IS THERE A CORRELATION BETWEEN HER-2/NEU STATUS AND GRADE OF THE PRIMARY BREAST CANCER?

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SUMMARY – HER-2/neu amplification is a marker of poor prognosis in breast cancer. Patients whose tumors show overexpression of HER-2/neu have shortened disease-free and overall survival. The aim of this study was to determinate immunohistochemistry HER-2/neu overexpression in 121 cases of primary breast cancer. In addition HER-2/neu status was correlated with hormone receptor status, grades and pTNM stage. The both groups of HER-2/neu negative and HER-2/neu positive patients were average ages of about 60 years. In all the HER-2/neu groups (negative, 1+, 2+ and 3+) estrogen and progesterone receptor were highly positive in a high percentage of cases. Also in both groups of HER-2/neu negative and HER-2/neu positive invasive ductal carcinoma the most common grades were 2, but in HER-2/neu 3+ group, there were also 30.0% of cases having grade 3. Our results show that there might be a correlation between HER-2/neu overexpression and higher grade of breast cancer. The two most common pTNM stages were T1N0MX and T2N1MX. HER-2/neu receptor is important therapeutic target and testing for HER-2/neu status is now recommended as a part of routine breast cancer diagnosis. The role of new biomarkers, such as HER2/neu and clinical value of its determination must be provided by prospective clinical studies.

Key words: *breast cancer, HER-2/neu, prognostic value*

Introduction

The established prognostic factors currently used in cases of primary breast cancer include axillary lymph node involvement, histologic subtype, tumor size, nuclear or histologic grade, estrogen and progesterone receptor status and proliferative index.Amplification of HER-2/neu with overexpression of the p185 HER2 receptor occurs in 20%-30% of breast cancers and has been established as an independent prognostic factor in numerous studies.[1, 2] Increasing evidence suggests that HER2 may be a predictive marker for response to chemotherapy and hormonal therapy.[3, 4] HER2 overexpression has provided a new target in breast cancer therapy, as evidenced by the development of trastuzumab (Herceptin(R)), a monoclonal antibody targeted against HER2. Detection of HER2 in the clinical setting is performed by immunohistochemistry or fluorescence in situ hybridization in tissue, and by detection of the shed extracellular domain in serum or plasma.[5, 6] HER-2/neu positive status may predict the likelihood of resistance or sensitivity to some conventional anticancer therapies. The high incidence of HER-2/neu overexpression on the cell surface of breast cancer cells and the recognized prognostic and potentially predictive value of HER-2/neu receptor represent a novel and important therapeutic target.[7]

Patients and methods

The research was done on 121 cases (43.7%) out of a total of 277 patients in whom breast cancer was diagnosed as the result of biopsies analyzed at the Ljudevit Jurak University Department of Pathology during the period

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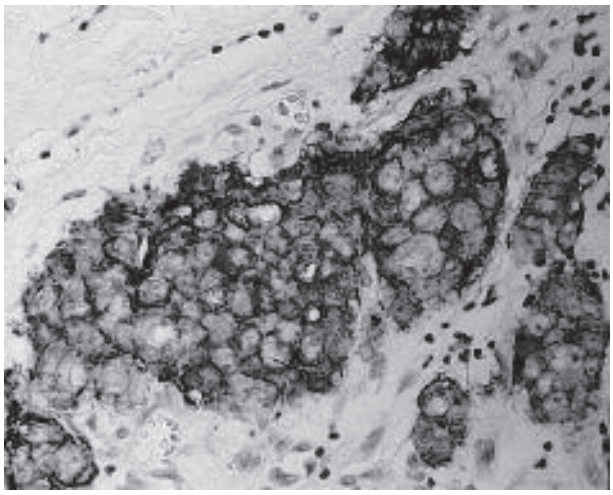

Figure 1. HER-2/neu 3+ invasive ductal carcinoma

from May 1st, 2000. to February 28th, 2003. Formalin fixed and paraffin embedded tumor tissues were cut into five mm sections, deparaffinized and stained with hematoxylin end eosin. Immunohistochemistry for estrogen (ER), progesterone (PgR) and HER-2/neu (DAKO, Copenhagen, Denmark; donated by La Roche Ltd) was performed on 121 (43.7%) cases (Figure 1). The tissue sections were subjected to antigen retrieval in microwave 2x5 min in citrate buffer solution pH 6,0 - Chem Mate Buffer for Antigen Retrival diluted 1:10. At the termination of the antigen retrieval step slides were left in the buffer for at least 20 minutes at room temperature. Slides were stained immunohistochemically by labeled streptavidin biotin method (LSAB) as visualization system on Dako Tech Mate automatic immunostainer using microwave streptavidin immunoperoxidase-MSIP protocol. Scoring for estrogen and progesterone receptor was done counting positive tumor cells in "hot spot" and grading as negative (<5% positive tumor cells), 1+ (5-10% positive tumor cells), 2+ (10-50% positive tumor cells) and 3+ (>50% positive tumor cells). Scoring for HER-2/neu was done using Guidelines for scoring HercepTest, Dako. Other information related to ER and PgR were received from Laboratory for Hormonal Receptors and Radiobiochemistry. In 94 cases (77.7%) from a total of 121 cases ER and PgR were attained by the common method of saturation analysis (Dextran Coated Charcoal; DCC). The concentrations of ER and PgR, determined by this method, were then grouped into three categories according to the probability of tumor-response to hormonal therapy. Concentrations lower then 25 fmol/mg are considered to have a low tumor-response, concentrations between 25-100 fmol/mg a mild tumor-response and concentrations above 100 a high tumor-response. Grading of the invasive ductal breast cancer was done using Scarff-Bloom-Richardson score system.[8]

Results

Immunohistochemistry were performed on 121 patients of whom 119 were female and 2 were male. One was a 71 years old man with immunohistochemicaly positive ER 3+, PgR 3+ and HER-2/neu 1+, with T1N0MX and grade 2. The other was 47 years old man, also with immunohistochemicaly positive ER3+, PgR3+, but in this case HER-2/neu was 0 score. The concentration of ER determined through DCC analysis was categorized as mild tumor-response, whereas the concentration of PgR was categorized as high tumor-response. pTNM stage was T3N1MX and grade was 2.

From a total of 119 female patients who were randomly chosen for HER-2/neu testing 115 had invasive ductal carcinoma. Of these 109 (91.6%) were NOS, 3 (2.5%) were mucinous carcinoma and there was also 1 (0.8%) medullary carcinoma, 1 (0.8%) papillary carcinoma and 1 (0.8%) inflammatory carcinoma. Both cases of male breast cancer were verified as invasive ductal carcinoma. Invasive lobular carcinoma was found in 4 cases (3.4%), (Table 1). The results of HER-2/neu testing in female patients with ductal carcinoma were: of 115 cases 81 (70.4%) have HER-2/neu score 0-negative and 16 cases (13.9%) have 1+ score which is also considered as negative overexpression. Positive overexpression were found in 18 patient (15.7%): 8 cases, or 7.0% were 2+ and 10 cases, or 8.7% were 3+. The testing in patients with lobular carcinoma HER-2/neu

Table 1. Sex distribution and histological subtypes of analyzed breast cancer

	Female		Male	
	Number	%	Number	%
Ductal carcinoma: NOS	109	91,6%	2	100,0%
mucinous	3	2,5%	0	0,0%
medullary	1	0,8%	0	0,0%
papillary	1	0,8%	0	0,0%
inflammatory	1	0,8%	0	0,0%
Total	115	96,6%	2	100,0%
Lobular carcinoma	4	3,4%	0	100,0%
Total	119	100,0%	2	100,0%

Table 2. Distribution of HER-2/neu status in analyzed breast cancer

Her-2/neu-score	Number of cases	%
0	84	70.1%
1+	18	14.5%
2+	9	6.8%
3+	10	8.6%
Total	121	100.0%

showed that from a total of 4 cases 3 were negative and 1 was positive, (Table 2).

The average age of HER-2/neu negative female patients with invasive ductal carcinoma was 60.7 years with a standard deviation of ±12.9 years. ER and PgR receptors, verified by immunohistochemistry were highly positive (3+) in most cases (ER: 44 cases, or 55.0% and PgR: 40 cases, or 50.0%), (Figure 2). Analyzing ER and PgR results determined by DCC testing in majority of cases (19 cases or 31.1%) ER was positive with concentrations belonging to the mild tumor-response category. The average concentration of ER was 100.5 fmol/mg. PgR in the majority of cases were negative (29 cases, or 47.5%). The average concentration of PgR was 41 fmol/mg. In the most cases was found grade 2 (60 case, or 78.9%), (Figure 3), and the most common pTNM stages were: T1N1MX (18 cases, or 23.4%), T2N1MX (17 cases, or 22.1%) and T1N0MX (16 cases, or 20.8 %).

Average age and the of HER-2/neu 1+ positive patients was 66.4±12.8 years. Immunohistochemicaly ER was 3+ positive in 12 cases (75.0%) and PgR was 3+ positive in 11 cases (68.8%), (Figure 2). DCC analyzed ER showed mild tumor-response in most cases (5 cases, or 33.3%). The average concentration of ER was 70.7 fmol/mg. PgR was on the other hand negative in most cases (8 cases, or 53.3%). The average concentration of PgR was 30.4 fmol/mg. In the majority of cases the grade 2 was found (14 cases, or 87.4%), (Figure 3). The most common pTNM stages were: T1N0MX (4 cases, or 25.0%), T1N1MX and T2N1MX (both with 3 cases, or 18.8%).

The majority of HER-2/neu 2+ patients, average age 58.9±10.9 years, were immunohistochemicaly ER and PgR 3+ positive, (Figure 2). There were 4 cases (50.0%) for both ER 3+ and PgR 3+. Using DCC analysis it was found that both ER and PgR were negative in the majority of cases (both negative in 4 cases, or 57.2%). The average concentration of ER was 20.9 fmol/mg and for PgR 53.6 fmol/mg. Again the most common grade was 2, (Figure 3), and the two most common pTNM stages being: T1N0MX (4 cases, or 50.0%) and T2N1MX (2 cases, or 25.0%).

Finally, in HER-2/neu 3+ positive patients, average age 58,8±7.9 years, ER was 3+ positive in the majority of cases (5 cases, or 50.0%), but unlike the above findings PgR was in most cases negative (4 cases, or 40.0%), (Figure 2). Again utilizing DCC analysis both ER and PgR were found to be negative in the majority of cases (ER: 3 cases, or 42.8%, PgR: 4 cases, or 57.1%). The average concentration of ER was 29.1 fmol/mg and 12.3 fmol/mg for PgR. The grade was 2 in most cases, but there were also 30.0% of cases having grade 3, (Figure 3). The most common pTNM stage was T1N1MX (6 cases, or 60.0%).

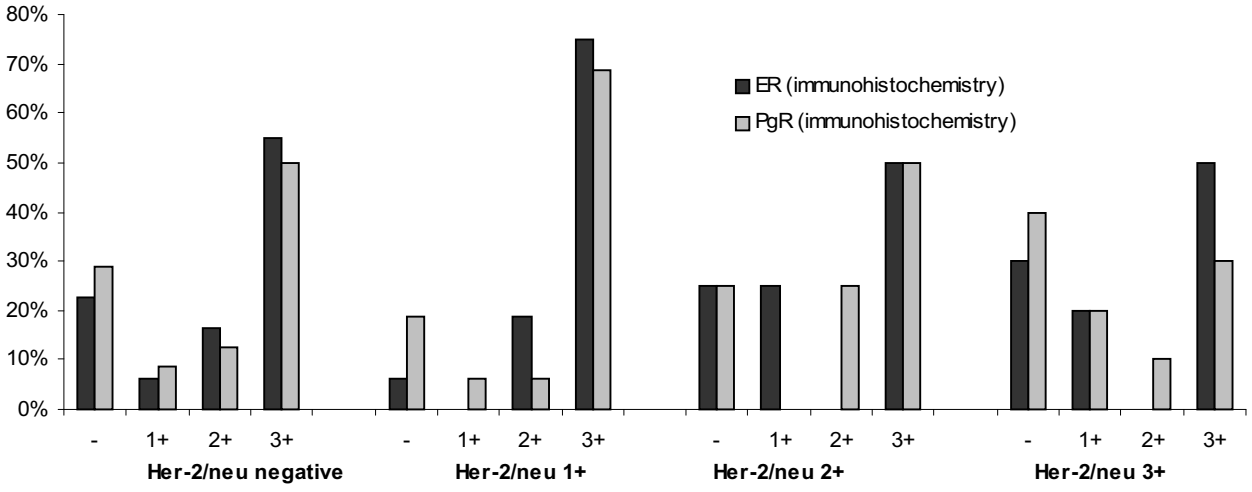

Figure 2. Correlation between HER-2/neu status and hormone receptor status determinated by immunohistochemistry

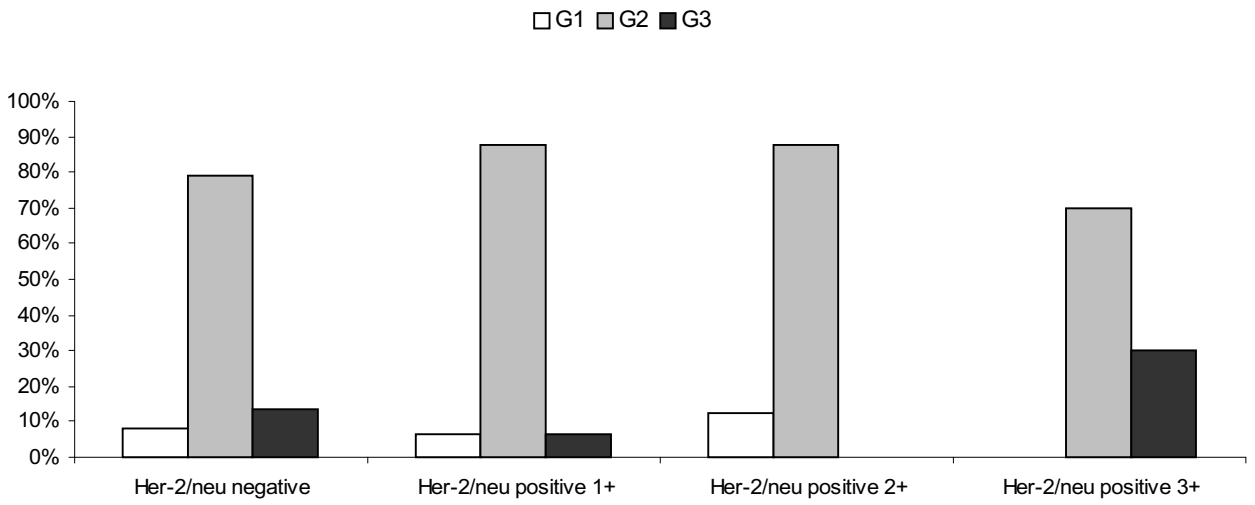

Figure 3. Correlation between HER-2/neu status and grade of the breast cancer

Discussion and conclusion

Immunohistochemistry used in 121 cases of primary breast cancer revealed that both groups of HER-2/neu negative and HER-2/neu positive patients were average ages of about 60 years which coincides with data from other literature (61 years).[9,10] A slight difference was found only with HER-2/neu 1+ patients whose average age was 66.4 years. Summarizing the results in terms of relationship between HER-2/neu positivety or negativity with ER and PgR positivity and negativity, in all the HER-2/neu groups (negative, 1+, 2+ and 3+), ER and PgR were found highly positive, in a high percentage of cases. The only findings that stand out was in the HER-2/neu 3+ group of patients, where PgR was negative in 40% of cases (the majority). In all analyzed cases the most common grade was 2, but in HER-2/neu 3+ group, there were also 30.0% of cases having grade 3. Our results show that there might be a correlation between HER-2/neu overexpression and higher grade of breast cancer, which coincides with findings of other authors.[11] The two most common pTNM stages were T1N0MX and T2N1MX.

Because of its biological heterogeneity and wide spectrum of responsiveness to different treatments, breast cancer is a complex disease with difficult clinical management. Axillary nodal status, age, tumor size, pathologic grade, and hormone receptor status are the established prognostic and/or predictive factors for selection of adjuvant treatments.[12,13] Evaluation of HER-2/neu status in combination with other prognostic markers enhances the accuracy and power of the predictive prognosis and should help to more clearly define the most appropriate treatment for each individual patient.[14,15] The role of new biomarkers, such as p53, HER2/neu, angiogenesis, and the proliferation index value, is promising; however, the clinical value of their determination must be provided by prospective clinical studies.[16]

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Sažetak

DA LI POSTOJI POVEZANOST IZMEĐU HER-2/NEU ST ATUSA I GRADUSA PRIMARNIH TUMORA DOJKE?

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HER-2/neu onkogen u tumorskim stanicama pokazatelj je loše prognoze karcinoma dojk e. Pacijenti u kojih HercepT est daje jako pozitivne vrijednosti (3+) statistički imaju kraće vrijeme bez pojave bolesti i općenito kraće preživljenje. Cilj ovog istraživanja bio je utvrditi vrijednosti HER-2/neu proteina u 121 bolesnika s primarnim karcinomom dojk e uz pomoć imunohistok emijske analize HercepTest-om. Nadalje su rezultati dobiveni HercepT est-om uspoređivani s vrijednostima estrogenskih i progesteronskih receptora, gradusom i pTNM stadijem tumora. U sk upini pacijenata s negativnim, kao i u sk upini s pozitivnim vrijednostima HercepTest-a prosječna starost bolesnika kretala se oko 60 godina. U svim grupama pacijenata (grupa HER-2/neu negativni, HER-2/neu 1+, HER-2/neu 2+ te HER-2/neu 3+) imunohistok emijski određeni estrogenski i progesteronski receptori bili su pozitivni u visokom postotk u. Najčešće nađeni gradus invazivnog duktalnog karcinoma bio je 2, ali je osim toga u grupi HER-2/neu 3+ karcinoma dojk e 30.0% karcinoma bilo gradusa 3. R ezultati pokazuju mogućnost korelacije između 3+ pozitivnog HercepTest-a i višeg gradusa tumora. P acijentima je bolest dijegnosticirana najčešće u stadiju T1N0MX i T2N1MX. HER-2/neu receptor važan je terapijski cilj u liječenju pacijenata s karcinomom dojk e te bi HercepTest trebao postati dio rutinsk e dijagnostik e karcinoma dojk e. Ulogu otkrivanja novih biomark era, kao što je HER-2/neu, te njihovu kliničk u važnost valja utvrditi prospektivnim kliničkim studijama.

Ključne riječi: *rak dojke, HER-2/neu, prognoza*

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