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CLINICAL STUDY

The effects of E-cadherin and bcl-2 on prognosis in patients with breast cancer

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Abstract: *Background:* Breast cancer is the most common malignancy in women. Axillary lymph node involvement and tumor size are the most significant prognostic factors in breast cancer. However, more factors are needed for evaluating the prognosis and individualizing the treatment in these patients. Intracellular adhesion molecule E-cadherin, antiapopitotic protein bcl-2, and p53 might have predictive and prognostic properties in breast cancer.
Objective: We have investigated the effects of E-cadherin, bcl-2, and p53 on disease free survival and overall survival in patients with breast cancer. Methods: Positivity of aforementioned genes was detected with immunohistochemistry staining. Seventy-six women patients with invasive ductal and lobular breast cancer who had received adjuvant therapy were included in the study.
Results: Bcl-2, E-cadherin, and p53 expression in tumor tissue specimens were found in 26.31 %, 35.52 %, and 9.21 %, respectively. Mean duration of follow-up was 93.58±3.40 months. In multivariate analyses, axillary lymph node presence and C-erb-B2 overexpression were a strong negative prognostic factor of disease free survival and overall survival. E-cadherin and bcl-2 failed to have an effect on disease free survival and overall survival in our study. In addition, p53 overexpression was determined in a limited number of patients.
Conclusions: The results of this study showed that E-cadherin, bcl-2, and p53 did not have any significant prognostic value for our patients. In order to arrive at a decision, we need studies, which include more patients and long follow-up *periods (Tab. 2, Fig. 2, Ref. 24)*. Full Text in free PDF *www.bmj.sk.*
Key words: E-cadherin, Bcl-2, breast cancer.

Breast cancer is the most frequent cancer in women. It develops in one of every nine women (1). Thus, it is an important morbidity and mortality factor. Breast cancer is a heterogeneous disease. In order to avoid problems like excessive or insufficient treatment, patient-based treatment plans should be made. Today various prognostic and predictive factors are used to make these treatment plans. Axillary lymph node metastasis is the most important prognostic factor (1, 2). Tumor size, grade, and lymphatic or vascular invasion are other accepted prognostic factors (2). Identifying new prognostic molecular markers has become the objective in translational research studies. Three of these potential markers, E-cadherin, bcl-2 and p53, have received growing attention as prognostic indicators in breast carcinoma.

E-cadherin, which plays an important role in intercellular epithelial adhesion, is a calcium-dependent transmembrane glycoprotein (3). Normal ductal epithelial cells found in breast gland strongly express E-cadherin protein in cytoplasmic membrane (3). The importance of E-cadherin expression remains poorly understood in breast cancer. The loss of E-cadherin might be the cause of the increase in invasive and metastatic abilities of breast cancer cells.

The inhibition of apoptosis plays a role in the development of malignancy. The most important antiapoptotic protein is bcl-2 (4). It causes treatment resistance in follicular lymphoma. It was frequently demonstrated in breast cancer. But its prognostic role is still controversial (5, 6). The p53 gene is the most important tumor suppressor gene. It either conducts the cell to apoptosis as a response to DNA damage or causes an arrest in G1 phase of cells. Uncontrolled cell reproduction could be seen as a result of inactivation of p53 (4). The p53 mutation is frequently seen in breast cancer (4, 7). The p53 mutant loses its activating effect on apoptosis and causes proliferation of malign cells.

The aim of this study was to determine the prognostic value of molecular markers such as bcl-2, E-cadherin, and p53 in non-metastatic breast carcinoma. Results might be helping us in defining the group of patients in need of more aggressive treatment.

Material and methods

Patient selection

Ninety patients referred to Department of Oncology, School of Medicine, Black Sea Technical University between January

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2000 and June 2004 were included in this study. Patients were operated andreceived adjuvant therapy (chemotherapy; doxorobucine 60 mg/m^2 + cyclophosphamide 600 mg/m^2 for node negative patients and cyclophosphamide 500 mg/m^2 + epirubucine 90 mg/m^2 +Flourouracil 500 mg/m^2 for node positive patients,+/– endocrine therapy, and +/– radiotherapy). Paraffin-embedded formalin-fixed tissues were obtained from the Pathology Department. Disease-free survival (DFS) was defined as the time from diagnosis to first recurrence (local, regional, or distant); overall survival (OS) was defined as the time from diagnosis to death from the disease.

Inclusion Criteria

Patients were diagnosed with invasive ductal or lobular carcinoma according to pathology results. Six weeks after the operation, they began receiving adjuvant therapy, and during the diagnosis, they were administered with antimetastatic therapy.

Exclusion criteria

Patients, who did not show compliance with the treatment protocol during adjuvant therapy, failed to attend follow-up visits, did not have pathology samples for immunohistochemistry (IHC) staining, did not have enough tumor tissue for IHC staining, or those not receiving adjuvant treatment.

The study protocol

Patients who met the study criteria were followed up after the adjuvant therapy once every three months for the first two years, once every six months for the next two years, and once a year for the following years. Patients were checked for recurrence and/or metastasis of the disease. At each visit, physical examination, blood analysis (total blood count, blood biochemistry, CEA, and CA 15–3 levels), yearly mammography and/or breast ultrasonography, yearly abdominal ultrasonography, thoracic and abdominal CT (in case of indication), and bone radiography with bone scintigraphy were performed. Patients were followed according to this protocol until October 2008. This study was approved by the institutional ethics committee and informed consent was taken from the patients.

IHC staining

IHC was performed with automatic staining device (Nexes IHC, Ventana). ER (Clon SPI, NeoMarkers, California, USA), C-erb-B2 (Clon e2-4001+3B5, Neomarkers, California, USA), bcl-2 (Clon 100/D5, NeoMarkers, California, USA), E-cadherin (Clon 36B5, Neomarkers, California, USA), and p53 (Clon SP5, NeoMarkers, California, USA) were used as primary antibodies. Formalin-fixed paraffin-embedded blocks containing tumor tissue were obtained either with excisional biopsy or with surgical operation. The samples were cut into slices with a thickness of 5 ěm. After deparafinization, blocks were boiled in citrate buffer with pH of 6.0 for 15 minutes at 200 W by microwave method. Then the slides were cooled for 10 minutes. Automated staining device added endogen peroxidase inhibitor, primer antibodies, biotinized secondary antibodies, peroxidase with avidin-biotin complex, 3,3'-diaminobenzidin tetrahydrocloride, and rich copper at 36 °C. Next IHC stained slides were evaluated with Olympus BH50 light microscope. After evaluating the slides, percentages of stained cells were calculated by counting at least 1,000 cells (8,9). For ER, C-erb-B2, E-cadherin, bcl-2, and p53, a slide staining of more than 10 % was accepted as a positive expression (1, 5, 10, 11). Nuclear staining for ER and p53, cytoplasmic staining for bcl-2, membranous staining for C-erb-B2 and E-cadherin were accepted as positive (5, 10, 11). C-erb-B2 was graded between +1 and +3 according to the degree of membranous staining. Three findings of positive staining were accepted as overexpression (12). Slides of normal lymph node and colon mucosa positive for bcl-2 and p53, respectively and those of normal breast tissue positive for E-cadherin, ER and C-erb-B2 were used as a positive control (3, 8, 13, 14).

Statistical methods

Statistical calculations were done with SPSS 13 (License number: 31.133.001). Chi-square test for the comparison of qualitative data, log-rank test for the comparison of variables that were used, Kaplan–Meier method for the evaluation of the relationship of these variables with DFS and OS, and Cox-Regression test for multivariate analysis were used.

Results

Representative material was identified for 90 patients with nonmetastatic breast carcinomas. Fourteen patients were excluded from the study due to exclusion criteria, thus the final analysis was made in 76 patients. The mean (±SD) age of patients was 50.76 (±10.46) years. Mean follow-up was 93.58±3.40 months. Sixty-six patients (86.8 %) were diagnosed with invasive ductal carcinoma, 10 patients (13.2 %) with invasive lobular carcinoma. Median DFS (25 %) and OS (16 %) could not be reached. At the time of data analysis, there were 19 recurrences and 12 deaths in the cohort of 76 patients. Of the 19 recurrences, 4 were local and 15 systemic, with 1 patient having both, local and systemic failures.

Data related with patient characteristics and relationships between variables are given in Table 1. There was no statistically significant relationship between bcl-2, E-cadherin, and other variables.

In univariate analyses, we could not find any statistical significance between DFS and all parameters. The only statistical significance was found between OS and both, lymph node indicator and C-erb-B2 ($p=0.029$ and $p=0.031$).

C-erb-B2 overexpression was found only in six of twelve patients who were C-erb-B2 positive. Of these six patients, there were three recurrences at the time of data analysis, and all of them died due to disease progression.

The p53 mutation positivity was observed in seven patients (9.2 %), while no effect on prognostic parameters was encountered ($p=0.419$ for DFS and $p=0.218$ for OS).

Positive bcl-2 and E-cadherin immunostaining were noted in 26.31 % (20 of 76) and 35.52 % (27 of 76) of the specimens. In bcl-2 expressed group, 4 recurrences and 3 deaths were ob-

Tab. 1.Characteristics of patients and relationships Bcl-2 and E--cadherin.

Characteristic	Number	Bcl-2 positive (%)	E-cadherin positive (%)
Age (years)			
=50	42	12 (28.6)	16 (38.0)
<50	34	8 (23.5)	11 (32.3)
Menauposal status			
Post	36	8 (22.2)	11 (30.5)
Pre	40	12 (30.0)	16 (40.0)
Grading			
I	21	4 (19.0)	7 (33.3)
II–III	55	16 (29.0)	20 (36.3)
Tumor size (cm)			
=2	25	7 (28.0)	7 (28.0)
>2	51	13 (25.4)	20 (39.2)
Nodal status			
Positive	49	11 (22.4)	21 (42.9)
Negative	27	9 (33.3)	6 (22.2)
ER			
Positive	30	11 (36.7)	9 (30.0)
Negative	46	9 (19.6)	18 (39.1)
Bcl-2			
Positive	20	–	9 (45.0)
Negative	56	–	18 (32.1)
E-cadherin			
Positive	27	9 (36.0)	–
Negative	49	11 (22.4)	–

ER – estrogen receptor

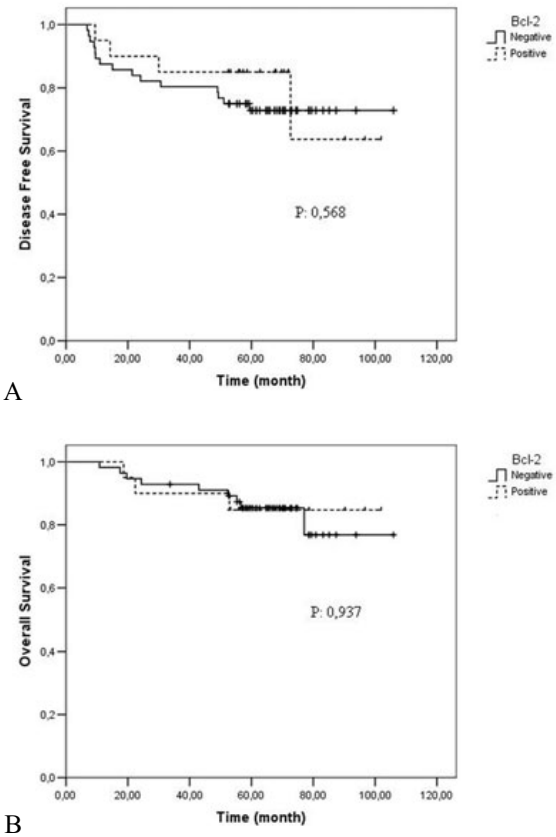


Fig. 1. Kaplan–Meier curves of (a) disease-free survival and (b) overall survival E-cadherin positive and negative breast cancer patients.

served. Recurrence and death ratio were not different from the non-expressed group (recurrence of 20 % versus 27 %; p=0.568 and death of 15 % versus 16 %; p=0.937). In E-cadherin positive group, 6 recurrences were observed and all of these patients died. Recurrence and death ratios at the time of analyses were not different from those in non-expressed group (recurrence of 23 % versus 27 %; p=0.760 and death of 23 % versus 13 %; p=0.281). Kaplan–Meier curves for DFS and OS are presented for the expression of both, bcl-2 and E-cadherin (Figs 1 a, b and Figs 2 a, b) while the expression of bcl-2 and E-cadherin was linked neither to recurrence nor death (p=0.568, p=0.937 for bcl-2, and p=0.760, p=0.281 for E-Cadherin).

A multivariate analysis was performed with variables that were between p=0.05 and p=0.10 with univariate analysis. Effects of variables of multivariate analysis on DFS and OS are shown in Table 2.

There was a significant relationship between lymph node involvement and both, DFS and OS. The recurrence was increased 3.22 times and death was increased 9.74 times in cases with positive lymph nodes.

Discussion

Metastases are seen in one third of breast cancer cases not receiving adjuvant chemotherapy. The researches on prognostic factors are aimed at assessment of patients at risk of metastasis development as well as at protection from toxicity of chemotherapies, which may be unnecessary due to the lack of potential benefit. It is clear that we need new prognostic indicators in addition to those currently used.

We found only six patients (7.89 %) who were C-erb-B2-positive in our study. Recurrences were observed in half of these cases, and all of these patients died. Some studies stated that C-erb-B2 positivity was an independent prognostic factor (12, 15). Nevertheless, it is usually emphasized that C-erb-B2 positivity has a strong predictive value because of the insensitivity to cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) therapy as well as due to response to trastuzumab. We could not use trastuzumab because it was not licensed in our country at the time of adjuvant settings. Thus, it seems it might have a strong prognostic value for our patients.

We determined E-cadherin expression in 27 cases (35.52 %) in our study. We did not determine a relationship between E-cadherin mutation and outcome of our patients. We did not determine a relationship between E-cadherin mutation and DFS and/or OS in our patients. In the subgroup analysis, there was no relationship between E-cadherin expression and other variables. It is stated that E-cadherin mutation can cause an increase in the development of invasion and metastasis because of the weakening of cell-cell connections (16). Rahka et al. (13) followed 1665 patients for a mean of 56 months and suggested that the E-cadherin mutation could be an independent factor for DFS and

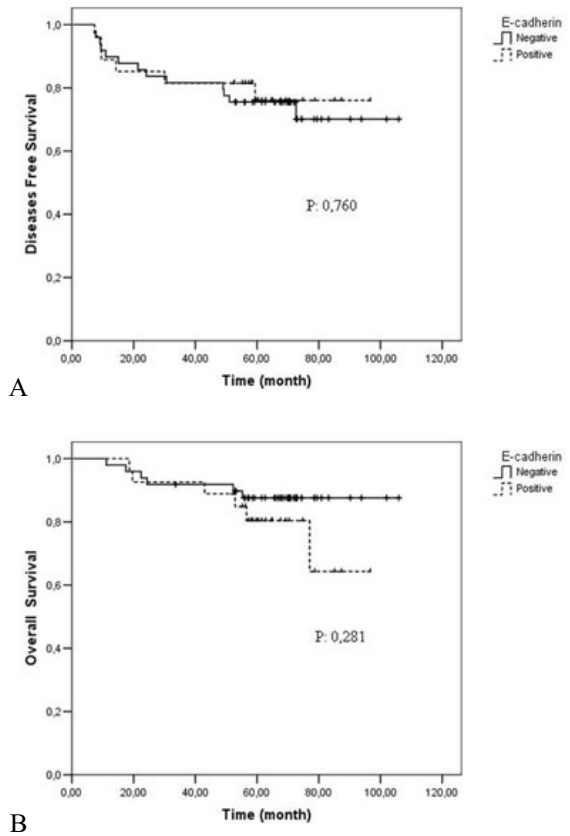


Fig. 2. Kaplan–Meier curves of (a) disease-free survival and (b) overall survival Bcl–2 positive and negative breast-cancer patients.

OS. Nevertheless, similarly as in our study, most studies determined no relationship (17–19). Anyhow, a fourteen-year follow-up study reported that E-cadherin expression was associated with DFS decrease, and a subgroup analysis determined a relationship between tumor grade and advanced age (14). Patients without lymph node involvement were enrolled into the study and they received no adjuvant therapy. We postulated that the treatment could have decreased the difference due to E-cadherin mutation. Although a high rate of E-cadherin mutation is seen in breast cancer patients, according to our study and contradictions in literature, we think that it might not be a prognostic factor in patients receiving adjuvant chemotherapy. Although, we could find no statistical difference in E-cadherin mutated patients in our study and all patients with recurrence died, we consider the presented facts very interesting.

The resistance to apoptosis could cause neoplastic development (20). Bcl-2 is the most important antiapoptotic protein. In our study, bcl-2 expression was determined positive in 20 patients (26.31 %) but it was of no effect on the prognosis. Our results were generally compatible with literature (21, 22). In a retrospective study where CMF were used for adjuvant therapy, it was reported that bcl-2 expression was an independent prognostic factor (8). In our study, anthracycline-based adjuvant chemotherapy might eliminate this importance. In another positive

Tab. 2. The effects of variables on disease-free survival and overall survival in multivariate analysis.

Variables	Disease free survival	Overall survival
	RR (%95 CI) p	RR (%95 CI) p
Estrogen receptors	0.54 (0.20–1.49) 0.23	0.64 (0.18–2.24) 0.49
Bcl-2	0.97 (0.31–3.00) 0.96	1.49 (0.37–5.92) 0.56
E-cadherin	0.60 (0.22–1.64) 0.32	1.18 (0.37–3.78) 0.77
C-erb-B2	3.94 (1.09–14.13) 0.03*	7.17 (1.69–30.37) 0.007*
Nodal status	3.22 (1.02–10.10) 0.04*	9.74 (1.15–82.07) 0.03*

* statistically significant

study, it was reported that bcl-2 expression caused a decrease in DFS and OS (23). However, in this study, cells stained more than 30 % were accepted as positive. Our study suggests that bcl-2 could not have had a prognostic value because 10 % staining had been accepted as positive. Recently, in a meta-analysis of 18 studies investigating the role of bcl-2 expression on a sample size greater than 100, the hazard ratios (HR) were 1.58 for DFS and 1.37 for OS (in our study HR: 1.49) (24). In our patients, the lack of prognostic importance may be due to the small sample size.

Similarly as bcl-2, another factor playing a role in apoptosis regulation is p-53, which activates the apoptotic path. Mutant type p53 allows cell proliferation by losing this function (4, 23). In our study, p-53 mutation was determined to be lower than in literature (9.21 %). We do not know that why different studies reported different p53 mutation rates. The reason could lie either in race differences or in the use of different techniques. We suggest that p53 could not be a prognostic factor for breast cancer because of different mutation rates in different studies.

According to the results of our small study, E-cadherin, bcl-2, and p53 were not effective in the prognosis of breast cancer patients. In addition, p53 overexpression was determined in a limited number patient. In order to individualize the treatment of patients with breast cancer, we need more studies that would evaluate factors of possibly greater prognostic value. Studies also should include more patients and long follow-up periods.

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