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ORIGINAL ARTICLE

Extracellular domain of HER2: A useful marker for the initial workup and follow-up of HER2-positive breast cancer

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Summary

Purpose: *The extracellular domain (ECD) of the HER2 receptor is proposed as a real-time marker of HER2-positive breast cancer (BC). In this study, ECD-HER2 levels were compared with standard clinical and pathological prognostic factors.*

Patients and methods: *In 247 consecutive patients (116 with early or localized BC, 116 with advanced or metastatic BC, and 16 with benign mastopathies), serum ECD-HER2 levels were measured. In 116 advanced-disease patients ECD-HER2 status was also studied by immunohistochemistry (IHC) and compared with established clinical and pathological variables.*

Results: *Mean serum ECD-HER2 value was 19.62 ng/ml (median 10.35, range 3 - >250). Mean value in benign mastopathies was 9.04 ng/ml, 9.4 ng/ml in early disease and 34.5 ng/ml in advanced disease. No difference between benign mastopathies and early BC was observed, while significant difference between early and advanced BC ($p<0.001$) was noted. However, in advanced-disease patients a positive correlation of ECD-HER2 with IHC ($p=0.002$), disease grade ($p=0.034$) and level II axillary node involvement ($p=0.011$) was noted, as well as a significant negative correlation with estrogen receptor (ER) and progesterone receptor (PR) ($p=0.035$ and $p=0.011$, respectively).*

Conclusion: *ECD-HER2 is a reliable marker for breast cancer, as suggested from the existing literature; therefore, its integration in the initial workup and follow-up routine of breast cancer, particularly the HER2-positive, is proposed.*

Key words: breast cancer, HER2, HER2-extracellular domain, trastuzumab

Introduction

HER2/neu proto-oncogene, or else c-erbB-2/neu, encodes for a protein with molecular weight 185000 Da (p185). The gene product is a tyrosine kinase transmembrane receptor of the EGFR family (Epidermal Growth Factor Receptors) [1,2]. Amplification of the proto-oncogene HER2 and overexpression of its protein in breast cancer have been correlated with poor prognosis, more aggressive disease and decreased survival [3] that may be associated with resistance to chemotherapy [4,5] and hormone therapy [6-9].

Of the various methods available for the assessment of tissue HER2 status, the most commonly used are IHC, detecting protein overexpression, and fluorescent and chromogenic *in situ* hybridization (FISH and CISH, respectively), detecting gene amplification.

The product of HER2 consists of a cytoplasmic domain exhibiting tyrosine kinase activity, a transmembrane domain and an extracellular domain (ECD). Cleavage and shedding of the ECD-HER2 may be regulated by proteolytic processes [10,11] and ECD-HER2 levels may be determined by ELISA. No significant cross-reaction is observed between ECD-HER2 and other members of the EGFR family, and measurement of ECD-HER2 levels is not affected by the therapeutic antibody trastuzumab, as the detection domains of trastuzumab and ELISA are different [12].

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ECD-HER2 is a useful marker, approved by both FDA (USA) and CE (EU) for $HER2^+$ BC follow-up.

The interest in the marker lies in its potential capacity to provide information about the presence or absence of disease and about disease burden in real-time, contrary to the assessments of the initial biopsy.

In this study, ECD-HER2 levels were compared with standard clinical and pathological variables.

Patients and methods

Patients

From 247 consecutive inpatients, blood samples (10 cc from each patient collected in non heparinized tubes) were obtained, centrifuged and stored at –80° C until assayed. One hundred and sixteen patients had early or localized disease (stage I-IIIA), 116 patients had locally very advanced or metastatic disease (stage IIIB-IV), and 15 had benign mastopathy. In the first and third group blood samples were obtained in the perioperative period (from one day preoperatively to two days postoperatively), while in the second group the samples were obtained prior to the initiation of any treatment.

Serum immunochemistry

Serum ECD-HER2 levels were measured by ELISA (Immuno-1 assay for HER2, Bayer Co) and compared with clinical and laboratory variables (clinical stage, pathological and biological data).

Immunohistochemistry

All the available hematoxylin & eosin stained slides of surgical specimens of patients with advanced or metastatic disease (n=116) were reviewed and representative paraffin blocks for each case were selected for immunohistochemical study. Two serial sections of 3 μm thick were cut from each block and placed onto Super Frost Plus glass-slides.

HERCEP-test (Dako Co., Carpinteria) for the assessment of HER2/neu protein overexpression was used. HER2 membranous staining was scored semi-quantitatively according to the following USA FDA-approved scoring system: 0: no immunostaining or membrane staining in <10% of the tumor cells; 1+: incomplete membrane staining of >10% of the tumor cells; 2+: weak to moderate complete membrane staining of >10% of the tumor cells; 3+: moderate to strong complete membrane staining of >10% of the tumor cells. Scores of 0 or 1+ indicated a negative tumor, while scores of 2+ and 3+ were regarded as positive expression of HER2.

Statistical considerations

Statistical data processing involved nonparametric methods of analysis. The Kruskal-Wallis test was used to assess relations between ECD-HER2 and median variables. The Spierman coefficient was used to compute correlations between continuous variables.

Results

IHC in 116 samples of patients with advanced or metastatic disease revealed the following results in respect to the membranous expression of HER2: 0: 63 (54.31%) cases, +1: 11 (9.48%) cases, +2: 15 (12.93%) cases, +3: 27 (23.27%) cases.

247 serum samples from equal number of patients were assessed with the following results: mean ECD-HER2 in all patients was 19.62 ng/ml (standard error/SE=2.34), with median 10.35 and range 3 - >250 ng/ml. Mean value in benign mastopathies was 9.04 ng/ml (SE=0.72) and 9.4 ng/ml (SE=0.2) in early BC. Mean ECD-HER2 in advanced BC was 34.5 ng/ml (SE=6.05) (Table 1). The difference between early and advanced disease was statistically significant ($p<0.001$). No statistically significant difference was noted in ECD-HER2 levels between benign mastopathies (controls) and early BC (Figure 1).

A clear positive correlation was observed when serum ECD-HER2 was compared with IHC positivity ($p= 0.002$; Figure 2). There was a clear positive correla-

Table 1. Disease stage and relative mean and median serum values of ECD-HER2

Disease stage	*Patients, no.*	*Mean value ECD-HER2 (ng/ml)*	*Median value ECD-HER2 (ng/ml)*	*+SE*
Benign mastopathies	15	9.04	9.0	0.72
Early BC	116	9.40	9.5	0.20
Advanced BC (stage IIIB and IV)	116	34.50	36.0	6.05

BC: breast cancer, SE: standard error, early vs. advanced BC $p<0.001$

tion between ECD-HER2 and disease grade (p=0.034) (mean values 7.9, 11.39 and 29.9 ng/ml for grades I, II and III, respectively, evident in Figure 3. A statistically significant correlation was also observed between serum ECD-HER2 levels and level II axillary lymph node metastasis (p=0.011; Figure 4).

Furthermore, a negative relationship was established between ECD-HER2 levels and ER with r= –0.227 and p=0.035 (Figure 5), as well as with PR with r= –0.021 and p=0.011 (Figure 6).

Correlations between IHC score and serum ECD-HER2 levels are indicated in Table 2. It should be noted that in patients with IHC score of 0, 1 and 2+, the levels of ECD-HER2, with a cutoff value of 12.7 ng/ml, were negative in 88.9, 90.9 and 73.3% of the cases, respectively (p=0.009), whilst in patients with score 3+, the levels of ECD-HER2, with a cutoff value of 10 ng/ml, were positive in 59.3% of the cases (p=0.032).

Discussion

Both overexpression and/or amplification of HER2 and detection of increased ECD-HER2 levels have been

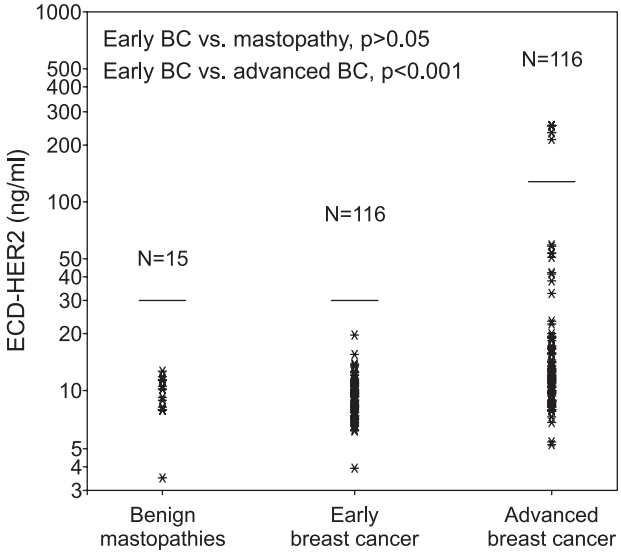

Figure 1. Relationship between ECD-HER2 levels and disease stage.

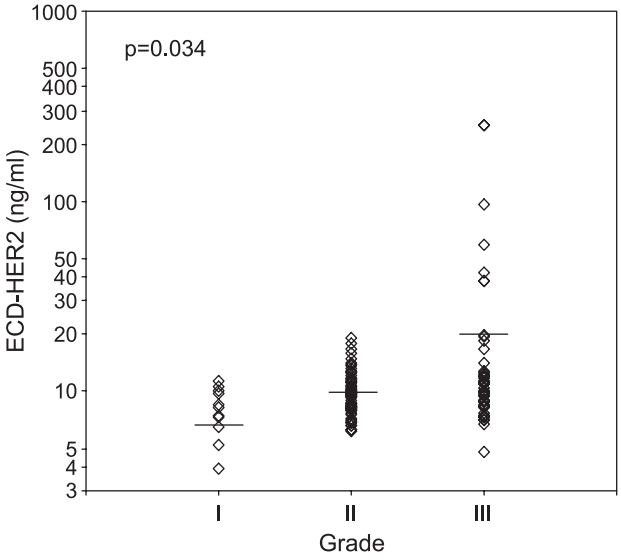

Figure 3. Comparison between ECD-HER2 levels and disease grade in advanced-stage patients (stage IIIB-IV).

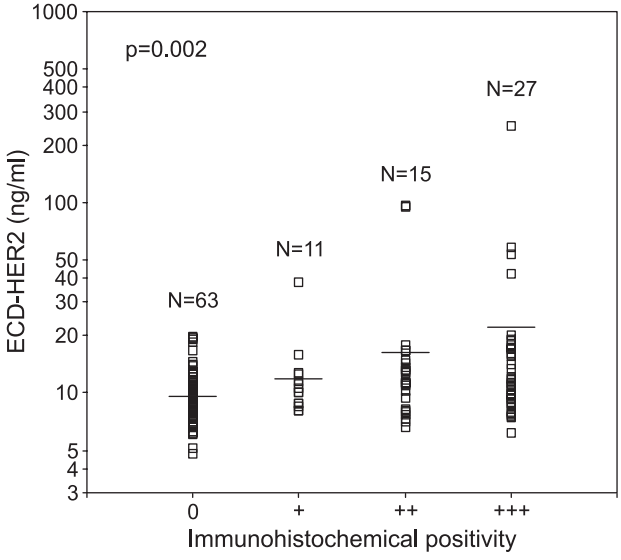

Figure 2. Comparison of immunohistochemical positivity with ECD-HER2 levels in advanced-stage patients (stage IIIB-IV).

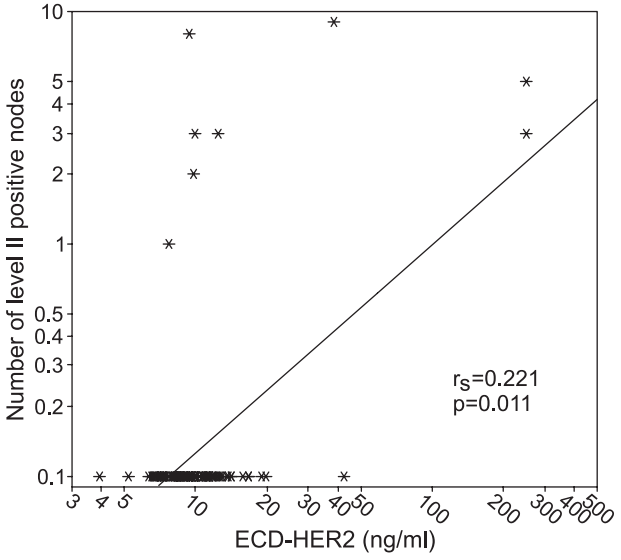

Figure 4. Relationship of ECD-HER2 with level II positive nodes in advanced-disease patients (stage IIIB-IV).

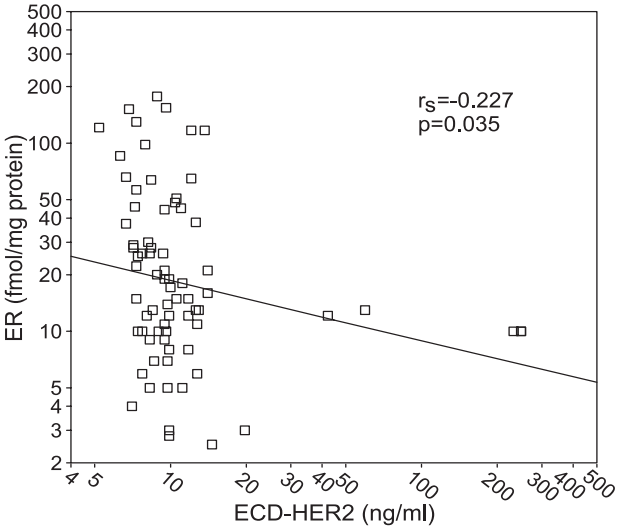

Figure 5. Relationship between ECD-HER2 and estrogen receptors in advanced-stage patients (stage IIIB-IV).

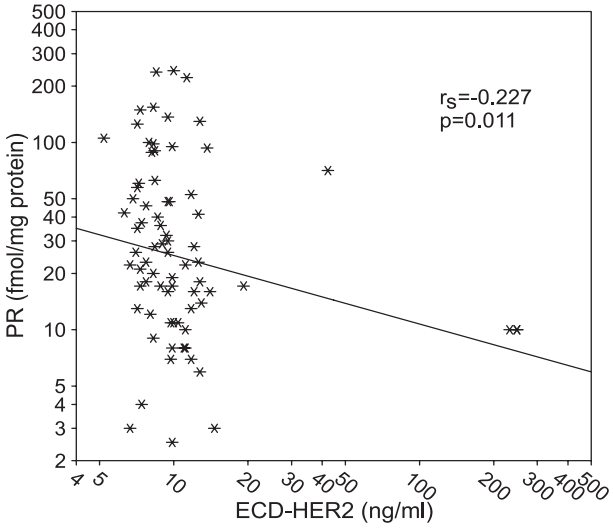

Figure 6. Relationship between ECD-HER2 and progesterone receptors in advanced-stage patients (stage IIIB-IV).

Table 2. Relationship between immunohistochemical status and ECD-HER2 levels in advanced-stage patients (stage IIIB-IV)

IHC	*No. of patients*	*ECD-negative*	*ECD-positive*	*p-value*
		No. of patients (%)		
		Cutoff = 10.0 ng/ml		
0	63	45 (71.4)	18 (28.6)	
+	11	7 (63.6)	4 (36.4)	
++	15	7 (46.7)	8 (53.3)	0.032
+++	27	11 (40.7)	16 (59.3)	
		Cutoff = 12.7 ng/ml		
0	63	56 (88.9)	7 (11.1)	
+	11	10 (90.9)	1 (9.1)	
++	15	11 (73.3)	4 (26.7)	0.009
+++	27	16 (59.3)	11 (40.7)	

IHC: immunohistochemistry

correlated with more aggressive disease and resistance to therapy with certain classes of chemotherapeutic agents, as well as to hormone therapy, resulting in decreased progression free survival and overall survival [8,13-16]. Patients with elevated ECD-HER2 levels show increased probability for response with trastuzumab treatment [17]. According to several trials, variations in ECD-HER2 levels run parallel to the course of the disease [18], and may help predict response to trastuzumab treatment since the first treatment sessions [19]. Therefore ECD-HER2 emerges as a very useful marker for the follow-up of BC and the assessment of its response to treatment [19,20].

Moreover, given the convenience of its determination, ECD-HER2 presumably offers the advantage of a "real-time biomarker" allowing the assessment of HER2-positive load at any time in the course of breast cancer as opposed to the "photographic" nature of IHC and FISH/CISH techniques, limited to a single documentation. The true HER2 status of some tumors might be underestimated, resulting in inappropriate prognostic evaluation and thus, inadequate treatment in the adjuvant setting or at early relapse [21].

The cutoff value of ECD-HER2 has not yet been clearly established, ranging from 15 to 37 ng/ml [19,22, 23]. In this study, analysis has established two different cutoff values. Higher specificity is observed at the level of 12.7 ng/ml, while higher sensitivity is observed at the level of 10.0 ng/ml (p=0.009 and p=0.032, respectively). Based on these findings and given the limited number of patients, no cutoff value may be suggested from the present study; however, we consider that further research is required to this end.

The results of the present study agree, in general,

with those published in the international literature. In particular, the clear positive correlation of ECD-HER2 levels with disease stage and grade and its negative correlation with ER and PR observed in this study are, with slight differences, common findings in the majority of the studies reviewed.

However, as far as we know, the finding that shows a correlation between ECD-HER2 levels and level II positive lymph nodes is not described in any other study, and probably suggests an unfavorable prognosis in this group of patients. It also shows the potential sensitivity of this marker in detecting such subgroups. Further confirmation of this finding in larger numbers of patients is required.

It should be specifically noted that the presence of increased ECD-HER2 levels in patients with tumors that do not exhibit overexpression or amplification of HER2, a finding described by other researchers too, supports the assumption that the two methods are complementary in detecting HER2-positive disease.

As a conclusion, we believe that the results of this study, combined with those published in the literature, suggest that ECD-HER2 is a reliable marker in BC, and speak for the necessity of its integration both in the initial workup and in the routine follow-up of BC for early detection of relapse.

Acknowledgement

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