

ORIGINAL ARTICLE

Prognostic significance of estrogen receptor, progesterone receptor, HER2/neu, Ki-67, and nm23 expression in patients with invasive breast cancer

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Summary

Purpose: To determine the prognostic significance of estrogen receptor (ER), progesterone receptor (PR), HER2/neu, Ki-67, and nm23 immunohistochemical expression with respect to progression free survival (PFS) and overall survival (OS) in Turkish patients with invasive breast cancer (IBC).

Methods: Patients with IBC (n = 81; mean age = 51.9 ± 11.1 years) were prospectively enrolled at the Department of Oncology, Uludag University Medical Center, Bursa, Turkey. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue sections.

Results: We did not find any significant association between immunohistochemical expression of ER, PR, HER2/neu, Ki-67, and nm23 and the baseline characteristics of IBC patients. The median patient PFS was 30 months (range 22-45), and the median OS was 32 months (range

23-46). Stratification of the patient population according to nm23 immunohistochemical expression revealed a statistically significant difference in terms of both OS (p < 0.05) and DFS (p < 0.05). Multivariate Cox regression analysis indicated that tumor grade, axillary lymph node status, and nm23 immunohistochemical expression were the 3 main independent prognostic factors for PFS and OS in IBC patients.

Conclusion: Reduced nm23 immunohistochemical expression is an independent negative prognostic factor for OS and PFS. Patients with negative nm23 expression may require a more intensive follow-up.

Key words: immunohistochemistry, invasive breast cancer, nm23, prognosis, survival

Introduction

IBC continues to be one of the most common cancers and a major cause of death among women worldwide [1]. IBC represents a heterogeneous disease in terms of clinical course and pathology [2]. Most of the IBCs (about 80%) are invasive ductal carcinomas [1,2]. These tumors start in the duct of the breast, break through the duct wall and invade the surrounding fatty tissue, from where they can spread through the lymphatic system or bloodstream [3]. The other main type of IBC (about 10-15%) is the invasive lobular carcinoma [4]. These cancers begin in the lobules of the

breast and act then similarly to the invasive ductal carcinomas. Less frequent than the invasive ductal carcinomas are the medullary carcinomas, ranging between 5 and 10%. Other rare types of IBC are inflammatory, tubular, and mucinous carcinomas [5-8]. Age, tumor size, tumor extension, tumor histopathological characteristics and tumor markers are highly associated with survival of cancer patients and are considered as important prognostic factors in IBC [3,9,10]. Some other factors, such as treatment modalities, also contribute to the prognosis and survival of patients with breast cancer [11].

Classical immunohistochemistry markers in

IBC include the expression of ER and PR [12,13]. The presence of these hormone receptors is generally correlated with a better outcome and is an important predictor of response to anti-estrogen therapy [14]. About 80% of carcinomas that are ER and PR positive are responsive to hormonal manipulation, whereas only about 40% of those with either ER or PR alone respond [15]. Importantly, ER-positive cancers are generally less likely to respond to chemotherapy [16]. Conversely, cancers that fail to express ER or PR have a less than 10% likelihood of responding to hormonal therapy but are more likely to respond to chemotherapy [17]. Besides ER and PR, overexpression of HER2/neu(c-erbB2) has been associated with poorer survival in IBC, but its main clinical importance is currently as a predictor of response to agents that target this transmembrane protein (e.g., trastuzumab) [18,19]. Proliferative indexes which reflect the proliferative activity of cancer cells have been also proposed as prognostic factors in IBC. In particular, Ki-67 is a non-histone nucleolar protein tightly linked to cell-cycle, whose expression is universal in cells going through the cell cycle, whereas it is absent in cells in G0 [20]. The feasibility of its determination in frozen and paraffin-embedded tissues using immunohistochemistry techniques and its high efficacy in reporting proliferation has prompted its extensive use in breast cancer research [21]. Finally, the expression of the non metastatic (nm) 23 protein in breast epithelial lesions has been shown to play a role in the progression of IBC by suppressing the metastatic phenotype [22,23].

The aim of this study was to determine the prognostic significance of ER, PR, HER2/neu, Ki-67, and nm23 immunohistochemical expression with respect to OS and PFS in Turkish patients with IBC.

Methods

Participants

Patients with IBC (N = 81; mean age = 51.9 ± 11.1 years) were prospectively enrolled at the Department of Oncology, Uludag University Medical Center, Bursa, Turkey. The clinicopathological characteristics such as age, menopausal status, and tumor necrosis were evaluated based on pathological reports and medical records. 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 [24]. Prognostic factors including tumor size, histological grade, histological type, nodal status, type of surgery, radiotherapy,

and chemotherapy were evaluated in all participants. All patients had undergone complete or partial mastectomies with fully resected axillary lymph nodes. Patients received anthracycline- and taxane-containing adjuvant chemotherapy in the presence of positive axillary nodes. Disease stage was assessed according to the 6th edition of the American Joint Committee on Cancer (AJCC) staging manual for breast cancer. The study protocol was approved by the institutional review committee of the Uludag University Medical Center, Bursa, Turkey. All patients gave written informed consent.

Immunohistochemistry

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded breast cancer tissue. ER and PR status were considered as positive if more than 10% of tumor cells showed staining [25]. Immunohistochemical score of 3+ or fluorescence in situ hybridization (FISH) for HER2/neu was accepted as HER2/neu positivity [26]. The immunohistochemical detection of Ki-67 (clone MIB-1, DAKO M7240, Dako Corporation, Carpinteria, CA, USA; dilution 1:70) was carried out as previously described [27]. Immunohistochemistry for nm23 was performed using the avidin-biotin method [28]. The antibody used was a rabbit anti-human monoclonal antibody nm23 (DAKO A0485, Dako Corporation; dilution 1:40). The nm23 staining in breast cancer tissue was classified as negative or positive [28].

Statistics

The sample size was calculated by using GraphPad StatMate version 2.00 for Windows, (GraphPad Software, San Diego, CA, USA). Our study had a 95% power to detect a hazard ratio (HR) of death of 0.34 with a significance level (alpha) of 0.05 (two-tailed) between patients with positive (vs negative) nm23 immunohistochemical expression. Variables were expressed as means \pm standard deviations or as numbers (percentages) if categorical. PFS was defined as freedom from breast cancer progression. OS was defined as freedom from breast cancer death or other causes of death. Cumulative survival rates of IBC cases were analyzed by the Kaplan-Meier method. The differences of cumulative survival were assessed using the log-rank method. We assessed the association of each risk factor with PFS and OS by multivariate Cox proportional hazard regression analysis. 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 and tested as per Grambsch and Therneau [29,30]. The assumption of linearity for the Cox models was examined through visual inspection, and no violation was found. 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 17.0, SPSS Inc., Chicago, IL, USA).

Two-tailed p values < 0.05 were considered statistically significant.

Results

The general characteristics of the IBC patients are depicted in Table 1. Of the 81 study participants, 78 (96.4%) had ductal carcinoma, 1 (1.2%) medullary carcinoma, 1 (1.2%) mucinous carcinoma, and the remaining 1 (1.2%) small cell carcinoma. The pathological axillary lymph node status was as follows: pN0 in 39 patients (48.1%), pN1 in 27 (33.3%), pN2 in 9 (11.1%), and pN3 in 6 patients (7.5%). Fifty-four patients (66.7%) underwent total and 27 (33.3%) partial mastectomies. A total of 41 patients (50.6%) received anthracycline-based adjuvant chemotherapy, whereas the remaining 40 (49.4%) received anthracycline-taxane combinations. Besides chemotherapy, 77 patients (95.1%) received radiotherapy.

Immunohistochemical characteristics of patients and prognosis

The immunohistochemical characteristics of the study participants are shown in Table 2. We did not find any significant association between immunohistochemical expression of ER, PR, HER2/neu, Ki-67, and nm23 and the baseline characteristics of IBC patients (data not shown). The median patient PFS was 30 months (range 22-45) and the median OS 32 months (range 23-46). Stratification of the patient population according to nm23 immunohistochemical expression revealed a statistically significant difference in terms of both OS ($p < 0.05$; Figure 1) and PFS ($p < 0.05$; Figure 2). We did not find significant associations between ER, PR, HER2/neu, and Ki-67 expression with the study endpoints.

Multivariate analysis

The results of multivariate Cox regression analysis (Table 3) indicated that tumor grade, axillary lymph node status, and nm23 expression were the 3 main independent prognostic factors for OS and PFS in our patients with IBC.

Discussion

The results of this study provide evidence that tumor grade, axillary lymph node status, and nm23 expression are the main independent prognostic factors for OS and PFS in Turkish

Table 1. General characteristics of the patients with invasive breast cancer (N=81)

Characteristics	N (%)
Age (years)	51.9 ± 11.1*
Postmenopausal	44 (54.3)
Presence of necrosis	37 (45.7)
Tumor size (cm)	
< 2	23 (28.4)
2-5	46 (56.8)
> 5	12 (14.8)
Tumor grade	
1	5 (6.2)
2	23 (28.4)
3	53 (65.4)

*mean± standard deviation

Table 2. Results of immunohistochemistry in patients with invasive breast cancer (N=81)

	N (%)
Estrogen receptor (-) HER2/neu (-)	39 (48.1)
Estrogen receptor (+) HER2/neu (+)	20 (24.7)
Estrogen receptor (-) HER2/neu (+)	22 (27.2)
Estrogen receptor (+)	20 (24.7)
HER2/neu (+)	42 (51.8)
Ki-67	386 ± 216*
Nm23 (+)	56 (69.1)

*mean± standard deviation

patients with IBC. Axillary nodal status and pathological grade are some of the most common factors which have been considered as having prognostic value in connection with breast cancer recurrence and/or survival [31,32]. Although the above mentioned clinicopathologic factors are used currently by clinicians to help in the decision making regarding the need for, and type of adjuvant chemotherapy, these factors do not appear to reflect or take into account the underlying biology of breast cancer. By using immunohistochemical analysis, several molecular biomarkers have shown prognostic value in IBC irrespective of therapy and have been shown to be predictive factors for outcome after conventional or high-dose chemotherapy [33]. In the present study, we have tested multiple immunohistochemical markers to identify predictive classifiers which would be robustly and independently associated with clinical outcomes.

The main finding of the present report is that a negative immunohistochemical expression of nm23 is an independent adverse prognostic factor

for OS and PFS in our series of IBC patients. Nm23 was the first identified metastasis suppressor gene by screening cDNA libraries of matched metastatic/non-metastatic murine melanoma cell lines by subtraction cloning [34]. From a molecular standpoint, nm23 possesses at least 3 enzymatic activities, including nucleoside diphosphate kinase, histidine kinase, and a 3'-5' exonuclease [35]. Such enzymatic activities are closely associated with DNA repair functions and the maintenance of genomic integrity, and their loss would be expected to cause increased mutation rates, suggesting nm23 may have the potential to suppress mutations required for metastasis [36]. Several studies have shown that the nm23 protein acts as an inhibitor of metastatic progression in several human solid cancers and its downregulation has been associated with increased metastatic rate and reduced survival [37-39]. In particular, Youn et al. [40] have reported that nm23 is inversely related to breast cancer metastasis and angiogenesis by interacting with many proteins involved in cellular signal transduction in angiogenesis and tumorigenesis. Zhao and colleagues [41] demonstrated that the nm23 gene plays a major role in metastasis of breast cancer and its mechanism of action of metastasis suppression may involve downregulation of genes associated with cell adhesion, motility, and possibly certain tumor/metastasis suppressors. By studying several different breast lesions, Royds et al. [42] concluded that nm23 protein expression decreases with advancing histological grade. Similarly, Bal and coworkers [28] reported that nm23 expression showed a progressive downregulation with increasing neoplastic transformation of breast cancer lesions. In a study from China, Dong et al. [43] have recently suggested that a positive expression of nm23 is associated with less lymph node metastasis in ER-negative premenopausal patients with invasive ductal carcinoma. Similarly, Terasaki-Fukuzawa et al. [44] have reported that a decrease in nm23 expression is significantly correlated with lymph node metastasis of breast invasive ductal carcinoma. Although the existing evidence on the prognostic significance of nm23 expression in human primary breast cancer is still unclear, it can be speculated that previous negative findings could be due to different patient characteristics and the lack of standardization for conventional section analyses of nm23 immunohistochemical expression. The literature on the protein expression

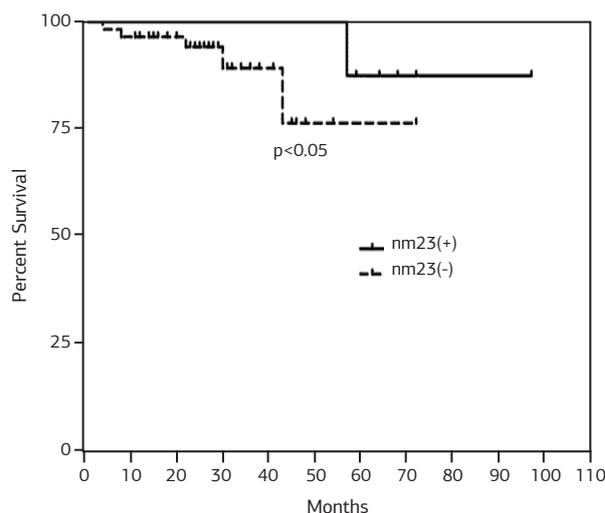


Figure 1. Kaplan–Meier plots for overall survival according to nm23 immunohistochemical expression.

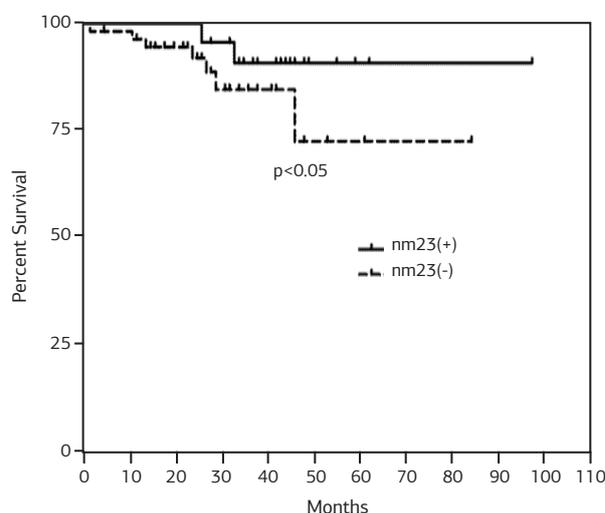


Figure 2. Kaplan–Meier plots for progression-free survival according to nm23 immunohistochemical expression.

in solid tumors is often conflicting. Variations in antibodies used, staining protocols, fixation of tissues, selection of patients, and criteria for interpretation of staining are routinely discussed as possible sources for discrepancy regarding results.

Several caveats 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, in breast cancer research, some other important risk factors should be considered, such as the patient's social-economic status [45]. Unfortunately, gross household income and education levels were not

Table 3. Predictors of progression-free survival and overall survival (Cox regression analysis) in patients with invasive breast cancer (N = 81)

Variables	Progression-free survival				Overall survival			
	Univariate HR (95% CI)	p-value	Multivariate HR (95% CI)	p-value	Univariate HR (95% CI)	p-value	Multivariate HR (95% CI)	p-value
Age	1.11 (0.98-1.19)	0.21	1.21 (0.93-1.23)	0.38	1.14 (0.94-1.22)	0.27	1.17 (0.93-1.29)	0.48
Postmenopausal status	2.45 (0.94-4.12)	0.55	2.33 (0.89-3.62)	0.76	2.89 (0.84-3.22)	0.42	2.42 (0.74-3.67)	0.71
Presence of necrosis	1.21 (0.89-1.73)	0.48	1.27 (0.93-1.81)	0.67	1.18 (0.89-1.39)	0.64	1.23 (0.87-1.47)	0.79
Tumor size	1.13 (0.81-2.01)	0.75	1.22 (0.85-2.85)	0.89	1.32 (0.90-1.87)	0.74	1.37 (0.94-1.75)	0.63
Tumor grade	2.57 (1.14-3.52)	<0.01	2.21 (1.11-3.66)	<0.05	2.12 (1.11-2.97)	<0.01	1.94 (1.07-2.57)	<0.05
Tumor stage	1.94 (0.96-2.32)	0.21	2.17 (0.89-2.55)	0.56	1.74 (0.94-1.91)	0.39	1.56 (0.87-1.84)	0.51
Axillary lymph node status	3.80 (1.66-5.32)	<0.001	3.43 (1.37-5.62)	<0.001	3.22 (1.61-4.44)	<0.01	2.91 (1.22-3.94)	<0.01
Estrogen receptor (-) HER2/neu (-)	1.67 (0.92-1.88)	0.34	1.56 (0.90-1.96)	0.61	1.44 (0.81-1.79)	0.52	1.30 (0.71-1.22)	0.77
Estrogen receptor (+) HER2/neu (+)	1.23 (0.79-1.56)	0.61	1.11 (0.69-1.67)	0.79	1.11 (0.69-1.14)	0.71	1.04 (0.89-1.44)	0.83
Estrogen receptor (-) HER2/neu (+)	1.41 (0.84-1.98)	0.52	1.31 (0.89-2.58)	0.65	1.58 (0.90-2.01)	0.31	1.41 (0.87-1.88)	0.49
Estrogen receptor (+)	0.89 (0.69-1.14)	0.48	0.95 (0.78-1.23)	0.54	0.78 (0.55-1.12)	0.33	0.84 (0.61-1.09)	0.42
HER2/neu (+)	1.22 (0.97-1.98)	0.11	1.26 (0.98-2.11)	0.09	1.35 (0.94-1.64)	0.24	1.24 (0.97-1.51)	0.19
Ki-67	1.69 (0.89-2.81)	0.88	1.71 (0.84-2.99)	0.91	1.89 (0.67-2.22)	0.81	1.76 (0.75-2.47)	0.89
Nm23 (+)	0.85 (0.49-0.94)	< 0.05	0.76 (0.55-0.97)	< 0.05	0.79 (0.68-0.95)	<0.05	0.80 (0.71-0.96)	<0.05

HR: hazard ratio, CI: confidence interval

recorded in this study. Finally, we did not measure nm23 gene expression in the histopathological specimens. Therefore, we cannot exclude that a reduced expression of nm23 at the mRNA level can be more useful in the risk assessment of IBC patients than its immunohistochemical expression.

In summary, the main finding of the present report is that a negative nm23 immunohistochemical expression is an adverse prognostic factor in Turkish

IBC patients due to an increased risk of tumor recurrence and death. Therefore, patients with negative nm23 expression may require more intensive follow-up. Our findings suggest that nm23 negative tumors can present distinct clinical and biological challenges among IBC. The significance of nm23 immunohistochemical expression in breast cancer should be further studied in future translational and clinical research.

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