RESEARCH ARTICLE

Prognostic Significance of Human Epidermal Receptor (HER)-3 Immunohistochemical Expression in Patients with Metastatic Breast Cancer

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Abstract

Background: Previous reports have shown that human epidermal receptor (HER)-3 overexpression may be associated with poor prognosis in patients with breast cancer, but results have been conflicting. In this study, we sought to investigate the prognostic significance of HER-3 immunohistochemical expression in patients with metastatic breast cancer. Methods: We retrospectively analyzed HER-3 immunohistochemical expression profiles in 45 paraffin-embedded specimens from patients who had been treated between 1996 and 2006 in the Department of Oncology of the Uludag University School of Medicine, Bursa, Turkey. Membranous or cytoplasmic dominant expression patterns of HER-3 were analyzed using the Rajkumar score and a cytoplasmic 4-point scoring system, respectively. Progression-free survival (PFS) and overall survival (OS) served as the main outcome measures. Results: The median PFS in the study participants was 9 months (interquartile range: 4.5-13 months), whereas the median OS was 20 months (interquartile range: 7.5-28 months). Categorization of the patient population according to HER-3 positive immunohistochemical expression did not reveal any statistically significant difference in terms of both PFS (p=0.70) and OS (p=0.81). The results of multivariable Cox regression analysis indicated that tumor size was the only independent predictor of PFS, whereas estrogen and progesterone receptor status was independently associated with OS. Conclusions: HER-3 immunohistochemical expression did not correlate with outcomes in Turkish patients with metastatic breast cancer. Although our results suggest that HER-3 expression in cancer specimens is not of prognostic significance, further prospective studies are warranted to confirm these results.

Keywords: Metastatic breast cancer - human epidermal receptor-3 - immunohistochemistry - survival

Asian Pac J Cancer Prev, **14 (7)**, 4115-4119

Introduction

Although patients with breast cancer metastases generally have high mortality rates and a very poor prognosis, over the last decades the progresses in medical management have improved clinical outcomes, so women are living longer with progressive disease (Arslan et al., 2011; Lyman et al., 2012). When breast cancer patients develop distant metastases, the choice of systemic treatment with chemotherapy, hormonal therapy, antiangiogenesis therapy, or human epidermal growth factor receptor 2 (HER2)-targeted therapy is based on assessment of specific cancer receptor types in the primary tumor using routine immunohistochemistry (IHC) and/or molecular analysis (Cortazar et al., 2012; Eckhardt et al., 2012). The most common receptors that play a key role in metastatic breast cancer are estrogen receptor (ER) (Chaudhri et al., 2012), progesterone receptor (PR) (Sumida et al., 2004), and the human epidermal growth factor-2 receptor (HER-2) (Orphanos and Kountourakis, 2012). HER-2 is a member of the tyrosine kinase growth factor receptor family, which is believed to form heterodimers with other members of the receptor family and takes part in the cellular response to epidermal growth factor (Nanda, 2007; Tafe and Tsongalis, 2011). HER-2 overexpression as detected by IHC can be found in about 25 to 30% of breast cancers and is associated with an aggressive form of disease (Allred, 2010). Fortunately, HER-2 receptor positive cancers generally respond to trastuzumab, a monoclonal antibody directed against the external domain of the HER-2 protein (Brufsky, 2010; Goel et al., 2011).

Besides HER-2, HER-3 has been recently put into the centre of attention and investigation as a prognostic factor

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in patients with breast cancer (Way and Lin, 2005; Vaught et al., 2012). The HER-3 receptor lacks the intracellular kinase activity and acts only as a key dimerization partner for other HER family members (Gullick, 1996; Amler, 2010). In particular, evidence suggests that the HER2/HER3 partnership may represent the most oncogenic unit (Stern, 2008). Some reports have shown that HER3 overexpression may be associated with poor prognosis in patients with breast cancer, whereas other studies have indicated that an increased expression of HER-3 may be a favorable prognostic factor (Koutras et al., 2010). Notably, the biological and clinical importance of HER-3 signaling in human malignancies has been recently highlighted by the potential utility of fully human anti-HER-3 monoclonal antibodies as a novel therapeutic strategy in solid malignancies (Lorusso et al., 2013). Because the expression of HER-3 may have a significant impact of survival in patients with solid tumors due to its influence on tumor biology, we sought to investigate the prognostic significance of HER-3 immunohistochemical expression in patients with metastatic breast cancer. To this aim, we retrospectively analyzed the membranous or cytoplasmic dominant expression patterns of HER-3 in 45 paraffin-embedded specimens from Turkish women with metastatic breast cancer.

Materials and Methods

Participants

We retrospectively analyzed HER-3 immunohisto chemical expression profiles in 45 paraffin-embedded specimens from Turkish women with metastatic breast cancer who had been treated between 1996 and 2006 in the Department of Oncology of the Uludag University School of Medicine, Bursa, Turkey. Prognostic factors including age, menopausal status, tumor size, nodal status, and histological grade, were evaluated in all participants. Lesion staging was assessed according to the sixth edition of the American Joint Committee on Cancer (AJCC) staging manual for breast cancer. Tumor necrosis was defined as the presence of necrosis of any dimension in a section of invasive cancer. Histological grading was performed using the criteria of Bloom and Richardson (1957). In all participants, the expression of ER, PR, and HER-2 was immunohistochemically assessed in paraffin-embedded tissue specimens. The study protocol was approved by the local research ethics committee and written informed consent was obtained from all participants.

Immunohistochemistry for HER-2

IHC for HER-2 was performed on paraffin-embedded tissue sections (3-4 μm thick) placed on poly-L-Lysine coated slides. After deparaffinization and blocking of endogenous peroxidase, HER-2 immunostaining was performed using rabbit anti-human HER-2 oncoprotein as primary antibody (Dako, Copenhagen, Denmark) at 1:100 dilution. The binding of the primary antibody was checked using the Dako Quick-Staining, Labelled Streptavidin-Biotin System (LSAB; Dako), followed by the addition of diaminobenzidine (DAB) as a chromogen. Each slide was scored in a blinded fashion by two pathologists according to the manufacturer's recommended criteria. The immunostaining was read in a semiquantitative manner and graded as follows: 0, 1+, 2+, and 3+. Intensity scores of 0 or 1+ were designated as negative expression, whereas scores of 2+ and 3+ were considered as equivocal and positive, respectively (Liu et al., 2013).

Immunohistochemistry for HER-3

The immunohistochemical expression of HER-3 was evaluated on paraffin-embedded tissue sections (3-4 μm thick) fixed in 10% (volume/volume) neutral buffered formalin. The sections were deparaffinized in xylene, rehydrated in graded ethanol, washed in phosphate-buffered saline, and heated in a microwave at 98°C with buffer (pH=9) for 40 min. Peroxide blocking was performed with 3% H_2O_2 at room temperature for 10 min. The samples were then incubated overnight at 4°C with a primary mouse anti-human monoclonal antibody specific to HER-3 (RTJ-1) (1:40 dilution; GeneTex, San Antonio, TX, USA). Incubation with the secondary antibody (GeneTex) was performed for 30 min, followed by the application of DAB for 5 min. The slides were counterstained with Meyer's hematoxylin for 1 min, dehydrated in a graded series of alcohol, treated with xylene, coverslipped, and evaluated independently using light microscopy by two expert pathologists. HER-3 expression was generally detected as a homogeneous fine granular cytoplasmatic staining of neoplastic cells and, more rarely, membrane staining.

Rajkumar score for HER-3 immunohistochemical expression

The sections were scored for the following parameters: (a) the intensity of cytoplasmic staining (score 0: no staining, score 1: +, score 2: ++, score 3: +++), (b) the percentage of HER-3 positive cells (score 1: 1-25%, score 2: 26-50%, score 3: 51-75%, and score 4: >75%), (c) presence or absence of membrane staining (score 0: absent; score 1: present). The total Rajkumar score was calculated as the sum of the three separate scores and ranged from 0 to 7. A score of 5 or higher was considered as indicating a positive HER-3 immunostaining (Tanner et al., 2006) (Figure 1).

Cytoplasmatic score for HER-3 immunohistochemical expression

The sections were scored from 0 to 4 for the percentage of HER-3 positive cells, as follows: score 1: 1-25%, score

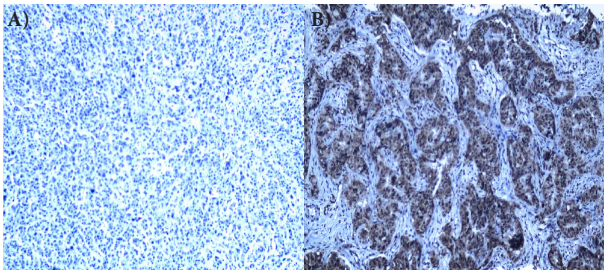

Figure 1. Negative (a) and Positive (b) Immunostaining of HER-3 Immunohistochemical Expression in Breast Cancer Specimens; Magnification ×100 and ×200

2: 26-50%, score 3: 51-75%, and score 4: >75%.

Statistical analysis

Variables were expressed as means±standard deviation, medians (lower quartile-upper quartile) or as numbers (percentages) if categorical. The correlations between the study variables were investigated using the Spearman's correlation coefficient. PFS was defined as freedom from breast cancer recurrence. OS was defined as freedom from breast cancer death or other causes of death. We assessed the association of each risk factor with (DSF) PFS and OS by multivariable Cox proportional hazard regression analysis. Cumulative survival rates of breast cancer cases were analyzed by the Kaplan-Meier method. The differences of cumulative survival were assessed using the log-rank method. The multivariable Cox model included all the demographical, clinical, and biochemical characteristics of the study participants. The appropriateness of the proportional hazards assumption was verified using graphical methods. The assumption of linearity for the Cox models was examined through visual inspection, and no violation was found. Hazard ratios (HRs) and their 95%CIs were calculated with the estimated regression coefficients and their standard errors in the Cox models. Statistical analyses were performed using SPSS software (version 14.0, SPSS Inc., Chicago, IL, USA). Two-tailed p values<0.05 were considered statistically significant.

Results

The general characteristics of the 45 study participants are reported in Table 1. Of the study patients, 5 had bone metastases (11%), 1 (2%) had distant lymph node metastasis, and the remaining 39 (87%) had visceral metastases. Chemotherapy was based on anthracyclines in 15 patients (33%), taxanes in 15 patients (33%), trastuzumab in 9 patients (20%), and other agents in 6 patients (14%). The median PFS in the study participants was 9 months (interquartile range: 4.5-13 months), whereas the median OS was 20 months (interquartile range: 7.5-28 months).

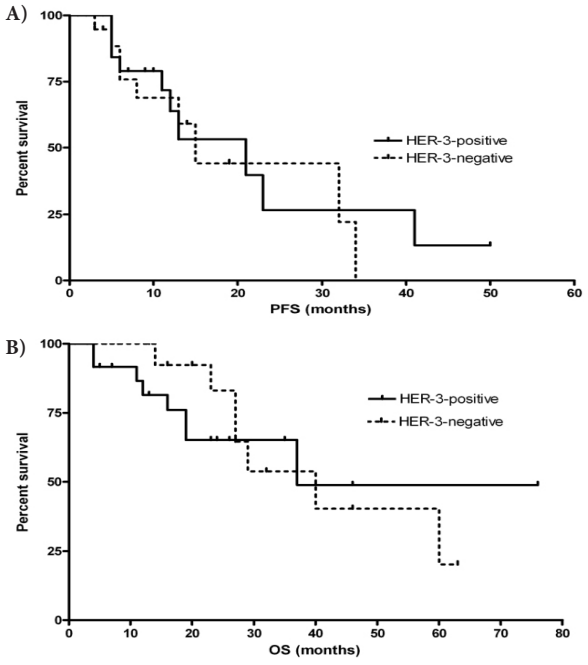

Figure 2. Kaplan–Meier Plots for Progression-free Survival (A) and Overall Survival (B) According to HER-3 Immunohistochemical Expression

HER-3 immunohistochemical expression and prognosis

The mean Rajkumar score for HER-3 immunohistochemical expression was 4.4±2.1, whereas the mean citoplasmatic score was 2.6±1.5. Using 5 as a cut-off for the Rajkumar score, there were 26 patients who were HER-3-positive (58%), and 19 subjects (42%) who were HER-3-negative. There was no significant association between the Rajkumar score for HER-3 immunohistochemical expression and the general characteristics of the study subjects (Table 2). Similarly, the cytoplasmatic score for HER-3 immunohistochemical expression was not associated with the study variables

Table 1. Characteristics of Women with Metastatic Breast Cancer (n=45)

		n %
Age (years)		50±12
Postmenopausal status		22 (49)
Tumor localization	Right breast	24 (53)
	Left breast	17 (38)
	Bilateral	4 (9)
Tumor size	<2 cm	4 (9)
	2-5 cm	22 (49)
	>5 cm	5 (11)
Any size with direct extension to the chest		14 (31)
Histological grade	1	5 (11)
	2	18 (40)
	3	22 (49)
Nodal status		
Absence of axillary node metastasis		7 (16)
Metastases in 1-3 axillary nodes		13 (29)
Metastases in 4-9 axillary nodes		23 (51)
Metastases in ≥ 10 axillary nodes		2 (4)
Estrogen receptor status	Positive	29 (64)
	Negative	12 (27)
	Unknown	4 (9)
Progesterone receptor status	Positive	25 (56)
	Negative	16 (35)
	Unknown	4 (9)
HER-2 status	Positive	23 (51)
	Negative	22 (49)

*Data are means±standard deviation or number of patients (%)

Table 2. Correlations between the Rajkumar Score for HER-3 Immunohistochemical Expression and the General Characteristics of the Study Participants

Variable	Spearman's ρ	p value
Age	-0.21	0.17
Postmenopausal status	-0.15	0.3
Tumor localization	-0.07	0.66
Tumor size	-0.25	0.09
Histological grade	0.01	0.98
Nodal status	-0.02	0.87
Estrogen receptor status	-0.03	0.85
Progesterone receptor status	-0.13	0.4
HER-2 status	-0.25	0.09

(data not shown).

Categorization of the patient population according to HER-3 positive immunohistochemical expression (Rajkumar score ≥5) did not reveal any statistically significant difference in terms of both PFS (p=0.70, Figure 2a) and OS (p=0.81, Figure 2b). The results of multivariable Cox regression analysis indicated that tumor size was the only independent predictor of PFS (HR=1.7, 95%CI=1.3-3.5, p<0.01), whereas ER (HR=1.5, 95%CI=1.2-2.7, p<0.01) and PR status (HR=1.3, 95%CI=1.1-3.2, p<0.05) were independently associated with OS.

Discussion

In the present study, we did not find any significant association between HER-3 immunohistochemical expression and baseline characteristics of patients with metastatic breast cancer. In addition, HER-3 immunohistochemical expression did not predict prognosis in our sample of Turkish patients. We also found no significant differences in terms of prognostic significance between cytoplasmic and membranous HER-3 staining.

Recent attention has focused on the potential significance of HER-3 in influencing tumor biology and clinical outcomes in breast cancer. Levels of HER-3 overexpression in solid cancers range between 20% and 60% (Ocana et al., 2013), which is line with the figure observed in our study. Interestingly, patients with HER-2-positive metastatic breast cancer have been shown to benefit from pertuzumab, a humanized monoclonal antibody which acts a dimerization inhibitor and prevents the formation of HER-2 homodimers or HER-2/HER-3 heterodimers (Keating, 2012). Although HER-3 is believed to act as a potent mitogenic signaling partner for HER-2 (Stern, 2008), the prognostic significance of its expression in breast cancer is still controversial (Koutras et al., 2010). Witton et al. (2003) have shown that the immunohistochemical expression of HER-3 in breast carcinomas was a significant and independent adverse prognostic factor. In line with these findings, HER-3 gene amplification has been associated with a reduced disease-free survival and shorter relapse-free survival rates (Bieche et al., 2003; Sassen et al., 2008). However, at least other three studies have reported opposite findings, with better outcomes associated with a positive HER-3 expression (Pawlowski et al., 2000; Lee et al., 2007; Koutras et al., 2008). One potential explanation for the discrepant findings regarding the prognostic value of HER-3 expression levels may be that clinical outcomes can also be influenced by its phosphorylation level or the expression of HER-3 ligands (Koutras et al., 2010).

The lack of prognostic value of HER-3 expression in women with metastatic breast cancer observed in our study can be also explained by the fact that the outcomes of this patient group are significantly poorer compared with other forms of breast cancer (Arslan et al., 2011; Lyman et al., 2012). In our sample of women with metastatic breast cancer, we have found that tumor size, ER, and PR status were the main predictors of prognosis. Our results on tumor size are in keeping with previous studies showing that size is associated with increased lethality in breast cancer, such that each milimeter of tumor diameter is associated with an additional ~1% chance of death (Michaelson et al., 2003). Similarly, several previous studies have shown that the presence or absence of ER and PR is a significant predictor of ultimate clinical outcomes in metastatic breast cancer (Ravdin et al., 1992; Lower et al., 2005).

Some limitations of our study merit consideration. First, our population consisted exclusively of Turkish subjects without ethnical diversity. Therefore, extrapolation of any conclusions from the present investigation may be incorrect and future studies in different clinical cohorts are needed to confirm and expand our findings. Second, we did not measure HER-3 phosphorylation levels and/or its ligand. Third, this study has a retrospective nature and the number of patients is relatively small. However, we believe that our negative results are interesting because they raise the possibility that anti-HER-3 strategies may not be a valuable treatment for patients with metastatic breast cancer.

In summary, HER-3 immunohistochemical expression does not correlate with outcomes in Turkish patients with metastatic breast cancer. Although our results suggest that HER-3 expression in cancer specimens is not of prognostic significance, further prospective studies are warranted to confirm these results.

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