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Serum HER2/ECD value in stage I and II early breast cancer – need of a lower cut-off?

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HER2/ECD Serumwerte bei Stadium I und II Brustkrebs – brauchen wir niedrigere Grenzwerte?

Zusammenfassung. *Grundlagen:* Die Überexpression von HER2 ist ein gut bekannter Risikofaktor einer schlechten Prognose beim metastasierten und frühen Brustkrebs. Um eine HER2-Positivität nachzuweisen, kann Tumorgewebe immunohistochemisch gefärbt werden oder durch Fluoreszenz-in-situ-Hybridisierung nachgewiesen werden. Es ist auch möglich, die extrazelluläre Domäne des HER2 (HER2/ECD)-Rezeptors durch eine Serumuntersuchung zu bestätigen. HER2/ECD korreliert gut mit einer schlechteren Prognose beim metastasierten und lokal fortgeschrittenen (Stadium III) Krebs, wenn die Serumkonzentration höher als 15 ng/ml ist, allerdings gibt es keine übereinstimmenden Daten für Patientinnen mit Brustkrebs in frühen Stadien.

Methodik und Ergebnisse: 41 Personen mit Brustkrebs Stadium I und II und 52 gesunde Personen als Kontrollgruppe haben an der Studie teilgenommen. Vor der Operation wurde HER2/ECD ermittelt und mit HER2/neu-Überexpression, Ki67, Hormonrezeptorstatus und mit dem Stadium der Krankheit verglichen. Die durchschnittliche Serumkonzentration des HER2/ECD war bei den Patienten 8,62 ng/ml und 5,78 ng/ml bei der Kontrollgruppe, die Differenz war von statistischer Bedeutung ($p = 0{,}000061$). Der beste diagnostische Grenzwert war 7,7 ng/ml mit einer Sensitivität von 76,92 % und einer Spezifität von 72,92 %. Der positive Vorhersagewert des Tests war 69,77 %, der negative Vorhersagewert war 79,55 %. 74,71 % der Patienten waren richtig eingestuft. Serum HER2/ECD korrelierte gut mit dem Hormonrezeptorstatus, hatte aber eine negative Korrelation mit der histologischen Überexpression.

Schlussfolgerung: Eine höhere Serumkonzentration als 7,7 ng/ml von HER2/ECD hat einen möglichen diagnostischen Wert im Stadium I und II bei Brustkrebs. Bei der Ermittlung von HER2-Positivität kann es nicht als ausschlaggebender Faktor verwendet werden. Die prognostische Bedeutung von HER2/ECD beim frühen Brustkrebs, die Korrelation mit dem Hormonrezeptorstatus und der Zusammenhang zwischen dem Hormonrezeptor und der HER2-Rezeptorsignalgebung müssen weiter analysiert werden, da diese therapeutische Implikationen haben könnte.

Summary. *Background:* HER2 overexpression is well-established risk factor of worse prognosis in metastatic and early breast cancer. HER2 positivity can be determined from tumor tissue by immunohistochemical staining or by fluorescent *in situ* hybridization, or from serum by measuring concentration of HER2 receptor extracellular domain (HER2/ECD). HER2/ECD correlates well with worse prognosis in metastatic and locally advanced (stage III) disease if serum concentration is >15 ng/ml, but there are no consistent data for patients with early breast cancer.

Methods and results: 41 patients with stage I and II breast cancer and 52 healthy controls were included into the study. HER2/ECD was determined before surgery and correlated with HER2/neu overexpression, Ki67, hormone receptor status and disease stage, and compared with value in healthy controls. Mean serum HER2/ECD concentration in patients was 8.62 ng/ml and 5.78 ng/ml in controls, and the difference was statistically significant ($p = 0.000061$). The best diagnostic cut-off value was 7.7 ng/ml, with 76.92% sensitivity and 72.92% specificity. Positive predictive value of the test was 69.77% and negative predictive value was 79.55%, with 74.71% of patients correctly classified. Serum HER2/ECD correlated with hormone receptors status, and no correlation with histological overexpression has been observed.

Conclusion. Serum HER2/ECD concentration of ≥7.7 ng/ml has possible diagnostic value in stage I and II

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breast cancer. It should not be used as a determinant of HER2 positivity. Prognostic significance of HER2/ECD in early breast cancer, its correlation with hormone receptor status, and interconnection between hormone receptors and HER2 receptor signaling should be further analyzed, since it may have therapeutic implications.

Key words: Breast cancer, HER2/ECD, tumor markers.

Introduction

Breast cancer is the most common malignant disease in women in western countries, the most common cause of cancer mortality in women 20–59 years of age, and the second most common cause of cancer death in women >60 years of age. Constitutive expression of damaged oncogenes (e.g. cyclin D1, Wnt1), loss of function of tumor suppressors (e.g. p53), as well as expression of antiapoptotic (Bad/Bcl-2) and loss of function of proapoptotic genes lead to uncontrolled cell growth and division, and after gaining additional genome damage with consequent cell immortalization breast cancer develops [1–8]. Estrogens and progesterone induce expression of cyclin D1 and c-myc as well as Bad/Bcl-2, which is proposed mechanism of hormone-dependent breast cancer development, seen in about 80% of breast cancer patients [9, 10]. In the remaining 20% of patients, the proposed mechanism is overexpression of epidermal growth factor (EGF) receptor amplifier, i.e. HER2/neu (or c-erb-B2). Of course, exceptions can be found in minority of patients, known as triple-negative and triple-positive breast cancer. HER2/neu is one of four identified EGF receptors so far (besides to EGFR/HER1, c-erb-B3 and c-erb-B4). Unlike other EGF receptors which bind numerous paracrine ligands like EGF, transforming growth factor alpha (TGFα), hergulin, heparin-binding EGF-like growth factor, beta-cellulin and epiregulin, HER2/neu has no specific ligand; it serves as a modulator of growth and division signal received by EGFR [11, 12]. When overexpressed on the cell membrane, it can spontaneously form homodimers which have strong signal activity without binding specific ligands by EGFR and, thus, lead to autonomous cell growth stimulation [13]. Despite continuous controversies regarding the biology, prognostic significance and methods used for its determination, it is generally accepted that HER2/neu overexpression correlates with higher histological tumor grade, non-expression of hormone receptors, aneuploidy, higher proliferation index, tumor size and, consequently, worse clinical outcome because of higher aggressiveness, dissemination potential and treatment resistance of overexpressing tumors.

HER2/neu receptor is a transmembrane protein 185 kDA in size. It consists of three parts: intracellular tyrosine kinase which activates intracellular cell growth and division pathways, small intramembranous part and extracellular domain (HER2/ECD) 105 kDA in size. It can commonly be found on lungs, urinary bladder, pancreas, breast and prostate epithelial cells. Metalloproteases clear HER2/ECD from tumor cell surface, and it can be detected in sera of patients with primary or metastatic breast cancer using specific antibodies [14–17]. Quantification of HER2/neu overexpression can be performed by immunohistochemical staining (IHC) or by direct determination of gene amplification by fluorescence in situ hybridization (FISH) in tumor tissue samples, or by determination of serum concentration of HER2/ECD.

Numerous clinical studies confirmed prognostic significance of HER2/ECD for overall survival (OS) progression-free survival (PFS), response to taxane-based chemotherapy, hormone therapy and anti-HER2 targeted therapy in metastatic breast cancer [18–23]. In patients with locally advanced breast cancer (stage III disease, LABC) it has been shown that HER2/ECD has prognostic significance for OS and disease-free survival (DFS), as well as for response to anthracycline-based chemotherapy and endocrine therapy [24]. It has also been shown that it can be used as a tumor marker for detection of local and metastatic relapse of LABC [25–27]. Switch in HER2/neu positivity has been verified in about 20% of patients after primary therapy probably due to therapy-related clonal selection, suggesting the change in the biology of the disease when metastatic relapse occurs [28]. Logically, higher HER2/neu positivity has been found in patients with metastatic relapse than during primary therapy [29].

Since no consistent data exist on the value of HER2/ECD testing in patients with early breast cancer, we conducted this study to determine its prevalence and correlation with established breast cancer prognostic factors, as well as to preliminary screen for possible diagnostic and prognostic value of the test in early breast cancer patients.

Materials and methods

Patients and sampling

A group of 66 women presenting to the Department of Oncology at University Hospital Center Zagreb from January 2009 till February 2010 with cytologically or histologically newly diagnosed localized breast cancer have been screened into prospective observational study, 41 of which were classified as stage I and II disease and therefore have been candidates for initial breast-conserving surgery, whose specimens have been used in further analysis for purpose of this study, enough to ensure good statistical power. Median follow-up was 13.8 months and two patients were lost to follow-up. 66% (27/41) of included patients had T1N0 disease, and 34% (14/41) had T1N1 disease. Age range of patients was 35 to 82 with an average of 60. To increase negative predictive value of the test, 52 age- and gender-matched healthy controls with no family history of breast cancer and/or presence of fibrocystic proliferative breast diseases have also been included into the study.

Patients were consented prior to surgery by study staff according to the local ethics committee approved protocol. Patients had to have early-stage breast cancer and to be eligible for initial surgery, i.e. stage I and II. Patients have been treated by breast-conserving surgery and adjuvant radiotherapy, chemotherapy, endocrine therapy and targeted anti-HER2 therapy according to standard protocols. Patients' demographic data, treatment and outcome are shown in Table 1.

HER2/neu and HER2/ECD determination

Blood samples were obtained before surgery for HER2/ECD determination. Samples have been centrifuged at 1250 g, the sera

Table 1. Patients' demographic data, treatment and outcome

	No	%
Age		
median	61	
range	36–83	
PHD[1]		
CDI[1]	32	78.05
CLI[1]	5	12.19
Ca medullare	1	2.44
Ca papillare	1	2.44
DCIS[1]	2	4.87
TNM		
T1	21	51.22
T2	20	48.78
N0	26	63.42
N1	15	36.59
ER/PR[1]		
ER^+/PR^+	21	51.22
ER^+/PR^-	4	9.76
ER^-/PR^+	0	0
ER^-/PR^-	16	39.02
HER2/neu	5	12.2
Ki67	26	63.42
Adjuvant treatment		
anthracycline based	22	53.66
taxanes	3	7.32
hormone therapy	28	68.29
trastuzumab	5	12.2
RT[1] only	4	9.76
Outcome		
DFS[1] HER2/ECD <7.7 ng/ml	22.8	months
DFS HER2/ECD ≥7.7 ng/ml	13.5	months
	*$p = 0.369$	

[1] *PHD* pathohistological diagnosis; *CDI* carcinoma ductale invasivum; *CLI* carcinoma lobulare invasivum; *DCIS* ductal cancer *in situ*; *ER* estrogen receptors; *PR* progesterone receptors; *RT* radiotherapy; *DFS* disease-free survival.

were detached and stored at the temperature of –20°C or lower. Tumor tissue samples have been taken at the time of surgery and tumor type, histological grade, Ki67 proliferation index, hormone receptor status and HER2/neu status have been obtained. HER2/neu determination has been performed by IHC using HercepTest kit protocol (Dako Ltd., Denmark House, Angel Drove, Ely, Cambridgeshire, CB7 4ET, UK) or by FISH (Abbot-Vysis PathVysion HER-2 DNA Probe kit, Abbot Laboratories, Abbot Park, IL, USA) in equivocal cases. Tumors scored 3+ on IHC were considered HER2 positive, 0+ and 1+ were considered negative, and in 2+ FISH was performed, with results scoring >2.0 signal ratio between the average number of copies of the HER2/neu gene and centromere of chromosome 17 (analyzing 60 neoplastic nuclei) considered positive. HER2/ECD determination has been performed by using FDA-approved ELISA method (Human Neu Oncoprotein ELISA, Siemens Diagnostics, Oncogene Science/Bayer Diagnostics) [30].

Statistical analysis

Comparison of HER2/ECD values in patients and controls has been performed using *t*-test. HER2/neu and HER2/ECD values were correlated with known prognostic factors using Kendall's test. For determination of diagnostic value of HER2/ECD in early breast cancer patients we used receiver operating characteristic curve (ROC), with area under curve (AUC) as measurement of diagnostic value, and for selection of the best cut-off value TG-ROC curve has been used. Measurements of test performance (sensitivity, specificity) were calculated using STATA 10.1 (Stata Press, College station, Texas, USA) according to REMARK criteria [31]. Test subjects have then been stratified according to DFS, defined as time interval between treatment of breast cancer and disease relapse, and Kaplan-Meier survival estimates have been calculated to determine prognostic value of HER2/ECD determination in early breast cancer.

Results

Mean HER2/ECD concentration in patients and in healthy controls was 8.62 ng/ml (SD ± 1.486) and 5.78 ng/ml (SD ± 3.99), respectively, and the difference between groups was statistically significant ($p = 0.000061$); Box and Whisker plot is shown in Fig. 1. The best diagnostic cut-off value for HER2/ECD concentration between patients and

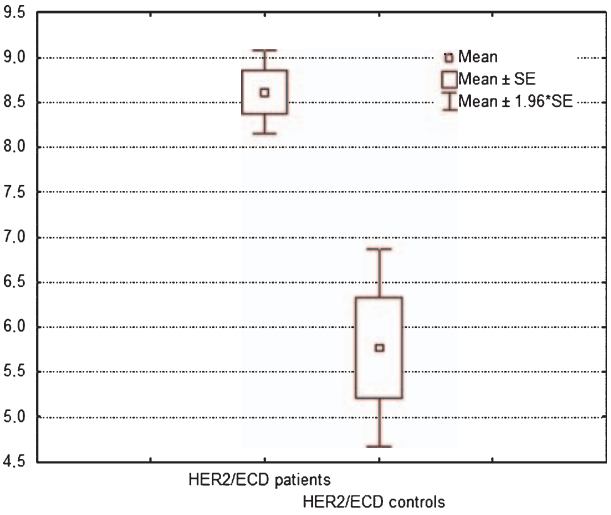

Fig. 1. The Box and Whisker plot shows statistically significant difference of the mean serum HER2/ECD concentration in patients with early breast cancer and healthy controls. X-axis: compared groups; Y-axis: serum HER2/ECD concentration in ng/ml

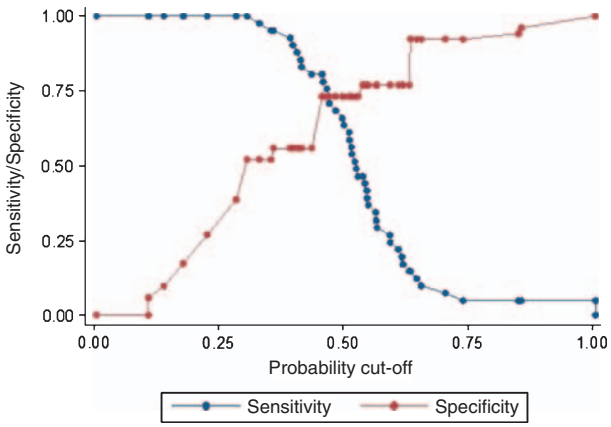

Fig. 2. TG-ROC curve used for selection of the best serum HER2/ECD concentration in early breast cancer patients. Cut-off value of 7.7 ng/ml resulted in 74.71% of patients correctly classified. X-axis probability cut-off; Y-axis sensitivity/specificity

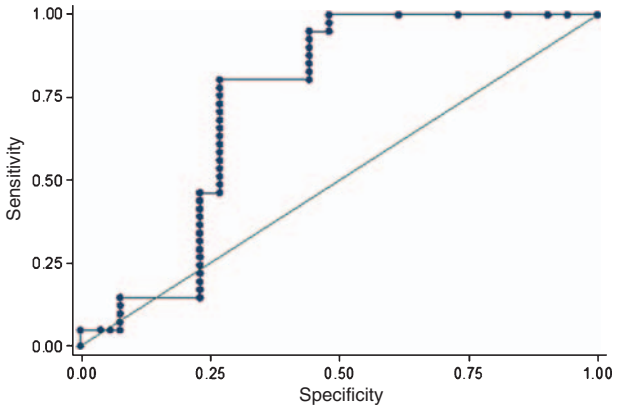

Fig. 3. ROC diagram showing an area under curve (AUC) of 0.739 as a measure of good diagnostic value of serum HER2/ECD concentration in early breast cancer patients. X-axis: specificity; Y-axis: sensitivity

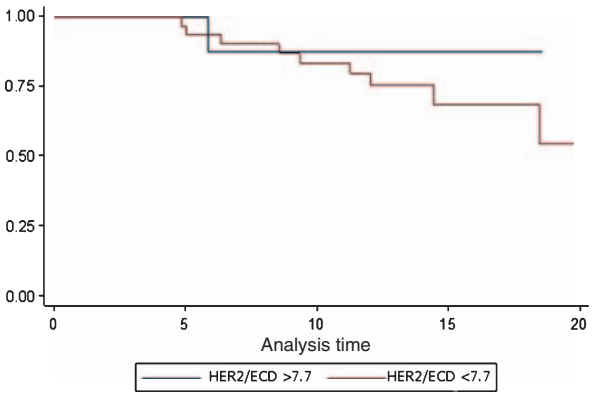

Fig. 4. Kaplan-Meier survival estimates for early breast cancer patients stratified by cut-off serum HER2/ECD value of 7.7 ng/ml show no statistically significant difference in disease-free survival. X-axis: analysis time in months; Y-axis: patients surviving

healthy controls, which equaled 7.7 ng/ml, has been calculated and patients were stratified into two groups accordingly, with ≥7.7 ng/ml considered positive (N=31) and <7.7 ng/ml (N=10) considered negative (TG-ROC curve is shown in Fig. 2). Positive correlation has been found between HER2/ECD positivity and tumor grade, higher Ki67 proliferation index, ER and PR positivity. No correlation of HER2/ECD with disease stage has been found, as well as with histological HER2/neu positivity. The test has actually shown good diagnostic value for early breast cancer, with AUC = 0.739 (ROC diagram is shown in Fig. 3). Sensitivity of the test using this cut-off value was 76.92%, specificity was 72.92%, positive predictive value 69.77% and negative predictive value 79.55%, with 74.71% of patients correctly classified. After a median follow-up of 13.8 months, Kaplan-Meier survival estimates were calculated using DFS as endpoint. Median survival times were 13.8 months in patients with serum HER2/ECD <7.7 ng/ml and 13.5 months in patients with HER2/ECD ≥7.7 ng/ml, and there was no statistical significance between two groups of patients (Log rank p = 0.369) as shown in Fig. 4.

Patient's HER2/neu positivity correlated with tumor grade, higher Ki67 proliferation index and number of positive axillary lymph nodes, while there was no correlation with hormone receptor status and disease stage.

Discussion

Because of unsuitability of determination of HER2/neu expression during the breast cancer treatment, serum concentration of HER2/ECD as a surrogate of histological marker has been widely used in clinical practice. It has been shown to be more practical and less invasive than IHC and FISH methods, while providing additional information about dynamics of tumor biology and evolution during chemotherapy, as proven in patients with metastatic disease [32–34]. However, although it was initially presumed that serum HER2 positivity equaled histological HER2 positivity, HER2/ECD has been recognized as an independent breast cancer risk factor of worse overall survival, PFS and DFS, since some recent studies have not shown correlation of histological HER2/neu and serum HER2/ECD positivity [35]. Possible logical explanation lies in interindividual genetic differences in metalloproteases activity, which are responsible for HER2/ECD elevation in serum [36]. Previously published results considered HER2 positivity based on serum HER2/ECD determination in metastatic breast cancer patients if measured HER2/ECD concentration was >15 ng/ml, while other authors simply translated the metastatic cut-off value onto early breast cancer patients (inclusively stage III) [37, 38].

In our study no correlation was found between HER2/neu and HER2/ECD as well, and that result also supports HER2/ECD as an independent risk factor which correlates with other established risk factors *per se*, and that it should not be used solely as designation of HER2 positivity as previously suggested. Moreover, necessity of a lower cut-off value arises in early breast cancer patients, whereby showing newly recognized diagnostic value of the test. Even Kaplan-Meier analysis showed a trend of curve separation favoring HER2/ECD value <7.7 ng/ml, although statistically insignificant to provide good statistical power for prognosis evaluation due to sample size limitation and too short follow-up. Results of longer follow-up and influence on overall survival will be published afterwards.

Correlation between hormone receptor positivity and growth factor receptors has recently been demonstrated by multiple studies, and receptor cross-talk has even been suggested as the underlying mechanism of endocrine and lapatinib therapy resistance [39–43]. Our study is the first to show positive correlation between serum HER2/ECD positivity and hormone receptors status.

Non-correlation between disease stage and both histological and serum HER2 positivity can easily be explained by the fact that patients were diagnosed during routine breast cancer screening, allowing its detection in earliest stages of the disease.

To conclude, HER2/ECD value ≥7.7 ng/ml in the moment of diagnosis of stage I and II breast cancer suggests the presence of high-risk cancer since it is complementary to other established histological risk factors. Furthermore,

it has possible diagnostic value in these patients. Apparently, serum HER2/ECD is an independent breast cancer risk factor, and serum positivity cannot be used as a measure of HER2 positivity. Its correlation with hormone receptor status should be assessed in metastatic breast cancer patients using biopsies, since it may have therapeutic implications. It is necessary to elucidate the interconnection between hormone receptors and HER2 receptor signaling, since similar concordance like that found in our study has been shown by other authors as well [44]. Further evaluation of prognostic significance of HER2/ECD in early breast cancer is necessary, but a cut-off value of 7.7 ng/ml in patients with stage I and II disease should be used. Further studies are warranted.

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Conflict of interest

Authors declare no financial relationship with the organization that sponsored the research.

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