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ORIGINAL ARTICLE

Immunohistochemical expression of human epidermal growth factor receptor (HER)-4 and prognosis in patients with metastatic breast cancer

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Summary

Purpose: *The clinical value of HER4 - a cell surface receptor that belongs to the human epidermal growth factor receptor family - for predicting survival outcomes in patients with breast cancer remains controversial. Herein, we sought to investigate the prognostic significance of HER4 immunohistochemical expression with respect to progression-free survival (PFS) and overall survival (OS) in Turkish patients with metastatic breast cancer (MBC).*

Methods: *MBC patients (N=45; mean age=50.5±12.7 years) were consecutively enrolled between 2000 and 2006 in the Department of Oncology at the Uludag University Medical Center, Bursa, Turkey. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded sections. The predictive value of HER4 expression was investigated by multivariate analysis after allowance for potential confounders.*

Results: *The mean PFS in the study participants was 11.35 months (range:1-50), whereas the median OS was 22.18 months (range:1-76). The mean PFS in patients with a HER4 immunohistochemical score of 0, 1+, 2+, and 3+ was 11.0 ± 4.8, 11.3 ± 7.7, 11.7 ± 8.1, and 10.4 ± 7.4 months, respectively (p=0.99) . The mean OS in patients with a HER4 score of 0, 1+, 2+, and 3+ was 13.3 ± 6.8, 25.6 ± 10.8, 22.9 ± 10.7, and 13.5 ± 9.9, months, respectively (p=0.44). The results of multivariate Cox regression analysis indicated that the presence of visceral metastases was the only independent prognostic factor for both OS (HR=3.01, 95% CI=1.56-3.99, p <0.01) and PFS (HR=2.91, 95% CI=1.51–3.78, p <0.01).*

Conclusion: *HER4 immunohistochemical expression is not an independent predictor of OS and PFS in Turkish MBC patients.*

Key words: *HER4, immunochemistry, metastatic breast cancer, prognosis, survival*

Introduction

MBC portends a poor prognosis (median survival time: 18-24 months) and continues to represent a significant public health issue [1-3]. Approximately 10% of all women with breast cancer present with metastatic disease at their initial diagnosis [3]. Moreover, 20-85% of all patients with breast malignancies can develop metastatic disease after years or even decades from the diagnosis of the primary tumor [3]. The main therapeutic goals in MBC are disease control and palliation [1]. Although the treatment strategy mainly depends on disease progression and patient preference, the absence or presence of specific receptor types in tumor cells may influence the therapeutic choices [4-6]. The most common receptors that can play a role in this setting are the estrogen receptor (ER) [4,5], the progesterone receptor (PR) [4,5], and the human epidermal growth factor 2 (HER2/neu) receptor [6]. Approximately 80% of ER- and PR-positive carcinomas are responsive

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to hormonal manipulation, whereas only about 40% of cancers that express either ER or PR alone respond successfully [4,5]. Notably, ER-positive cancers are generally less responsive to chemotherapy [7]. Conversely, malignancies that fail to express ER or PR have a <10% likelihood of responding to hormonal therapy but are more likely to respond to chemotherapy [7]. Besides ER and PR, overexpression of HER2/neu has been related to poorer survival rates in patients with breast cancer [8]. Moreover, HER2/neu expression is considered a key predictor of response to agents that target this transmembrane protein (e.g. trastuzumab) [9,10].

Human HER4 is a cognate of HER2/neu that contains both a ligand-binding domain and a tyrosine kinase domain [11,12]. In normal breast tissue, HER4 may be involved in tissue differentiation and can act as an ER coactivator [13]. Notably, preclinical studies suggested an ambivalent HER4 function on cell survival, being either proapoptotic [14,15] or pro-proliferative [16,17]. Because of this apparent inconsistency, it is not surprising that the potential role of HER4 expression on the clinical course and outcomes of breast cancer remains unclear. Accordingly, some reports have shown a favorable effect of HER4 expression, whereas other studies found the opposite [18-24]. In this scenario, we designed the current study to investigate the prognostic significance of HER4 immunohistochemical expression with respect to PFS and OS in Turkish patients with MBC.

Methods

Study design and participants

The current study was designed as a retrospective review of prospectively collected data. Patients with MBC (N=45; mean age=50.5±12.7 years) were consecutively enrolled between 2000 and 2006 in the Department of Oncology at the Uludag University Medical Center, Bursa, Turkey. All of the study participants were of Turkish descent and had a histological diagnosis of invasive ductal carcinoma. The following prognostic factors were evaluated: age, menopausal status, tumor location, histological grade, sites of metastases, and type of chemotherapy. Histological grading was performed using the criteria of Bloom and Richardson [25]. The Institutional Review Board of the Uludag University Medical Center (Bursa, Turkey) approved the study protocol.

Immunohistochemistry

In all participants, the expression of ER, PR, HER-2/neu, and HER4 was immunohistochemically determined using formalin-fixed, paraffin-embedded breast cancer tissue specimens. ER and PR status were taken as positive if more than 10% of tumor cells showed positive staining [26]. An immunohistochemical score of 3+ or fluorescence in situ hybridization+ for HER2/neu was accepted as HER2/neu positivity [27]. Classification of HER4 expression status was determined by applying a pathologist-based semi-quantitative H-Score [28]. The chromogenic immunolabeling of HER4 was categorized into four distinct categories, as follows: 0 (no membrane or cytoplasmic labeling; Figure 1), 1+ (weak cytoplasmic labeling), 2+ (weak membranous and/or strong cytoplasmic labeling), and 3+ (strong membranous (observable with 10× objective); with or without cytoplasmic staining; Figure 2).

Outcome evaluation

PFS and OS served as the main parameters for outcome evaluation [29]. PFS was calculated as the time from the date of diagnosis until the first reported occurrence of tumor progression. OS was calculated from the date of diagnosis to the date of death; surviving patients were censored on the last follow-up.

Statistics

The study variables were expressed as means ± standard deviations or as numbers (percentages) if categorical. Intergroup comparisons were performed using one-way analysis of variance (ANOVA) followed by the Bonferroni's post-hoc test (continuous variables)

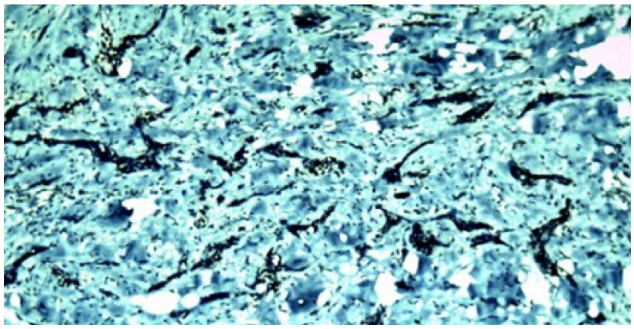

Figure 1. No membranous or cytoplasmic HER4 labeling (score 0) (HER4 x400).

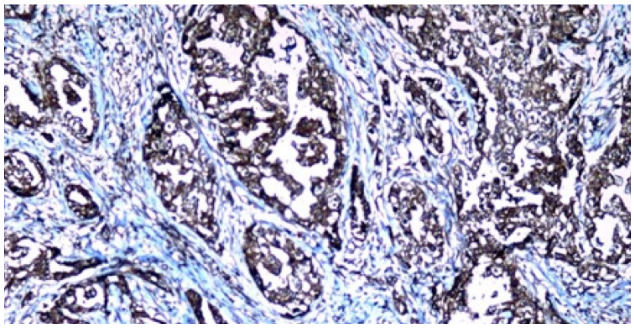

Figure 2. Strong membranous HER4 labeling (score 3+) (HER4 x400).

or the x^2 test (categorical variables). Multivariate Cox proportional hazard regression analysis was used to assess the association of each risk factor with PFS and OS. The multivariate Cox model included all the demographic, clinical, and immunohistochemical characteristics of the study participants. The appropriateness of the proportional hazards assumption was verified using graphical methods [30]. The assumption of linearity for the Cox models was examined through visual inspection [31], and no violation was found. Hazard ratios (HRs) and their 95% confidence intervals (CIs) were calculated with the estimated regression coefficients and their standard errors in the Cox models. All calculations were performed using the SPSS software package, version 17.0 (SPSS Inc., Chicago, IL, USA). A p value <0.05 (two-sided) was considered statistically significant.

Results

The general characteristics of MBC patients are summarized in Table 1. Of the 45 study participants, 24 (53.3%) had their primary tumor located in the right breast, 17 (37.8%) in the left breast, and 4 (8.9%) had bilateral malignancies. The sites of distant metastases were as follows: bone (N=5;11.1%), distant nodes (N=1;2.2%), and visceral organs (N=39;86.7%). A total of 13 patients (28.9%) received anthracycline-based chemotherapy, 12 (26.7%) received taxanes, and 6 (13.3%) other agents. In addition, 8 patients (17.8%) were treated with trastuzumab.

Immunohistochemical characteristics of patients and prognosis

The immunohistochemical characteristics of the study patients are displayed in Table 2. The mean PFS of the study participants was 11.35 months (range:1–50), whereas the median OS was 22.18 months (range:1-76). Categorization of the patient population according to HER4 immunohistochemical expression did not reveal a statistically significant difference in terms of both PFS and OS. Specifically, the mean PFS in patients with a HER4 immunohistochemical score of 0, 1+, 2+, and 3+ was 11.0±4.8, 11.3±7.7, 11.7±8.1, and 10.4±7.4 months, respectively (p=0.99, ANOVA) . The mean OS in patients with a HER4 score of 0, 1+, 2+, and 3+ was 13.3±6.8, 25.6±10.8, 22.9±10.7, and 13.5±9.9 months, respectively (p=0.44, ANOVA). We did not find any significant association between immunohistochemical expression of ER, PR, HER2/neu and HER4 and the baseline characteristics of the study patients (data not shown).

Table 1. General characteristics of patients with metastatic breast cancer (N=45)

Characteristics	*N (%)*
Age (years), mean±SD	50.5 ± 12.7
Postmenopausal status	23 (51.1)
Tumor size (cm)	
< 2	4 (8.9)
2-5	22 (48.9)
> 5	5 (11.1)
Chest wall invasion	14 (31.1)
Tumor grade	
1	5 (11.1)
2	18 (40.0)
3	22 (48.9)
Nodal involvement	
None	7 (15.6)
1-3 nodes	13 (28.9)
4-9 nodes	23 (51.1)
>9 nodes	2 (4.4)

Table 2. Results of immunohistochemistry in patients with metastatic breast cancer (N=45)

Results	*N (%)*
Estrogen receptor (+)	29 (64.4)
Progesterone receptor (+)	25 (55.6)
HER2/neu (+)	22 (48.9)
HER4, 0	4 (8.9)
HER4, 1+	16 (35.6)
HER4, 2+	21 (46.6)
HER4, 3+	4 (8.9)

Univariate and multivariate analysis

The results of univariate and multivariate Cox regression analysis (Table 3) indicated that the presence of visceral metastases was the only independent prognostic factor for both OS (HR=3.01, 95% CI=1.56-3.99, p<0.01) and PFS (HR=2.91, 95% CI=1.51-3.78, p<0.01) in our patients with MBC.

Discussion

Although loss of HER4 expression during the development of the metastatic phenotype has been reported in breast cancer, its prognostic significance in patients with breast cancer remains controversial [18-24]. A previous study conducted in MBC showed that HER4 expression was associated with a better OS in trastuzumab-treated patients [28]. Because the prognosis of patients with MBC is significantly poorer compared with

Table 3. Predictors of progression-free survival and overall survival (Cox regression analysis) in patients with metastatic breast cancer (N=45)

	Progression-free survival				*Overall survival*			
Variables	*Univariate*		*Multivariate*		*Univariate*		Multivariate	
	HR (95% CI)	*p value*	*HR (95% CI)*	*p value*	*HR (95% CI)*	*p value*	HR (95% CI)	*p* value
Age	1.24 (0.97–1.36)	0.60	1.29 (0.94–1.55)	0.71	1.56 (0.90–1.67)	0.75	1.50 (0.94–1.79)	0.82
Postmenopausal status	2.31 (0.90–5.23)	0.85	2.50 (0.87–5.56)	0.92	2.15 (0.81–3.16)	0.79	2.18 (0.85–3.89)	0.87
Tumor size	1.18 (0.90–2.14)	0.51	1.25 (0.89–2.12)	0.61	1.27 (0.88–1.55)	0.68	1.39 (0.91–1.64)	0.60
Tumor grade	1.34 (0.78–1.81)	0.72	1.45 (0.85–1.90)	0.79	1.58 (0.89–2.01)	0.57	1.51 (0.85–1.99)	0.55
Tumor location	1.89 (0.97–2.43)	0.25	2.10 (0.95–2.67)	0.45	1.73 (0.92–2.12)	0.51	1.63 (0.93–2.24)	0.62
Nodal involvement	2.75 (0.90–3.34)	0.56	2.13 (0.86–3.84)	0.76	2.56 (0.94–3.62)	0.25	2.66 (0.91–3.91)	0.31
Visceral metastases	2.91 (1.51–3.78)	< 0.01	2.68 (1.43–3.44)	< 0.01	3.13 (1.68–4.21)	< 0.01	3.01 (1.56–3.99)	< 0.01
Estrogen receptor (+)	1.55 (0.93–1.99)	0.69	1.68 (0.95–1.90)	0.56	1.89 (0.89–2.18)	0.84	1.97 (0.82–2.46)	0.93
Progesterone receptor (+)	1.35 (0.89–2.10)	0.87	1.38 (0.85–1.92)	0.70	1.34 (0.76–1.99)	0.70	1.67 (0.81–2.10)	0.84
HER2/neu (+)	1.59 (0.88–2.00)	0.80	1.54 (0.90–2.11)	0.85	1.40 (0.91–1.89)	0.38	1.48 (0.95–1.91)	0.47
HER4 expression	1.56 (0.70–1.90)	0.97	1.45 (0.74–1.99)	0.98	1.34 (0.68–1.56)	0.44	1.41 (0.61–1.60)	0.63

HR: hazard ratio, CI: confidence interval

non-metastatic patients, biomarker tools for improving the prognostic stratification of this high-risk group are eagerly awaited [32]. Unfortunately, our findings do not support an association of the HER4 immunohistochemical expression with either OS or PFS in Turkish patients with MBC. In contrast, we found that the presence of visceral metastases was the only variable independently associated with prognosis. The results on the adverse prognostic significance of visceral involvement are in accordance with previous studies analyzing MBC. In a retrospective analysis of 3-year breast cancer-specific survival rates in 294 patients treated for operable breast cancer, Imkampe et al. [33] demonstrated that prognosis was highly dependent on the site of first metastatic recurrence (with visceral metastases being associated with the lowest survival rates). Similarly, other studies have demonstrated an adverse prognostic impact of visceral involvement with OS by univariate and multivariate analyses [34].

HER4 is a member of the human epidermal growth factor receptor family and is frequently upregulated in various cancer tissues [12]. Some studies have shown that HER4 can promote tumor biological aggressiveness by activation on PI3K-AKT cascade and focal adhesion kinase [11-14]. In contrast, other reports demonstrated that HER4 signaling could have a protective function against carcinogenesis [11-14]. The significance of HER4 as a prognostic factor in patients with MBC is of potential clinical interest because established biomarkers in this entity are still lacking. Unfortunately, we did not identify a significant impact of HER4 immunohistochemical expression on clinical outcomes in this group of patients, indicating the limited value of this marker for risk stratification of MBC. In addition, no significant association between HER4 immunohistochemical expression and the general characteristics of the study participants was detected. Taken together, these findings suggest that HER4 expression is not directly related to the clinicopathological characteristics of MBC. Nonetheless, further investigation is needed to shed more light on the role of HER4 in breast cancer biology.

Some caveats of our study merit consideration. First, our population consisted exclusively of Turkish subjects, so that results may not be extrapolated to populations with different ethnic

background. Second, we did not measure HER4 gene expression in the histopathological specimens. We cannot therefore exclude that an altered expression of HER4 at the mRNA level could be more useful for risk assessment of MBC patients than its immunohistochemical expression. In this regard, it should be noted that at least four alternatively spliced HER4 isoforms exist [35], potentially being characterized by different function and signaling capabilities. Interestingly, there is also some evidence that a downregulation of HER4 mRNA is not invariably paralleled by a corresponding reduction in protein expression [36].

In summary, the results of our report do not support a significant association between HER4 immunohistochemical expression and survival endpoints in patients with MBC. Although the current findings suggest that HER4 expression in cancer specimens is not of prognostic significance for MBC, further research is warranted to determine the value of this immunohistochemical marker in patients with primary breast cancer.

Conflict of interests

The authors declare no confict of interests.

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