high amounts of soluble HER2 ECD may neutralize the biological activity of anti-HER2 antibodies by forming immune complexes and blocking the accessibility of the antibodies to the tumor [31]. This subgroup of tumors may have a more aggressive clinical course and the availability of a noninvasive, repeatable and reproducible technique to measure circulating HER2 ECD appears to be a useful tool for identifying high-risk breast cancer patients.

In conclusion, to our knowledge, this is the only prospective trial that has evaluated the prognostic role HER2 ECD in patients with operable breast cancer. A significant association with other poor prognostic factors has been found as well as with a worse DFS; however, due to limited percentage of patients with elevated serum levels of HER2 ECD in this series, these results must be interpreted cautiously and need to be confirmed in large prospective trials with serial measurements. It could also be worthwhile to evaluate in patients with high serum HER2 ECD levels the efficacy of different treatments targeting HER2 (receptor tyrosine kinase inhibitors) in comparison with trastuzumab.

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**references**


