Pathol. Oncol. Res. (2015) 21:589–595
DOI 10.1007/s12253-014-9859-6

RESEARCH

Circulating Her-2/Neu Extracellular Domain in Breast Cancer Patients-Correlation with Prognosis and Clinicopathological Parameters Including Steroid Receptor, Her-2/Neu Receptor Coexpression

Marina Barić · Ana Kulić · Maja Sirotković-Skerlev · Natalija Dedić Plavetić · Marina Vidović · Gordana Horvatić-Herceg · Damir Vrbanec

Received: 5 February 2014 / Accepted: 22 October 2014 / Published online: 4 November 2014

Summary HER-2/neu extracellular domain (ECD) can be detected in blood as a soluble circulating protein. The aim of this study was to analyze the relationship between HER-2/neu extracellular domain in the serum and the prognosis in breast cancer patients. We also correlated HER-2/neu ECD with various clinicopathological factors including steroid receptor, HER-2/neu receptor coexpression. The serum from seventy nine patients with invasive breast cancer and twenty individuals without malignancy was analyzed using the enzyme-linked immune adsorbent assay method. The cut-off value was estimated by the ROC curve analysis (15.86 μg/L). HER-2/neu ECD values in the serum of patients with breast cancer were significantly higher than in control subjects. Circulating HER-2/neu ECD was significantly associated with the histological grade of tumors and the status of axillary lymph nodes. Negative correlation was observed between HER-2/neu ECD in the serum and estrogen receptor positivity. When we analyzed HER-2/neu ECD in relation with coexpression of steroid receptor and HER-2/neu receptor in tissue, statistically higher values were found in the subgroup of patients with steroid receptor negative, HER-2/neu negative tumors than in the other subgroups. HER-2/neu ECD was not an independent factor in the univariate and multivariate analysis. However, elevated HER-2/neu ECD levels were found in patients with breast cancer possessing more aggressive phenotype.

Keywords HER-2/neu ECD · Breast cancer · Prognosis · Steroid receptor/HER-2/neu receptor coexpression

M. Barić (✉) · N. Dedić Plavetić · M. Vidović · D. Vrbanec
Department of Medical Oncology, Division of Oncology, University Hospital Center Zagreb and Zagreb Medical School, Kišpatićeva 12, HR- 10 000 Zagreb, Croatia
e-mail: marina.baric11@gmail.com

A. Kulić · M. Sirotković-Skerlev · N. Dedić Plavetić · D. Vrbanec
Department of Pathophysiology, Division of Oncology, University Hospital Center Zagreb and Zagreb Medical School, Zagreb, Croatia

G. Horvatić-Herceg
Clinical Department of Nuclear Medicine and Radiation Protection, University Hospital Center Zagreb, Zagreb, Croatia

Introduction

In the year 1987 Slamon et al. identified the correlation between HER-2/neu oncogene amplification and decreased survival in breast cancer patients [1]. Subsequent studies confirmed these results and nowadays HER-2/neu status is an established prognostic and predictive factor in breast cancer [2]. Approximately 15–20 % women with breast cancer have HER-2/neu positive tumor [2]. The amplification of HER-2/neu gene was also found in other non-breast related tumors such as lung [3,4], prostate [5], ovarian [6], bladder and gastric [7] cancers. In women with HER-2/neu positive tumors, HER-2/neu gene, located on 17th chromosome, multiplies, which increases synthesis of HER-2/neu protein and therefore tumor growth and proliferation.

Human epidermal growth factor receptor 2 (ErbB2/neu/HER-2) is a transmembrane glycoprotein that belongs to the ErbB family of receptor thyrosine kinases. Three other proteins, also termed ErbB1/HER-1, ErbB3/HER-3 and ErbB4/HER-4, belong to the same ErbB receptor family. HER-2/neu receptor is activated through dimerization with other ErbB family members while other three receptors are activated by a specific ligand binding.

HER-2/neu extracellular domain (ECD), or parts of it, can be detected in blood as soluble circulating proteins. They arise either by the process called shedding or by alternative splicing [8]. Shedding is a process in which HER-2/neu receptor releases its extracellular domain through the proteolytic mechanism leaving an active truncated intracellular receptor $p95^{HER2}$. Alternative splicing is a post transcriptional process that occurs prior to mRNA translation, by which one gene can encode several different proteins. Some of the HER-2/neu splice variants that can be detected in blood act as inhibitors of cancer cell proliferation, e.g. p100, while others promote cancer activity, e.g. Δ16HER-2. The exact quantification of each variant is not known [9]. Current FDA approved tests, as the test used in our study, use antigens that specifically bind to the extracellular domain, but they cannot discern the origin of circulating HER-2/neu protein.

The aim of our study was to correlate HER-2/neu ECD levels with the clinical outcome in breast cancer patients and to analyze the relationship of HER-2/neu ECD levels with various clinical, biochemical and pathological parameters, especially tissue steroid receptor and HER-2/neu status.

Materials and Methods

The study included seventy nine patients with breast cancer (61 with primary invasive carcinoma (stage I-III) and 18 patients with metastatic disease (stage IV), (Table 1), and twenty individuals without malignancy. All specimens were obtained through routine surgery at the University Hospital Center Zagreb between March and November 2003. Pathological and clinical data were obtained from pathological reports and medical records respectively. No patient received chemotherapy or irradiation treatment before surgery. The patients initially underwent either the modified radical mastectomy or lumpectomy with complete axillary node dissection followed by radiation therapy of residual breast tissue. All hormone receptor positive patients received endocrine treatment following surgery. Lymph node-positive patients received adjuvant chemotherapy. Lymph node-negative patients received adjuvant chemotherapy only if adverse prognostic factors were present. Approval for the study was obtained from the ethics committee of the Zagreb University Hospital. The follow up period was 60 months. The following parameters of each patient were taken into account: age, tumor size, axillary lymph node metastases, the concentrations of estrogen and progesterone receptor in the cytosol and HER's-2/neu receptor levels in tumor tissue. Clinical, pathological and biochemical parameters of the patients and their tumors are shown in Table 1.

Table 1 Distribution of prognostic and predictive markers in a group of 79 patients with breast carcinoma

Variable	No. of patients	Frequency [%]
All patients	79	
Age [years]		
≤50	29	36.70
>50	50	63.30
Tumor size		
≤2 cm	60	75.94
>2 cm	19	24.06
Axillary lymph nodes *		
N 0–2	67	84.81
N>2	12	15.19
Histological grade		
Well differentiated [I]	22	27.84
Moderately differentiated [II]	29	36.70
Poorly differentiated [III]	28	35.46
Estrogen receptor [ER]		
<10 fmol/mg proteins	34	43.03
≥10 fmol/mg proteins	45	56.97
Progesteron receptor [PgR]		
<20 fmol/mg proteins	40	50.63
≥20 fmol/mg proteins	39	49.37
HER-2/neu		
Negative	63	79.74
Positive	16	20.26
Stage		
I–III	61	77.22
IV	18	22.78

*number of axillary lymph nodes involved

Blood Samples

Blood samples from patients were retrieved before operation. Bloods were collected and allowed to clot. After centrifugation (1250 g for 10 min), the serum was removed and stored at −20 °C until assay.

Analysis of HER-2/Neu ECD in the Serum

The serum HER-2/neu ECD levels were measured by enzyme-linked immunoadsorbent assay (ELISA), (Human neu Quantitative ELISA Cat#QIA10 Oncogene research product, Cambridge, MA). The test was performed according to the manufacturer's instructions. The receiver operating curve (ROC) analysis was performed to determine the cut-off value. The quantification of HER-2/neu ECD in the serum was achieved by the construction of the standard curve.

Steroid Receptors

Steroid receptors (ER and PgR) were determined by using the ligand- binding method according to Horwitz and McGuire [10]. Tritium labeled steroid hormones were used: Estradiol (2,4,6,7 ^{3}H (N)) - NET 317 250 UC (Boston, USA) and Promegastone (17 alpha –methyl-^{3}H) - (R5020) - NET 555 250 UC (Boston, USA). A positive receptor status for ER and PgR was defined as the presence of 10 fmol/mg of cytosol proteins and 20 fmol/mg of cytosol proteins, respectively.

HER-2/Neu Status

HER-2/neu expression was determined immunohistochemically using antibodies against HER-2/neu (Herceptest, Dako, Glostrup, Denmark). Immunohistochemistry for HER-2/neu was done on whole slides using TechMate automatic stainer (Dako, Glostrup, Denmark). An immunohistochemical score of 3+ according to Herceptest criteria or fluorescence in situ hybridization (FISH; Dako, Glostrup, Denmark) with amplification ratio ≥2 was accepted as HER-2/neu positive result.

Histological Grading

Histological grading was done according to the Bloom-Richardson grading system [11].

Statistical Analysis

Statistical tests were performed using MedCalc software. Mann–Whitney test was used to evaluate differences between clinical and pathohistological factors. Comparison of more than two independent samples was done by the Kruskal-Wallis test. Survival curves were performed by the Kaplan-Meier method and the statistical significance was determined by the log-rank test. Cox proportional hazard regression was used for univariate and multivariate analysis in the forward fashion and in the stepwise fashion, respectively. The significance level was $p<0.05$.

Results

The ROC curve showed diagnostic efficiency of HER-2/neu ECD in the differentiation between breast cancer patients and the control group ($p=0.0168$). The area under the curve (AUC) was 0.635 (95 % confidence interval 0.532 to 0.730) with a sensitivity of 90 % and specificity of 44.3 % at a cut-off value of 15.86 μg/L (Fig. 1). The concentrations of HER-2/neu ECD in the serum ranged from 5.35 to 93.96 μg/L (Median=14.02; CI 95 % 12.3105–16.2316) in the serum from breast cancer patients and between 5.35 and 16.86 μg/L

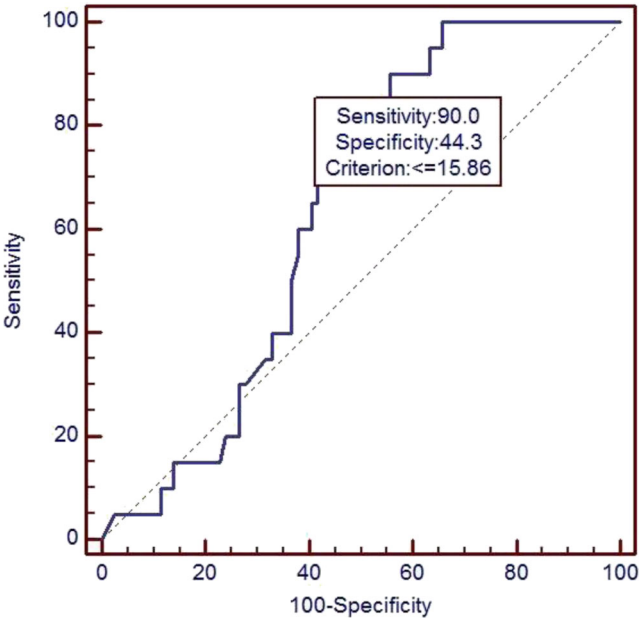

Fig. 1 Receiver operator characteristic (ROC) curve for HER-2/neu ECD

(Median=11.77; CI 95 % 10.956–12.9789) in the serum from healthy individuals. Thirty eight (48 %) out of 79 patients with breast carcinoma and 4 (20 %) out of 20 healthy controls had HER-2/neu ECD concentrations higher than 15.86 μg/L. HER-2/neu ECD values in the serum of patients with breast cancer were significantly higher than in the control subjects ($p=0.001$).

Circulating HER-2/neu ECD was significantly associated with the histological grade of tumors ($p=0.0021$; Fig. 2) and the status of axillary lymph nodes ($p=0.0425$; Fig. 3). Negative correlation was observed between HER-2/neu ECD in the serum and estrogen receptor positivity ($p=0.0091$; Fig. 4), while no association was found with the progesterone receptor status ($p=0.5714$). No correlation was found between

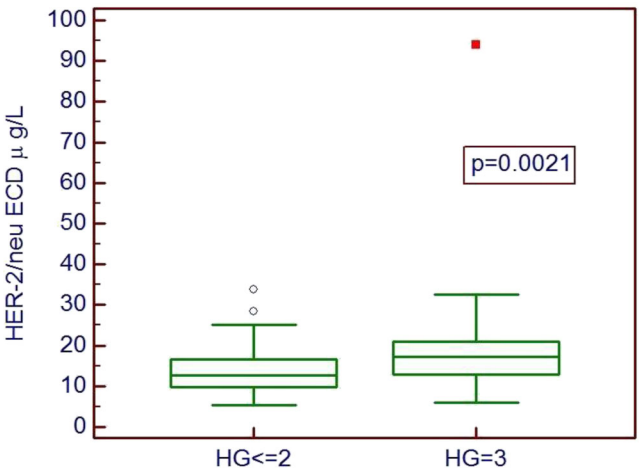

Fig. 2 Association between HER-2/neu ECD in serum (μg/L) and histological grade of tumors

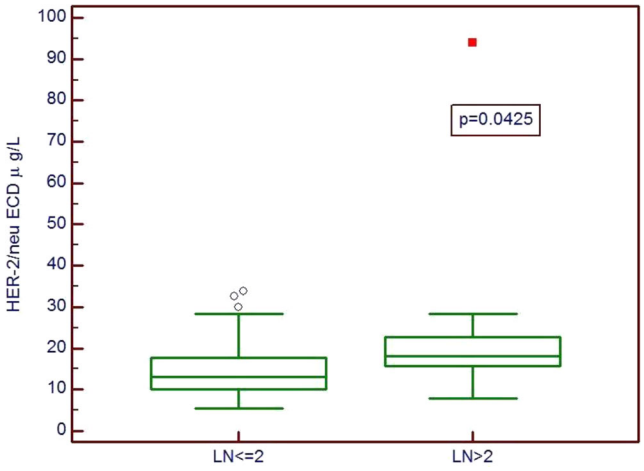

Fig. 3 Association between HER-2/neu ECD in serum (µg/L) and lymph nodes metastases (LN=lymph node)

circulating HER-2/neu ECD and the age of the patients (p=0.3190) or the size of the tumor (p=0.3378). Among 16 HER-2/neu positive cases, 11 were positive for HER-2/neu ECD. No significant association was observed between HER-2/neu ECD in the serum and HER-2/neu tissue positivity (p=0.0729). Circulating levels of HER-2/neu ECD were higher in the subgroup of patients with tissue negative steroid receptors and negative HER-2/neu receptor (6.62–93.96; median 16.8, N=17) than in the subgroup of patients with positive steroid receptors and negative HER-2 expression (5.35–20.93; median 11.83, N=18), (p=0.0477; Fig. 5). A significant difference in HER-2/neu ECD was also found between the steroid receptor negative, HER-2/neu negative subgroup of patients and the estrogen receptor positive, progesterone receptor negative, HER-2/neu negative subgroup of patients (5.35–33.73; median 11.055), (p=0.0153; Fig. 6).

The Kaplan-Meier analysis showed that patients with elevated HER-2/neu ECD in the serum had poorer prognosis (5-year survival) than patients with lower levels (p=0.0082; Fig. 7). Univariate and multivariate analyses showed that a high serum HER2/neu ECD level was not an independent prognostic factor of worse survival in this group of women with breast cancer (Table 2). The presence of HER-2/neu in

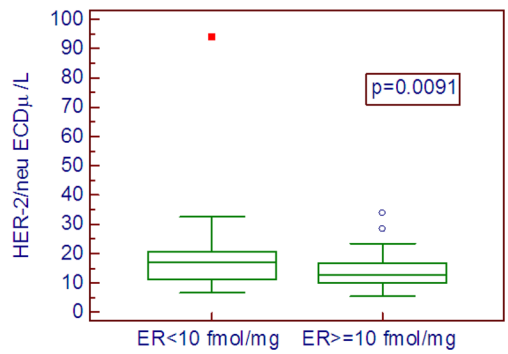

Fig. 4 Association between HER-2/neu ECD in serum (µg/L) and estrogen receptor (ER) status

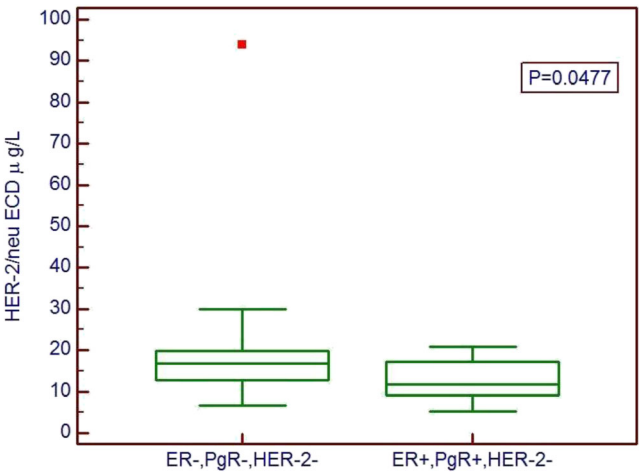

Fig. 5 Distribution of circulating HER-2/neu ECD for tissue ER negative, PgR negative, HER-2 negative in comparison with ER positive, PgR positive, HER-2 negative subgroup of patients

tissue and the tumor size were significant independent prognostic factors in the univariate and multivariate analysis (Table 2).

Discussion

In our study we analyzed the relationship between HER-2/neu ECD levels and the 5-year overall survival in breast cancer patients. We also correlated HER-2/neu ECD with various clinicopathological factors.

Different studies inquired the role of the serum HER-2 ECD as a plausible determinant of tissue HER-2/neu status. Many of them found a significant correlation between tissue HER-2/neu expression and serum HER-2/neu ECD concentrations [8]. But, the results depended greatly on cut-off values that were used [12]. Dresse et al. for example conducted a

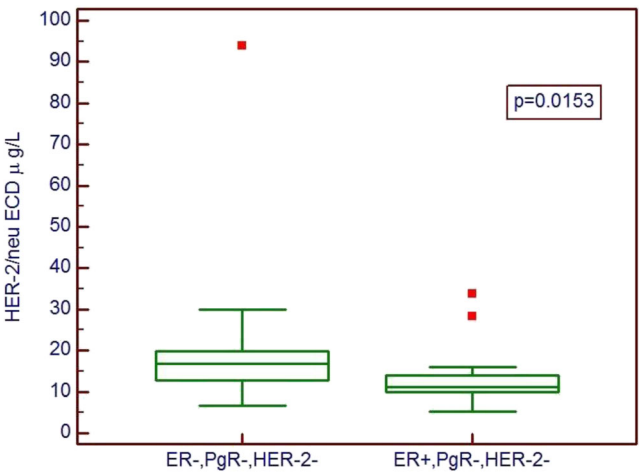

Fig. 6 Distribution of circulating HER-2/neu ECD for tissue ER negative, PgR negative, HER-2 negative in comparison with ER positive, PgR negative, HER-2 negative subgroup of patients

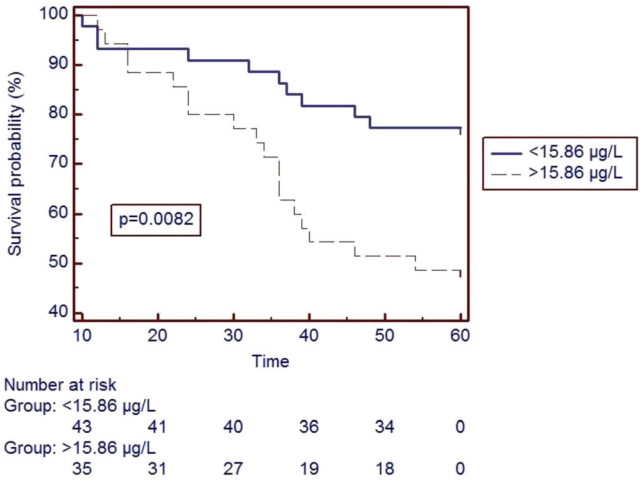

Fig. 7 Kaplan-Meier survival curves according to the serum concentrations of HER-2/neu ECD in breast cancer patients

study that involved 492 patients and found that serum HER-2/neu ECD values of 30 ng/ml or higher were only observed in HER-2/neu-positive patients, but the sensitivity, when using this cut off value, was only 7.7 % [13]. On the other hand, Leary et al. in their review, listed studies that found no correlation between tissue HER-2/neu expression and serum HER-2/neu ECD concentrations [14]. We did not find a correlation between tissue HER-2/neu status and circulating HER-2/neu ECD, either. The cut-off value in our study, estimated by the ROC analysis, was 15.86 µg/L, which is almost the same as the one recommended by FDA. Currently, there are two FDA approved tests for the detection of serum HER-2/neu, by which the cut-off is standardized at 15 µg/L. Carney et al. analyzed a large number of publications that used FDA approved tests and documented the potential value of serum HER-2 testing in both HER-2 positive and HER-2 negative patients [15].

Serum HER-2/neu ECD was also investigated in relation to age and other tumor characteristics such as histological grade, hormone receptor status, the lymph node status and tumor size. In our study, high histological grade and >2 metastatic axillary lymph node status were associated with higher serum HER-2/neu ECD values. These findings are in accordance with the majority of published studies [16,17]. Surprisingly, we did not find a connection between tumor size and serum HER-2/neu ECD levels although it had been previously established [18–20]. The difference in our study may be due to the possibility that certain tumor load has to be surpassed to show effect on results. Up to now, a referent value in tumor size, which would influence measurements, has not been established. Other factors that influence HER-2/neu ECD levels are not completely understood. They include the activity of matrix metalloproteinases and their inhibitors and physical factors such as tumor architecture and vascularity [21–23]. We did not find age to have significant coherence with serum HER-2 EDC levels. These results are in accordance with those of Ludovini et al. [24].

Studies that analyzed the connection between serum HER-2/neu ECD values and the hormone receptor status found significantly higher serum HER-2/neu ECD levels in estrogen and progesterone receptor negative tumors [17,24,25]. We also observed a negative correlation between serum HER-2/neu ECD and estrogen receptor positivity, but failed to find an association with the progesterone receptor status. Likewise, Molina et al. found higher values of serum HER-2/neu ECD only in estrogen receptor negative tumors, noting that it referred to patients with tissue overexpression [26]. When we analyzed HER-2/neu ECD in correlation with coexpression of steroid receptors and HER-2/neu in tissue, we found statistically higher values of HER-2/neu ECD in the subgroup of patients with ER and PgR receptor negative, HER-2/neu receptor negative tumors than in the subgroup of patients with ER positive, PgR positive, HER-2/neu negative tumors and

Table 2 Univariate and multivariate analysis of histopathological and biological factors for overall survival

Variable	Univariate			Multivariate		
	HR	95 % CI	P	HR	95%CI	P
ER	0.9902	0.9800–1.004	0.0607	NI		
PgR	1.0410	0.9875–1.015	0.8763	NI		
Age	1.0670	0.9847–1.049	0.3119	NI		
TS	1.5488	1.2428–1.930	0.0001	1.5765	1.231–1.9576	0.0002
HG	0.9070	0.9801–1.001	0.0907	NI		
LN	0.5359	0.6863–1.215	0.5359	NI		
HER-2/neu	1.4343	1.0961–1.942	0.0202	1.4765	1.088–2.0113	0.0140
HER-2/neu ECD	1.0014	0.9689–1.036	0.9362	NI		

ER Estrogen receptor, *PgR* Progesteron receptor, *TS* Tumor size, *HG* Histological grade, *LN* lymph nodes, *HR* hazard ratio, *CI* confidence interval, *NI* not included

the subgroup of patients with ER positive, PgR negative, HER-2/neu negative tumors. According to present knowledge, the tumor is a heterogeneous mass that consists of different populations of cancer cells, including tumor initiating cells (also called cancer stem cells). A positive correlation has been found between HER-2 overexpression and the number of tumor initiating cells. Additionally, HER 2 is considered to be a part of signaling pathway that is activated by cellular adaptive response to chemo and radiotherapy. This pathway may be activated not only in the group of HER-2 positive breast cancer but also in the group without HER-2 amplification [27]. Several studies showed that various external factors can facilitate the proliferation of tumor initiating cells increasing their population in the tumor mass. Lee et al. in their study showed that HER-2 signaling is functionally important in tumor initiating cells from triple negative breast cancers [28]. These results speak in favor of assessment of HER-2/neu ECD both in initially HER-2/neu positive and HER-2/neu negative patients. In HER-2/neu positive patients HER-2/neu ECD testing may be useful in monitoring the course of the disease, and in HER-2/neu negative patients assessment of HER-2/neu ECD may contribute to the identification of the subgroup of patients that may benefit from anti HER-2/neu therapy.

The results in our study, when analyzed with the Kaplan–Meier estimator, showed that patients with elevated HER-2/neu ECD in the serum had worse 5-year survival than those with lower concentrations. This indicates that serum HER-2/neu ECD could be used as a marker of poor prognosis. Other authors also found the association of HER-2/neu ECD with poor clinical outcome of breast cancer patients [20,24,29]. It has to be noted that methodology, follow-up period, cut-off values and parameters of prognosis vary between studies making the comparison difficult. HER-2/neu ECD was investigated as an independent factor with variable results, especially regarding the overall survival [20,24]. We did not find HER-2/neu ECD to be an independent prognostic factor.

In conclusion, our results support a negative prognostic role of HER-2/neu ECD in breast cancer patients and the relationship with the more aggressive phenotype. Additionally, we found significantly higher HER-2/neu ECD levels in the steroid receptor negative, tissue HER-2/neu negative subgroup of patients than in the steroid receptor positive or only estrogen receptor positive, HER-2/neu negative subgroup. Further studies on a larger number of patients are needed in order to validate these results.

Acknowledgments This work was supported by the Ministry of Science, Education and Sports, Republic of Croatia (grant 108-1080058-0046).

References

1. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, Mcguire WL (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235:177–182
2. Ross JS, Slodkowska EA, Symmans WF, Pusztai L, Ravdin PM, Hortobagyi GN (2009) The HER-2 receptor and breast cancer: 10 years of targeted anti-HER-2 therapy and personalized medicine. Oncologist 4:320–368
3. Suzuki M, Shigematsu H, Hiroshima K, Iizasa T, Nakatani Y, Minna JD et al (2005) Epidermal growth factor receptor expression status in lung cancer correlates with its mutation. Hum Pathol 36:1127–1134
4. Zeng S, Yang Y, Tan Y, Lu C, Pan Y, Chen L et al (2012) ERBB2-induced inflammation in lung carcinogenesis. Mol Biol Rep 39: 7911–7917
5. Minner S, Jessen B, Stiedenroth L, Burandt E, Köllermann J, Mirlacher M et al (2010) Low level HER2 overexpression is associated with rapid tumor cell proliferation and poor prognosis in prostate cancer. Clin Cancer Res 16:1553–1560
6. Bookman MA, Darcy KM, Clarke-Pearson D, Boothby RA, Horowitz IR (2003) Evaluation of monoclonal humanized anti-HER2 antibody, trastuzumab, in patients with recurrent or refractory ovarian or primary peritoneal carcinoma with overexpression of HER2: a phase II trial of the gynecologic oncology group. J Clin Oncol 21:283–290
7. Giuffrè G, Ieni A, Barresi V, Caruso RA, Tuccari G (2012) HER2 status in unusual histological variants of gastric adenocarcinomas. J Clin Pathol 65:237–241
8. Tsé C, Gauchez AS, Jacot W, Lamy PJ (2012) HER2 shedding and serum HER2 extracellular domain: biology and clinical utility in breast cancer. Cancer Treat Rev 38:133–142
9. Sasso M, Bianchi F, Ciravolo V, Tagliabue E, Campiglio M (2011) HER2 splice variants and their relevance in breast cancer. J Nucleic Acids Investig 2:e9
10. Horwitz KB, Mcguire WL (1977) Progesterone and progesterone receptors in experimental breast cancer. Cancer Res 37:1733–1738
11. Bloom HJ, Richardson WW (1957) Histological grading and prognosis in breast cancer; a study of 1409 cases of which 359 have been followed for 15 years. Br J Cancer 11(3):359–377
12. Carney WP, Neumann R, Lipton A, Leitzel K, Ali S, Price CP (2003) Potential clinical utility of serum HER-2/neu oncoprotein concentrations in patients with breast cancer. Clin Chem 49:1579–1598
13. Dresse M MD, Heinemann V, Kahlert S, Bauerfeind I, Nagel D, et al.. (2009) HER-2/neu in tissue and serum at time of primary breast cancer. J Clin Oncol Abstract #11500.
14. Leary AF, Hanna WM, van de Vijver MJ, Penault-Llorca F, Rüschoff J, Osamura RY et al (2009) Value and limitations of measuring HER-2 extracellular domain in the serum of breast cancer patients. J Clin Oncol 27:1694–1705
15. Carney WP, Bernhardt D, Jasani B (2013) Circulating HER2 extracellular domain: a specific and quantitative biomarker of prognostic value in all breast cancer patients? Biomark Cancer 5:31–39
16. Thriveni K, Deshmane V, Bapsy PP, Krishnamoorthy L, Ramaswamy G (2007) Clinical utility of serum human epidermal receptor-2/neu detection in breast cancer patients. Indian J Med Res 125:137–142
17. Garoufali A, Kyriakou F, Kountourakis P, Yioti I, Malliou S, Nikaki A et al (2008) Extracellular domain of HER2: a useful marker for the initial workup and follow-up of HER2-positive breast cancer. J BUON 13:409–413
18. Pallud C, Guinebretiere JM, Guepratte S, Hacene K, Neumann R, Carney W et al (2005) Tissue expression and serum levels of the oncoprotein HER-2/neu in 157 primary breast tumours. Anticancer Res 25:1433–1440

19. Saghatchian M, Guepratte S, Hacene K, Neumann R, Floiras JL, Pichon MF (2004) Serum HER-2 extracellular domain: relationship with clinicobiological presentation and prognostic value before and after primary treatment in 701 breast cancer patients. Int J Biol Markers 19:14–22
20. Kong Y, Dai S, Xie X, Xiao X, Lv N, Guo J et al (2012) High serum HER2 extracellular domain levels: correlation with a worse disease-free survival and overall survival in primary operable breast cancer patients. J Cancer Res Clin Oncol 138:275–284
21. Molina MA, Codony-Servat J, Albanell J, Rojo F, Arribas J, Baselga J (2001) Trastuzumab (herceptin), a humanized anti-Her2 receptor monoclonal antibody, inhibits basal and activated Her2 ectodomain cleavage in breast cancer cells. Cancer Res 61: 4744–4749
22. Codony-Servat J, Albanell J, Lopez-Talavera J, Arribas J, Baselga J (1999) Cleavage of the HER2 ectodomain is a pervanadate-activable process that is inhibited by the tissue inhibitor of metalloproteases-1 in breast cancer cells. Cancer Res 59:1196–1201
23. Quaranta M, Daniele A, Coviello M, Savonarola A, Abbate I, Venneri MT et al (2006) c-erbB-2 protein level in tissue and sera of breast cancer patients: a possibly useful clinical correlation. Tumori 92:311–317
24. Ludovini V, Gori S, Colozza M, Pistola L, Rulli E, Floriani I et al (2008) Evaluation of serum HER2 extracellular domain in early breast cancer patients: correlation with clinicopathological parameters and survival. Ann Oncol 19:883–890
25. Ma L, Yang HY, Han XH, Li J, Wang F, Zhang CL et al (2012) Relationship between serum HER2 extracellular domain levels, tissue HER2 expression, and clinico-pathological parameters in early stage breast cancer. Chin Med J 125:4104–4110
26. Molina R, Augé JM, Escudero JM, Filella X, Zanon G, Pahisa J et al (2010) Evaluation of tumor markers (HER-2/neuoncoprotein, CEA, and CA 15.3) in patients with locoregional breast cancer: prognostic value. Tumour Biol 31:171–180
27. Duru N, Candas D, Jiang G, Li JJ (2014) Breast cancer adaptive resistance: HER2 and cancer stem cell repopulation in a heterogeneous tumor society. J Cancer Res Clin Oncol 140(1):1–14
28. Lee CY, Lin Y, Bratman SV, Feng W, Kuo AH, Scheeren FA et al (2014) Neuregulin autocrine signaling promotes self-renewal of breast tumor-initiating cells by triggering HER2/HER3 activation. Cancer Res 74(1):341–352
29. Samy N, Ragab HM, El Maksoud NA, Shaalan M (2010) Prognostic significance of serum Her2/neu, BCL2, CA15-3 and CEA in breast cancer patients: a short follow-up. Cancer Biomark 6:63–72