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ORIGINAL ARTICLE

The significance of HER-2 amplification and the size and type of pathological unicentric, initially operable clinical stage I and IIA/IIB breast cancer, in determining the treatment strategy

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Summary

Purpose: *In order to determine the initial treatment strategies for primary operable unicentric breast cancer, the possible relationships of the amplification of human epidermal growth-factor receptor-2 (HER-2), with age, menstrual status, tumor pathological size (pT), histopathological tumor type (HP) and kind of surgical treatment were studied.*

Methods: *Analysed were 301 patients treated initially by surgery in the period 2006-2009. HP tumor type, pT and HER-2 status (using firstly immunohistochemistry and then chromogenic in situ hybridization/CISH) were determined. The patients were divided into 2 subgroups according to the presence ($CISH^+$)/absence ($CISH^-$) of HER-2 amplification.*

Results: *Data on pT and HER-2 analyses were available for 293/301 (98.3%) patients with ductal (DC) and lobular carcinoma (LC). Amplification of HER-2 was found in 66 (21.9%) patients. No significant difference between the two subgroups regarding age ($p=0.08$), menstrual status ($p>0.05$) and kind of operation ($p>0.05$) was found. HP showed statistically significant difference between DC (55; 83.3%) and LC (11; 16.7%) patients with HER-2 amplification ($p<0.01$). Further HP analysis of the type of cancer within the pT category as a subgroup showed significantly higher frequency of HER-2 amplification in DC patients for pT1 ($p<0.01$) and in pT2 + pT3pN0 ($p<0.05$) compared with patients with LC.*

Conclusion: *This study showed a significantly higher incidence of HER-2 amplification in DC tumors, especially in pT1 and pT2, than in LC, which may influence the options in treatment strategies in primary unicentric operable DC type of breast cancer.*

Key words: breast, carcinoma, ductal, HER-2, lobular, size

Introduction

Breast cancer is the most common malignancy in women [1]. In all breast cancer (clinical stages I-IV), 15-30% have overexpression (amplification) of HER-2 [2-11]. HER-2 belongs to the family of epidermal growth factor receptors (EGFR). It is often referred to as neu or erbB2 or erbB2 HER-2/neu, since it was first discovered in laboratory mice that developed neuroblastoma.

HER-2 is identified by immunohistochemistry (ICH) (which is less precise), FISH (fluorescent *in situ* hybridization), or CISH methods.

In primary operable breast cancer the current trend is to perform less aggressive surgery of the affected breast with sentinel node biopsy, if the margins of the resected tumor are not infiltrated by malignant cells [12,13]. Homolateral axillary lymph node dissection is dependent on the sentinel node biopsy result [12-14].

HER-2 positivity is a predictive factor indicating aggressive disease course, worse prognosis and shorter disease-free and overall survival. The 9th (2005) St. Gallen International Consensus Conference on primary therapy of early breast cancer defined HER-2 positivity as a new category in the assessment of risk of recurrence

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[6-11]. Therefore, we undertook this research with the aim to determine initial treatment strategies for primary operable unicentric breast cancer by examining the relationship of HER-2 amplification, using the CISH method and the patient and tumor characteristics.

Methods

Patients

This research was conducted on 301 women with carcinoma of the breast, clinical stage I and II of DC or LC histology, who were surgically treated between 2006-2009 at the Institute for Oncology and Radiology of Serbia, Belgrade. The preoperative clinical examinations included mammography, breast and liver ultrasound, lung radiography and basic serum biochemical laboratory tests. In some cases, additional tests such as magnetic resonance imaging of the breasts, bone scintigraphy, X-ray skeletal survey and CEA, CA 15-3 and Ki-67 determinations were carried out.

Methods

Patients and disease characteristics analysis included age, menstrual status, type of surgical intervention, HER-2 determination, HP tumor type and pT. The amplification of HER-2 was carried out using CISH [15,16]. For the purposes of this study, 2 groups were compared: a group with HER-2 CISH amplification ($CISH^+$) and without CISH amplification ($CISH^-$).

Statistical analyses

Statistical analyses included testing of sample distribution for normality (graphs: Normal Q-Q Plot and Histogram; tests: Kolmogorov-Smirnov and Shapiro-Wilk), parameters description (the measures of descriptive statistics: frequencies, percentages, mean, median, standard deviation [SD]) and range) and testing the differences between the parameters studied (tests: Pearson's x^2 test, Fisher's exact test and Wilcoxon rank sum test with continuity correction). The level of statistical significance was $p< 0.05$.

Data analysis was performed in the statistical program R version 2.13.1 (2011-07-08)

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Results

General patient characteristics, disease characteristics and surgical treatment techniques are shown in Tables 1 and 2.

pT information was lacking in 5/301 (1.7%) patients, because accidental surgical cut of the tumor due to its very small size (<0.5 cm). CISH analysis in 3/301 (1.0%) patients was not possible because of lack of material for testing.

There was no significant difference between HER-2 $CISH^+$ and $CISH^-$ regarding the patient and tumor characteristics and the kind of surgical treatment, while DC was significantly more frequent in the HER-2 $CISH^+$ group (Tables 3 and 4).

Table 1. Patient characteristics

Characteristics	*N (%)*
Age (years)	
Mean (±SD)	55.9 (±12.5)
Median (range)	56 (24-83)
Menstrual status	
Premenopausal	79 (26.2)
Perimenopausal	21 (7.0)
Postmenopausal	201 (66.8)
Total	301 (100)

SD: standard deviation

Table 2. Characteristics of the disease and the surgical treatment

Characteristics	*N (%)*
Type of operation	
Sparing*	150 (49.8)
Radical	151 (50.2)
HP findings	
Ductal	183 (60.8)
Lobular	118 (39.2)
Pathological T stage	
pT1	171 (56.8)
pT2	121 (40.2)
pT3	4 (1.3)
No data	5 (1.7)
HER-2 CISH	
Positive	232 (77.1)
Negative	66 (21.9)
No data	3 (1.0)

*with dissection of the homolateral axillary lymph nodes
HP: histopathology, CISH: chromogenic *in situ* hybridization

This result prompted for further analysis of the HP type (DC vs. LC) in the pT category, the results of which are shown in Table 5. Data on pT and CISH had 293/301 (98.3%) patients (178/183; 97.3% with DC and 115/115; 100% with LC).

$CISH^+$ was significantly more frequent in DC in pT1 and pT2 categories, while in the pT3 category this was not confirmed, perhaps due to the small number of patients (Table 5).

Also, $CISH^+$ was significantly more frequent in DC than LC in both the pT1 category (Pearson x^2 test; $x^2_1=12.16$, $p=4.9\cdot10^{-4}$) and pT2+pT3 category (Fisher's Exact test, p=0.019) (Figure 1).

Discussion

In our study, the amplification of HER-2 ($CISH^+$) was seen in 21.9% of the patients. This is in accordance

Table 3. Patient characteristics and HER-2 CISH categories

Characteristics	*HER-2 CISH$^-$ N (%)*	*HER-2 CISH$^+$ N (%)*	*Test*
Age (years)			
Mean (±SD)	56.5 (±12.4)	53.1 (±12.1)	Wilcoxon rank sum*
Median (range)	57 (30-83)	53 (24-80)	W=8737; p=0.08
Menstrual status			
Premenopausal	60 (25.9)	19 (28.8)	Fisher's exact test
Perimenopausal	18 (7.7)	3 (4.5)	p=0.68
Postmenopausal	154 (66.4)	44 (66.7)	
Total	232 (100.0)	66 (100.0)	–

*Wilcoxon Rank Sum Test with continuity correction
CISH: chromogenic *in situ* hybridization, SD: standard deviation

Table 4. Tumor and the surgical treatment characteristics and HER-2 CISH categories

Characteristics	*HER-2 CISH$^-$ N (%)*	*HER-2 CISH$^+$ N (%)*	*Test*
Type of operation			
Sparing*	116 (50.0)	31 (47.0)	Pearson's x^2 test
Radical	116 (50.0)	35 (53.0)	x^2_1=0.19; p=0.66
HP findings			
Ductal	125 (53.9)	55 (83.3)	Pearson's x^2 test
Lobular	107 (46.1)	11 (16.7)	x^2_1=18.64; p=2·10^{-5}
Pathological T stage	129 (55.6)	39 (59.1)	
pT2	99 (42.6)	22 (33.3)	Fisher's exact test
pT3	2 (0.9)	2 (3.0)	p=0.17
No data	2 (0.9)	3 (4.6)	
Total	232 (100)	66 (100)	–

*with dissection of the homolateral axillary lymph nodes
HP: histopathology

with other studies, in which this percentage ranged from 15-30% for all clinical stages [2-11]. These findings indicate more aggressive disease and poorer prognosis [2-11,17-21].

In locally advanced breast cancer, the neoadjuvant systemic approach is the standard treatment modality [22,23]. Since HER-2 overexpression is a risk factor in the prognosis of breast cancer, some articles

Table 5. HER-2 CISH categories in relation to the histopathological type and pT categories

Characteristics	*DC N (%)*	*LC N (%)*	*Test*
pT1	102 (100)	66 (100)	Pearson's x^2 test
HER-2 CISH$^-$	69 (67.6)	60 (90.9)	x^2_1=12.16
HER-2 CISH$^+$	33 (32.4)	6 (9.1)	p=4.9·10^{-4}
pT2	73 (100)	48 (100)	Fisher's exact test
HER-2 CISH$^-$	55 (75.3)	44 (91.7)	p=0.0294
HER-2 CISH$^+$	18 (24.7)	4 (8.3)	
pT3	3 (100)	1 (100)	Fisher's exact test
HER-2 CISH	1 (33.3)	1 (100)	p=1
HER-2 CISH$^+$	2 (66.7)	–	
Total	178 (100)	115 (100)	–

DC: ductal carcinoma, LC: lobular carcinoma, CISH: chromogenic *in situ* hybridization

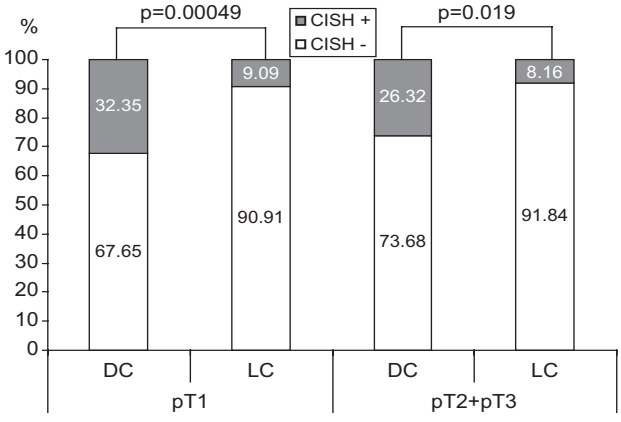

Figure 1. HER-2 CISH categories in relation to the histopathological type and pT categories. DC: ductal carcinoma, LC: lobular carcinoma, CISH: chromogenic *in situ* hybridization.

advocate the use of trastuzumab in the neoadjuvant setting [19,24].

Multidisciplinary approaches in the treatment of breast cancer show an extraordinary dynamism and changes in the treatment protocols, especially since the mid-1970s [25,26]. With the implementation of adequate chemotherapy, hormonotherapy and radiation therapy, disease-free interval was no longer depending on the type of surgical treatment. Breast-conserving surgery in clinical stages I and II gave the same therapeutic results compared with more extended operations and, therefore, led to much lesser permanent deformity, loss of body patterns and psychosocial consequences of treatment [27]. In the last 20 years, breast-conserving surgery and resection with free margins, which initially ranged from 2-5 cm, have been changed. Now, the appropriate operation is the one in which the free margins are reduced to 1-2 mm, a step forward in the therapy of early breast cancer [14].

Breast-conserving surgery in early breast cancer has long been the standard treatment modality [7,8,10, 11,14,23,26]. The neoadjuvant approach depends on the standard histological analysis. For a long time now, there is a possibility of preoperative diagnosis of the histological tumor type with core biopsy, and determination of steroid content and HER-2 status. However, some authors suggest that even in low clinical stages (I and II A and B), the initial preoperative neoadjuvant therapy should be initiated using chemotherapy and the monoclonal anti-HER-2 antibody trastuzumab. This approach does not exclude the postoperative systemic chemotherapy [13,19,24].

We showed a significantly higher incidence of HER-2 amplification in DC, especially in pT1 and pT2 tumors, compared with LC. The different surgical manipulations of breast-conserving operation may pose hazards for local dissemination. Addition of trastuzumab in the systemic neoadjuvant approach of initially operable, MRI-confirmed unicentric early DC could reduce the risk of local dissemination during the operation, as reported earlier [28].

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