Original Article

Human epidermal growth factor receptor 2 status in breast cancer: A comparison between borderline positive human epidermal growth factor receptor 2 and strongly positive human epidermal growth factor receptor 2 tumors

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ABSTRACT

Background: Human epidermal growth factor receptor 2 (*HER2*) status is an important biomarker and a molecular target for specific therapies such as humanized monoclonal antibody – trastuzumab. The aim of this study was to compare a group of the borderline positive *HER2* (++) status patients with a strongly positive *HER2* (+++) status group, according to clinicopathological features, cardiotoxicity, and treatment response. **Materials and Methods:** The analysis included medical records of 166 early and metastatic breast cancer patients treated with trastuzumab. **Results:** There were no significant differences between both groups in relation to patients' age at initial diagnosis and comorbid conditions; however, diabetes (4%) were observed only in tumors with strong *HER2* overexpression. Patients with *HER2* (+++) more frequently had a history of cigarette smoking in comparison with *HER2* borderline women (39% vs. 25%, $P = 0.06$). There was no association between overweight and *HER2* status. No statistically significant differences in steroid receptor status were detected between *HER* (++) and *HER2* (+++) positive tumors ($P = 1.00$). Borderline tumors were in earlier stage of disease (50% vs. 17%, $P = 0.002$). Lymph node metastases correlate with strongly positive breast cancer (58% vs. 33%, $P = 0.04$). Second neoplasm also more often developed in strongly positive *HER2* tumors and contralateral breast cancer in borderline. **Conclusion:** In the summary, borderline positive *HER2* breast cancer patients are in earlier advance stage and have a better outcome than strong positive *HER2* tumors. They are less predisposed to the development of cardiac side effects. Type 2 diabetes coexisted with strong *HER2* overexpression.

Key words: Breast cancer, human epidermal growth factor receptor 2 borderline overexpression, human epidermal growth factor receptor 2 strong overexpression

INTRODUCTION

The human epidermal growth factor receptor 2 (*HER2*)/neu (c-erbB-2) gene is localized to chromosome 17q and encodes a transmembrane tyrosine kinase receptor protein - a member of the epidermal growth factor receptor family. *HER2*/neu gene amplification and/or protein overexpression occur in 10–34% of invasive breast cancer tumors.[1-3] It is strongly associated with other prognostic factors such as a high proliferative index, a histopathological type, a negative steroid receptor status, and a presence of metastases in the lymph nodes.[4]

In recent years, *HER2* status has gained importance as an important biomarker and a molecular target for specific therapies such as humanized monoclonal antibody – trastuzumab that binds to the extracellular domain of the HER2 protein.[5,6] Food and Drug Administration and ASCO recommend either using

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immunohistochemistry (IHC) assays for initial evaluation of *HER2* status followed by reflex testing by fluorescent *in situ* hybridization (FISH) for some IHC categories (i.e., 2+) or using FISH in initial testing.[7] Patients with strong overexpression examine in IHC 3+ or positive FISH staining are qualified for trastuzumab therapy. The National Surgical Adjuvant Breast and Bowel Project B-31 trial and the North Central Cancer Treatment Group N9831 trial showed that adding trastuzumab to adjuvant chemotherapy improved disease-free survival and overall survival (OS) in women with early-stage *HER2*-positive breast cancer.[8] Trastuzumab improves also the outcome in patients with *HER2*-positive metastatic breast cancer.[9] New therapy directed at *HER2* – pertuzumab (a humanized monoclonal antibody that binds *HER2* at a different epitope of the *HER2* extracellular domain than that at which trastuzumab binds) in combination with trastuzumab and docetaxel significantly prolonged progression-free survival.[10]

As for now, authors have not found articles presenting the correlation between an intensity of *HER2* staining and the clinicopathological features. The aim of this study was to compare a group of borderline positive *HER2* (++) status patients with a strongly positive *HER2* (+++) status group, according to clinicopathological features (age, menopausal status, comorbid conditions, body mass index, history of smoking, steroid receptor status, tumor size, contralateral breast cancer, history of second neoplasm), cardiotoxicity, and treatment response. Authors also analyzed association between OS and *HER2* overexpression (borderline or strong overexpression).

MATERIALS AND METHODS

This retrospective study was conducted on medical records of *HER2*-positive breast cancer patients treated and followed-up in Clinical and Experimental Oncology Department, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology in Gliwice Branch in Poland. The analysis was performed according to national law regulation and included 166 early and metastatic breast cancer patients treated with trastuzumab between years 2006 and 2012. All patients were women. No ethical issues needed to be addressed as the study was retrospective.

Human epidermal growth factor receptor 2 overexpression was assessed using an IHC method in postoperative specimens or samples obtained by thick needle biopsy. *HER2* gene amplification was additionally assessed by FISH. IHC expression was scored as follows: 0, no staining or faint membrane staining; 1+, faint membrane staining in >10% of tumor cells, incomplete membrane staining; 2+, weak to moderate membrane staining in >10% of tumor cells; and 3+, intense circumferential membrane staining in >10% of tumor cells [Figures 1-3]. *HER2* scores of 0 and 1+ were considered negative. *HER2* IHC 3+ and FISH – tumors with amplification were considered positive. All IHC

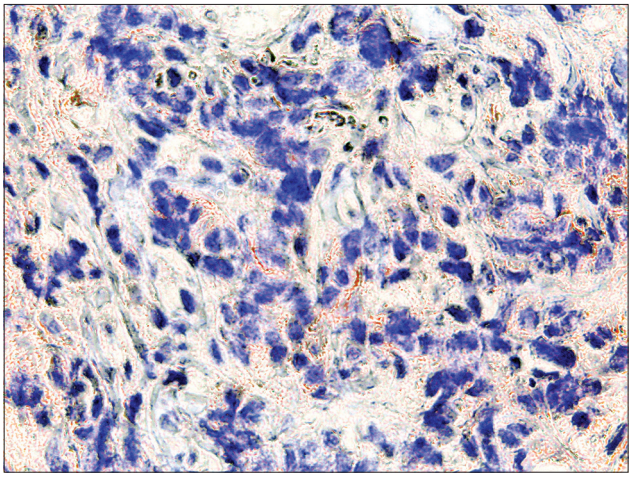


Figure 1: Immunohistochemical findings. No staining

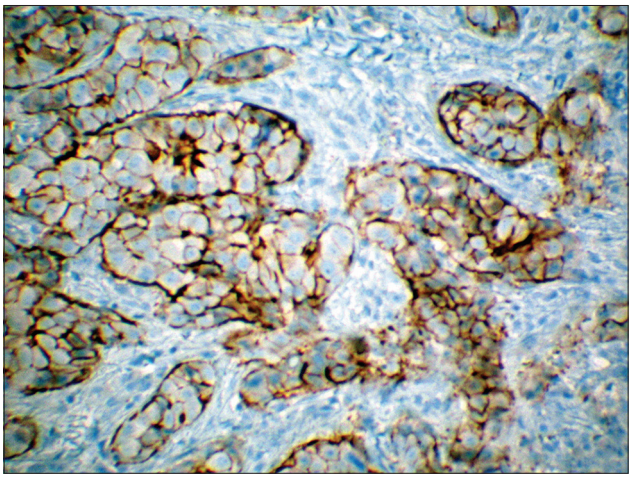


Figure 2: Immunohistochemical findings. Borderline positive human epidermal growth factor receptor 2 (++) staining

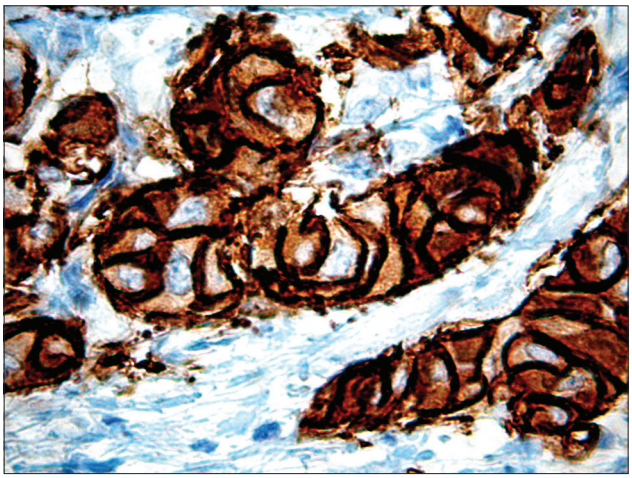


Figure 3: Immunohistochemical findings. Strongly positive human epidermal growth factor receptor 2 (+++) staining

2+ tumors and indeterminate tumors were tested for gene amplification by FISH.

The data including the age at onset, menopausal status, disease stage according to tumor node metastasis classification, surgical procedures, histology, estrogen and progesterone receptor (ER and PR) status, *HER2* status and contralateral breast cancer, were gathered from hospital records and pathology reports.

All patients were diagnosed and treated according to the same protocol. Echocardiography was performed every 3 months during trastuzumab therapy and also before and after the anthracyclines. Cardiac side effects were assessed in the New York Heart Association classification and in the CTCAE scale (version 4.0). The earliest manifestations of myocardial damage diagnosed by echocardiography were left ventricular ejection fraction (LVEF) decrease, abnormalities of right ventricular contractility, ventricular dilation, and abnormalities of left ventricular contractility.

Statistical analysis was carried out using STATISTICA 7 software (StatSoft Polska sp. zoo. 30-110 Kraków ul. Kraszewskiego 36). The frequency of side effects appearance was counted. The qualitative features were presented as the percentage of their occurrence and evaluated with Fisher's test. Survival evaluation was performed using the Kaplan–Meier estimator with log-rank test. Differences were considered as significant if the $P \leq 0.05$.

Table I: Patients characteristics

N	HER ++18 (11%)	HER +++148 (89%)	*P*
Age (from 24 to 76 years) median 54 years			
<65	17 (94)	133 (90)	0.459
>65	1 (6)	15 (10)	
Menopausal status			
Postmenopausal	13 (76)	101 (68)	0.347
Premenopausal	4 (24)	47 (32)	
Clinical staging			
I	9 (50)	25 (17)	0.002
II	5 (28)	65 (44)	
III	3 (17)	53 (35)	
IV	1 (6)	6 (4)	
Clinical staging nodes			
N0	12 (67)	62 (42)	0.04
N1-3	6 (33)	86 (58)	
Negative steroid receptor status			
Yes	9 (53)	67 (46)	1.00
No	8 (47)	80 (54)	
History of cigarette smoking			
Yes	4 (25)	57 (39)	0.06
No	14 (75)	91 (62)	
Breast cancer in family history			
Yes	3 (19)	17 (12)	0.309
No	13 (81)	130 (88)	
Contralateral breast cancer			
Yes	3 (17)	3 (2)	0.015
No	14 (83)	145 (98)	
Comorbid condition-diabetes			
Yes	0	6 (4)	0.496
No	18 (100)	142 (96)	
Comorbid condition-hypertension			
Yes	5 (28)	32 (22)	0.3690
No	13 (72)	116 (78)	

HER: Human epidermal growth factor receptor

RESULTS

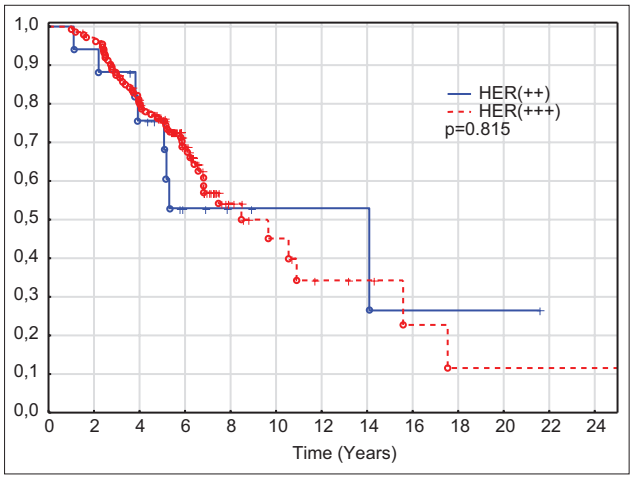
The analysis included a group of 166 *HER2*-positive breast cancer patients treated with trastuzumab. 108 (65%) of them received immunotherapy as adjuvant therapy and remaining 58 (35%) for metastatic disease. Median age of all women was 54 years (range from 24 to 76). 114 (69%) patients were postmenopausal. Baseline characteristics are shown in Table 1.

In the analyzed group, 18 (11%) tumors were borderline *HER2*-positive (*HER*++) tumors. In all tumors with borderline type gene amplification was assessed. There was no correlation between type of treatment (adjuvant therapy or treatment due to metastatic disease) and *HER2* overexpression ($P = 0.447$).

The results of this study demonstrated that all borderline tumors were no special type breast cancer by the WHO classification. No differences were detected between the both groups (*HER2* borderline and *HER2* strong overexpression) in menopausal status (76% vs. 68%) and patients age >65 years (6% vs. 10%) ($P = 0.347$) and ($P = 0.459$), respectively. Patients with *HER2* overexpression more frequently had a history of cigarette smoking in comparison with *HER2* baseline women (39% vs. 25%, $P = 0.06$). There was no association between hypertension and overweight, and *HER2* overexpression.

No differences were detected between the both groups in positive steroid receptor status (ER+/PR+) (47% vs. 46%, $P = 1.00$). The comparison between *HER* (++) and *HER* (+++) has shown that *HER* (++) tumors were in earlier stage of disease (50% vs. 17%, $P = 0.002$). Tumor size more than T2 was detected more often in *HER*+++ patients (37% s. 22%). Lymph node metastases were more frequently observed in strongly positive breast cancer (*HER*+++) (58% vs. 33%, $P = 0.04$). Distant metastases were only observed in strongly positive *HER2 patient*s (8%). Skin metastases also were more often detected in this group (14% vs. 6%).

Contralateral breast cancer occurred more often in borderline positive *HER2* (++) status patients in comparison with *HER2* strongly overexpression group (17% vs. 2%, $P = 0.015$). In contrary, the presence of second neoplasms such as thyroid cancer (1%), ovarian (1%), cervical cancer (1%), and basal cell carcinoma (1%) was observed only in strongly positive *HER2* tumors (4%, $P = 0.236$). Disease recurrence was more frequently observed in women with *HER2* overexpression



Graph 1: Correlation between human epidermal growth factor receptor 2 overexpression and overall survival

(72% vs. 36%, *P* = 0.006). Disease progression also occurred more often in this group of patients (57% vs. 27%, *P* = 0.02).

In our analysis, there was observed no association between OS and *HER2* overexpression (*HER2* borderline and *HER2* strong overexpression) (*P* = 0.815) [Graph 1].

The decrease of LVEF and cardiac side effects occurred at the same rate in both groups (6% vs. 5%). Acute cardiac side effects were presented only in strongly positive *HER2* (+++) status group (2%) *P* = 0.953.

DISCUSSION

The presence of *HER2* overexpression and *HER2* gene amplification is associated with a higher degree of malignancy, a high proliferative index, and a lower degree of differentiation.[11] *HER2* amplification was found to be a significant predictor of both OS (*P* = 0.0011) and time to disease relapse (*P* < 0.0001) in node-positive tumors.[2] Many clinical studies have found a positive correlation between *HER2* amplification/overexpression and poor disease outcome.[12,13] *HER2* status is also essential for the choice of treatment strategy. Patients with strong *HER2* overexpression (3+) in IHC staining or patients with *HER2* borderline (2+) with gene amplification in FISH or chromogenic *in situ* hybridization examination are qualified for trastuzumab therapy.

We included only the patients who received trastuzumab. We report that borderline positive *HER2* breast cancer patients are in earlier advance stage (*P* = 0.002) and have a better outcome (*P* = 0.006) than positive *HER2* tumors. However, they more often developed contralateral breast cancer (*P* = 0.015). Second neoplasm such as thyroid cancer, ovarian, cervical cancer, and basal cell carcinoma characterized strongly positive *HER2* tumors (*P* = 0.236).

In some studies, *HER2* status predicts development of metastases in node-negative breast cancer patients.[14-16] Our analysis has shown that lymph node metastases correlate with strongly positive breast cancer (*HER*+++). Borderline *HER2 patient*s more often were node negative. In several data, *HER2* overexpression has been associated with hormonotherapy resistance.[17,18] There was no association between *HER2* overexpression and steroid receptor status or hormone therapy efficacy.

Type 2 diabetes is a significant risk factor for developing breast cancer and breast cancer progression.[19,20] However, in some data, there was no statistical difference for the histological subgroup, grade, ER and PR status, *HER2-neu* overexpression rate and tumor size between diabetic and nondiabetic group.[21] In our study, the number of patients with diabetes was too small that shown association between diabetes and *HER2* overexpression and the same correlation with worse outcome. There was no significant difference for hypertension and overweight between patients with borderline and strong *HER2* overexpression.

The results of this study have many limitations mostly due to a small group of patients with borderline *HER2* overexpression. That is why the results should be taken into consideration with caution. Our data suggest that further research into the association between *HER2* overexpression (borderline or strong) and clinicopathological factors is warranted.

CONCLUSION

Borderline positive *HER2* breast cancer patients are in earlier advance stage and have a better outcome than positive *HER2* tumors (*P* = 0.002). They are less predisposed to the development of cardiac side effects. Second neoplasm developed more often in strongly positive *HER2* tumors (*P* = 0.236) and contralateral breast cancer in borderline patients (*P* = 0.015). *HER2* overexpression (borderline or strong overexpression) did not influence OS.

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