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RESEARCH ARTICLE

# Prognostic value of bcl-2 expression among women with breast cancer in Libya

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**Abstract** We studied the association of the immunohistochemical bcl-2 expression in Libyan breast cancer with clinicopathological variables and patient outcome. Histological samples from 170 previously untreated primary Libyan breast carcinoma patients were examined. In immunohistochemistry, the NCL-L-bcl-2-486 monoclonal antibody was used. Positive expression of bcl-2 was found in 106 patients (62.4 %). The bcl-2 expression was significantly associated with estrogen receptor ($p<0.0001$) and progesterone receptor positive tumors ($p=0.002$), small tumor size ($p<0.0001$), low tumor grade ($p<0.0001$), negative axillary lymph nodes ($p<0.0001$), early stages ($p=0.001$), and low risk of metastasis ($p<0.0001$). Positive expression was also associated with older patients (>50 years; $p=0.04$). Histological subtypes and family history of breast cancer did not have significant relationship with bcl-2. Patients with positive expression of bcl-2 had lower recurrence rate than bcl-2-negative patients and better survival after median follow-up of 47 months. Patients with high bcl-2 staining were associated with the best survival. The role of bcl-2 as an independent predictor of disease-specific survival was assessed in a multivariate survival (Cox) analysis, including age, hormonal status, recurrence, histological grade, and clinical stage variables. Bcl-2 ($p<0.0001$) and clinical stage ($p=0.016$) were independent predicators of disease-specific survival. For analysis of disease-free survival, the same variables were entered to the model and only bcl-2 proved to be an independent predictor ($p=0.002$). Patients with positive expression of bcl-2 were associated with low grade of malignancy, with lower recurrence rate, with lower rate of death, and with longer survival time. Bcl-2 is an independent predictor of breast cancer outcome, and it provides useful prognostic information in Libyan breast cancer. Thus, it could be used with classical clinicopathological factors to improve patient selection for therapy.

**Keywords** bcl-2 expression · Libyan female breast cancer · Prognosis

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## Introduction

In Libya, breast carcinoma is the most common malignant tumor in women [1]. The patients often present with advanced disease, have early disease recurrence, and are associated with high mortality [1, 2]. The mean age of breast

cancer patients in Libya was 46 years which was almost identical to that of Africa or Middle East and North Africa region but much lower than in Europe [3–5].

The clinical course of breast carcinoma is partially unpredictable despite improvements in diagnosis and therapy. The patient outcome with neoplastic disease in terms of local recurrence, distant metastases, and progression, is the major focus of several studies aimed to identifying the most reliable prognostic factors.

At present, breast cancer prognosis is evaluated on the basis of clinicopathological features. These are powerful independent prognosticators [6] but may be only crude measures of the biological behavior of a tumor. Therefore, it is valuable to find other prognosticators which can support these factors and can then be used to help in evaluating patient risks and selection of treatment.

Apoptosis (programmed cell death) is a physiological process in which cells die after various types of damage. Tumor cells may interfere with this mechanism by activating genes which inhibit apoptosis [7, 8]. The most important antiapoptotic protein is bcl-2 [9]. Bcl-2 is expressed in many types of normal tissue and belongs to a family of proteins which regulate apoptosis [10].

Bcl-2 expression in human tumors could be expected to be associated with an aggressive phenotype and to confer resistance to those forms of treatments inducing cell death. This is actually true for some human tumor types, such as aggressive non-Hodgkin's lymphoma [11]. However, in other human tumors including breast carcinoma, bcl-2 expression has been mainly associated with favorable prognosis [12–20].

The aim of this study was to investigate bcl-2 protein immunohistochemistry (IHC) and its association with clinicopathological variables and patients outcome in Libyan breast carcinoma.

## Patients and methods

### Clinicopathological data

The eligibility criteria for patients included the availability of paraffin blocks for analysis of immunostaining and complete data on follow-up.

There were 215 eligible patients with breast cancer diagnosed between years 2000 and 2007 in the National Oncology Institute, Sabratha, and Tripoli Medical Center, Tripoli, Libya.

Immunohistochemical staining could not be evaluated in 45 patients because histological samples were used for diagnostic purposes or sections cut for IHC had detached from slides before or during immunohistochemical staining.

The mean age at diagnosis of the remaining 170 patients was 45.1 years (range, 21–80 years). Age distribution is shown in Fig. 1.

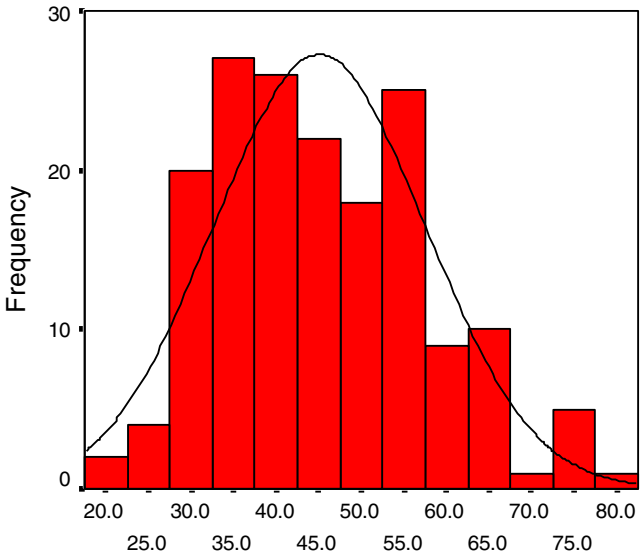


**Fig. 1** Age distribution among women with breast cancer in Libya ($n$=170)

The clinicopathological and clinical data were collected from the patient's files. The collected data included age, menopausal status, family history, hormonal status, histological type, tumor size, lymph node status, tumor stage, histological grade, treatment type, and follow-up.

Tumor staging of breast carcinoma was evaluated according to TNM classification data [21]. The series comprised 136 (80 %) invasive ductal carcinomas and 34 (20 %) breast carcinomas of other histological types. The hormone receptor status of the tumors was tested at the time of surgery. Monoclonal antibody was used for the determination of hormone receptors. Interpretation was done with Allred score method [22]. Tumors were positive for estrogen and progesterone receptors in 126 (74.1 %) and 103 (60.6 %) patients, respectively. The details of clinicopathological variables are shown in Table 2.

### Treatment and follow-up

One hundred twenty-six (74.1 %) patients were treated by modified radical mastectomy and axillary dissection, 21 (12.4 %) patients received neoadjuvant chemotherapy with modified radical mastectomy and axillary lymph node dissection. Lumpectomy was done in three patients, and simple mastectomy in one patient. No therapeutic surgical intervention was done for 19 (11.2 %) patients with metastasis at time of diagnosis (diagnosis with core biopsy). Adjuvant and neoadjuvant chemotherapy with anthracycline was given to 131 (77.1 %) patients while 29 (17.1 %) patients received combined chemotherapy based on anthracycline and taxans, and three patients received chemotherapy regime based on cyclophosphamide, methotrexate, and 5-FU. No chemotherapy was given to five patients with early

stage, and two patients were unfit to receive chemotherapy. Hormonal treatment (tamoxifen) was given to 126 (74.1 %) hormone receptor-positive patients. Axillary radiotherapy was given to node-positive patients ($n$=130).

The patients were followed up until death or to the end of the observation period. The median follow-up duration was 47.2 months (range, 5–125 months). The patients were seen at 3- to 6-month intervals. The bone isotope, chest, and abdominopelvic CT scans were performed every 6–12 months.

At the end of the follow-up period, 101 (59.4 %) patients were alive, 60 (35.5 %) had experienced disease recurrence, and 69 (40.6 %) had died.

### Immunohistochemical staining

Bcl-2 immunostaining was performed for all specimens using tissues obtained before treatment. Formalin-fixed, paraffin-embedded tissues were sectioned at 3 μm. The sections were de-waxed in xylene and rehydrated in graded ethanol. Novocastra peroxidase (3 % hydrogen peroxide) was used to neutralize endogenous peroxidase activity of the samples for 10 min. Bcl-2 staining was carried out by using mouse monoclonal antibody (bcl-2 oncoprotein: clone NCL-L-bcl-2-486, Novocastra Laboratories, Newcastle upon Tyne, NE 12 BEW, UK) at a 1:100 dilution, and the samples were incubated for 30 min at 25 °C. To reveal the binding of primary antibody by peroxidase staining, the substrate/chromogen, 3,3-diaminobenzidine (DAP), prepared from Novocastra DAP Chromogen and NovaLink DAP Substrate Buffer (Polymer) were used. Finally, the sections were counterstained with hematoxylin, dehydrated, and cleared with xylene, and the sections were mounted for examination. T cell lymphocytes were used as positive internal control and fibroblasts as negative internal control.

### Staining assessment

All slides were evaluated without knowledge of the patient's outcome. The slides were examined by one pathologist (BK) in a Nikon microscope (Eclipse E 600; Japan). Malignant cells with cytoplasmic staining were considered to be positive. A minimum of 500 cells were evaluated and counted per slide. The evaluation of bcl-2 expression was done accordingly to the following quantification model: bcl-2 negative—no tumor cells stain or weak heterogenous positive stain in less than 10 % of tumor cells and bcl-2 positive—more than 10 % of tumor cells stained [23]. In addition to the evaluation of the proportion of stained cells, bcl-2 staining intensity was evaluated as the average intensity of staining: 0=no bcl-2 staining, 1=weak staining intensity, 2=moderate staining intensity, and 3=strong staining intensity.

To test the reproducibility of expression profiling of bcl-2, another investigator has evaluated the same samples without knowledge of the first evaluation or other data. The majority of samples were stained for bcl-2 expression. The concordance rate between two evaluators was 90.4 %, which indicated high reproducibility rate between both investigators either as the proportion of stained cells (− or +) and/or staining intensity (0 to 3). The evaluation of the pathologist (BK) was used in statistical analysis. Laboratory work for bcl-2 immunostaining was done at the Department of Pathology, Salah Azaiz Cancer Institute, Tunis, Tunisia.

### Ethical consideration

This study is part of the breast cancer studies, which have gotten permission from the local ethical committee at the National Cancer Institute at Sabratha, Libya.

### Statistical analysis

The variables of the material were grouped into logical classes and descriptive statistics calculated for the continuous variables using SPSS 19.0 for Windows (SPSS, Inc., Chicago, IL). Frequency tables were analyzed using the Chi-square test, with likelihood ratio (LR), or Fisher's exact test to assess the significance of the correlation between the categorical variables. For survival analysis, only 151 cases with stages 1, 2, and 3 were included and stage 4 was excluded to justify the comparison with other studies. Kaplan–Meier curves were plotted, and differences between the curves analyzed using the log-rank test. Student's $t$ tests and ANOVA were also used to test differences between the groups.

Multivariate survival analysis for the outcome measure (overall survival, disease-specific survival (DSS) and disease-free survival (DFS)) was carried out using Cox's proportional hazards model in a backward stepwise manner with the log-LR significance test, using the default values for enter and exclusion criteria.

The assumption of proportional hazards was controlled by log-minus-log survival plots. In all tests, values of $p<0.05$ were regarded as statistically significant.

## Results

### Bcl-2 expression

Bcl-2 expression variables ($n$=170) is shown in Table 1. Positive bcl-2 expression was found in 62.4 % of tumors. Of the positive samples, 72.2 % were estrogen receptor positive (ER+), and 71.8 % were progesterone receptor positive (PR+). The majority of patients with positive bcl-2 expression demonstrated moderate-to-strong bcl-2 staining intensity. Examples of bcl-2

**Table 1** Bcl-2 expression in Libyan breast cancer ($n$=170)

| Variables | Number of patients | Percent |
|---|---|---|
| Bcl-2 expression | | |
| Negative | 64 | 37.6 |
| Positive | 106 | 62.4 |
| Bcl-2 staining intensity | | |
| Weak | 37 | 21.8 |
| Moderate | 35 | 20.6 |
| Strong | 34 | 20.0 |

expression (positive versus negative) and bcl-2 staining intensity are shown in Fig. 2.

## Bcl-2 expression with clinicopathological variables

The associations between bcl-2 expression and clinicopathological variables are shown in Table 2. Positive expression of bcl-2 was significantly associated with estrogen receptor-positive tumors ($p$<0.0001), progesterone receptor positive tumors ($p$=0.002), small tumor size ($p$<0.0001), negative lymph nodes ($p$<0.0001), low tumor grade ($p$<0.0001), early stages ($p$=0.001), low risk of metastasis ($p$<0.0001), and low rate of recurrence ($p$<0.0001).

Older patients (>50 years) tended to have tumors with positive bcl-2 expression than younger patients ($p$=0.04). Menopausal status, family history of breast cancer, histological subtypes, or type of surgery did not have significant relationship with bcl-2.

## Patient outcome

### *Bcl-2 expression and survival outcome*

Univariate survival analysis (survival rates) with bcl-2 expression is shown in Table 3. The survival rate was 89.3 % in patients with positive expression of bcl-2 and 16.7 % in patients with negative expression of bcl-2 ($p$<0.0001).

The analysis using Kaplan–Meier curves on immunohistochemical bcl-2 indicated that longer survival time was associated with positive expression of bcl-2 (Fig. 3a).

### *Bcl-2 expression levels and survival outcome*

A direct association between the intensity of bcl-2 staining and survival was identified (Fig. 3b). Patients who had tumors with high bcl-2 staining intensity (moderate-to-strong bcl-2 staining intensity) were associated with the best survival and showed no death during the follow-up period.

### *Multivariate survival analysis*

Survival analysis was done according to the Cox model for all breast cancer patients with lymph node status excluded from analysis.

The role of bcl-2 as an independent predictor of DSS was assessed in a multivariate survival (Cox) analysis, including

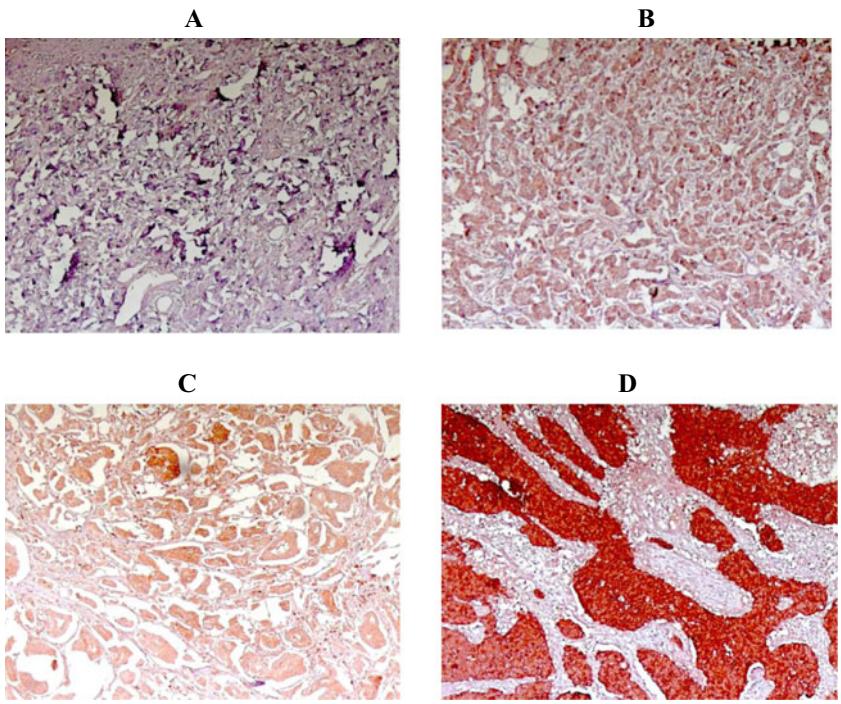


**Fig. 2** Immunohistochemical evaluation of bcl-2 staining intensity: **a** no bcl-2 staining, **b** weak staining intensity, **c** moderate staining intensity, and **d** strong staining intensity

**Table 2** The association between immunohistochemical bcl-2 expression and clinicopathological variables ($n$=170)

| Clinicopathological variables | Number of patients | Bcl-2 expression (%) | | $p$ value | Bcl-2 staining intensity (%) | | | $p$ value |
|---|---|---|---|---|---|---|---|---|
| | | Negative | Positive | | Weak | Moderate | Strong | |
| Age (years) | | | | | | | | |
| <50 | 109 | 43.1 | 56.9 | 0.04 | 21.1 | 21.1 | 14.7 | 0.081 |
| ≥50 | 61 | 27.9 | 72.1 | | 23.0 | 19.7 | 29.5 | |
| Menopausal status | | | | | | | | |
| Premenopausal | 106 | 41.5 | 58.5 | 0.17 | 21.7 | 21.7 | 15.1 | 0.20 |
| Postmenopausal | 64 | 31.2 | 68.8 | | 21.9 | 18.8 | 28.1 | |
| Hormonal status (ER) | | | | | | | | |
| Positive | 126 | 27.8 | 72.2 | <0.0001 | 23.8 | 23.0 | 25.4 | <0.0001 |
| Negative | 44 | 65.9 | 34.1 | | 15.9 | 13.6 | 4.5 | |
| Hormonal status (PR) | | | | | | | | |
| Positive | 103 | 28.2 | 71.8 | 0.002 | 25.2 | 22.3 | 24.3 | 0.015 |
| Negative | 67 | 52.2 | 47.8 | | 16.4 | 17.9 | 13.4 | |
| Histopathology type | | | | | | | | |
| IDC | 136 | 36.8 | 63.2 | 0.63 | 20.6 | 19.9 | 22.8 | 0.27 |
| Other types | 34 | 41.2 | 58.8 | | 26.5 | 23.5 | 8.8 | |
| Tumor size | | | | | | | | |
| T1 | 15 | 6.7 | 93.3 | <0.0001 | 6.2 | 46.7 | 40.4 | <0.0001 |
| T2 | 58 | 13.8 | 86.2 | | 19.0 | 27.5 | 39.7 | |
| T3 | 61 | 52.5 | 47.5 | | 22.6 | 15.2 | 9.7 | |
| T4 | 36 | 63.9 | 36.1 | | 30.5 | 5.6 | 0.0 | |
| Lymph node status[a] | | | | | | | | |
| Positive | 130 | 43.8 | 56.2 | <0.0001 | 23.8 | 16.2 | 16.2 | <0.0001 |
| Negative | 35 | 8.6 | 91.4 | | 14.3 | 40.0 | 37.1 | |
| Histological grade | | | | | | | | |
| Grade 1 | 22 | 13.6 | 86.4 | <0.0001 | 22.7 | 31.8 | 31.8 | <0.001 |
| Grade 2 | 79 | 29.1 | 70.9 | | 20.3 | 25.3 | 25.3 | |
| Grade 3 | 69 | 55.1 | 44.9 | | 23.2 | 11.6 | 10.1 | |
| Clinical stage | | | | | | | | |
| Stage 1 | 11 | 0.0 | 100.0 | <0.0001 | 9.1 | 36.4 | 54.5 | <0.0001 |
| Stage 2 | 48 | 8.3 | 91.7 | | 20.8 | 41.7 | 29.2 | |
| Stage 3 | 92 | 47.8 | 52.2 | | 27.2 | 9.8 | 15.2 | |
| Stage 4 | 19 | 84.2 | 15.8 | | 10.5 | 5.3 | 0.0 | |
| Metastases at diagnosis | | | | | | | | |
| M0 | 151 | 31.8 | 68.2 | <0.0001 | 23.8 | 21.9 | 22.5 | <0.0001 |
| M1 | 19 | 84.2 | 15.8 | | 10.5 | 5.3 | 0.0 | |
| Recurrence during follow-up (local or distant) | | | | | | | | |
| No | 91 | 6.6 | 93.4 | <0.0001 | 27.5 | 31.9 | 34.1 | <0.0001 |
| Yes | 60 | 70.0 | 30.0 | | 13.3 | 11.7 | 5.0 | |

[a] One hundred sixty-five patients had lymph node status histologically evaluated

age, hormonal status, recurrence, histological grade, and clinical stage variables. In this multivariate model, the two gates approach of bcl-2 (positive vs. negative) retained its significance as an independent predictor of DSS with hazard ratio (HR)=12.566 (95 % CI, 4.365–36.172; $p$<0.0001; negative expression as reference), which was independently predicted also by stage (HR=62.5 (95 % CI, 0.061–0.752); $p$=0.016; high stage as reference). All other variables were removed from the model in stepwise backward approach. When the same model was used (omitting recurrence) to assess the role of bcl-2 as an independent predictor of DFS, again bcl-2 proved to be an independent predictor with HR= 5.974 (95 % CI, 1.918–18.614); $p$=0.002; negative expression as reference) and stage (HR=2.75 (95 % CI, 8.795–36.202); $p$<0.001; high stage as reference).

In Libyan material, survival rate significantly associated with clinical stage, lymph node status, tumor size, and histological grade as shown in Table 4.

**Table 3** Univariate survival analysis of bcl-2 expression variables (only 151 patients with stages 1, 2, and 3 were included)

| Variables | Number of patients | Survival analysis | | | *p* value |
|---|---|---|---|---|---|
| | | Median survival (months) | Mean survival (months) | Survival rate (%) | |
| All patients | 151 | 47.45 | 54.30 | 61.3 | |
| Bcl-2 expression | | | | | |
| Negative | 48 | 27.00 | 27.66 | 16.7 | <0.0001 |
| Positive | 103 | 66.00 | 67.65 | 89.3 | |
| Bcl-2 staining intensity | | | | | |
| Negative | 48 | 27.00 | 27.66 | 16.7 | <0.0001 |
| Weak | 36 | 45.00 | 45.50 | 77.8 | |
| Moderate | 33 | 71.00 | 71.21 | 90.9 | |
| Strong | 34 | 85.50 | 88.24 | 100.0 | |

## Discussion

The identification of prognostic and predictive markers is clinically important, because breast cancer is heterogenous in respect to genetics, and variable in biological and clinical features.

At present, gene expression microarray studies have shown prognostic power, but IHC remains a suitable and powerful method of prognostication in a clinical situation because it is less expensive and more easily applied in clinical context [24].

Bcl-2 expression in breast cancer is associated with favorable prognostic factors, and it predicts a good outcome in early breast cancer and in metastatic disease [25]. Evaluation of bcl-2 in breast cancer may identify a subgroup of patients with good prognosis, who may not benefit from chemotherapy [26].

In the fact, this is the first study to investigate immunohistochemical bcl-2 expression in Libyan breast cancer. Several interesting and important observations were made, all implicating that the immunostaining analyses of bcl-2 in tumor cells provide significant prognostic information. Anyhow, material may be too small for final conclusions. The final conclusions can only be drawn after more intensive studies.

In the current study, positive expression of bcl-2 was found in 62.4 % cancers. These results are in line with the results of other studies [23, 27, 28]. These data show that Libyan cancers have about the same fraction of bcl-2 positive tumors. However, as Libyan tumors have worse prognosis and bcl-2 appears as a superior prognosticator, it may be that from biological point of view we have two types of Libyan breast cancers: bcl-2-positive ones with good prognosis and bcl-2-negative tumors with poor prognosis. However, the bcl-2 expression in breast cancers is widely variable (45 to 93 %) [23, 29].

Population variations can be explained by different ways of quantification: intensity and percentage of positive cells [30] and positive immunostaining reaction of more than 10 % of tumor cells or over 25 % of staining tumor cells [23, 29].

This study shows that positive expression of bcl-2 was associated with a lower grade of malignancy as in hormone receptor-positive tumors, early stages, negative lymph nodes, small tumor size, and low tumor grade.

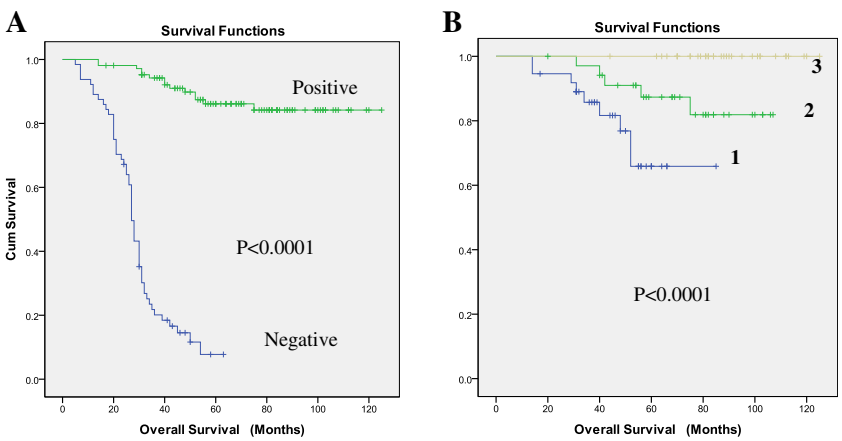


**Fig. 3** Overall survival according to analysis of bcl-2 expression in breast cancer (Kaplan–Meier curves). **a** Bcl-2 expression (positive and negative). **b** Bcl-2 staining intensity (*1*, weak staining intensity; *2*, moderate staining intensity; *3*, strong staining intensity). Only patients with stages 1, 2, and 3 were included ($n$=151)

**Table 4** Univariate survival analysis of clinicopathological variables (only 151 patients with stages 1, 2, and 3 were included)

| Variables | Threshold | Number of patients | Survival analysis | | | *p* value |
|---|---|---|---|---|---|---|
| | | | Median survival (months) | Mean survival (months) | Survival rate (%) | |
| All patients | | 151 | 47.45 | 54.30 | 61.3 | |
| Clinical stage | Stage 1 | 13 | 82.00 | 80.38 | 93.5. | <0.0001 |
| | Stage 2 | 46 | 72.50 | 74.63 | 90.3 | |
| | Stage 3 | 92 | 33.50 | 41.47 | 48.9 | |
| Lymph node status | Negative | 34 | 70.00 | 72.12 | 94.1 | <0.0001 |
| | Positive | 117 | 43.00 | 49.92 | 58.1 | |
| Tumor size | T1 | 15 | 79.20 | 82.00 | 89.7 | <0.0001 |
| | T2 | 58 | 67.00 | 67.41 | 86.7 | |
| | T3 | 56 | 39.00 | 44.07 | 53.6 | |
| | T4 | 22 | 28.50 | 33.05 | 22.7 | |
| Histology grade | Grade 1 | 22 | 61.45 | 63.00 | 81.8 | <0.0001 |
| | Grade 2 | 70 | 64.61 | 65.00 | 78.6 | |
| | Grade 3 | 59 | 32.00 | 40.98 | 45.8 | |

On other hand, bcl-2 negativity was more common with worse prognostic variables. This explains why bcl-2 expression has been consistently associated with better prognosis for breast cancer in previous studies [7, 16–20]. Additionally, these data suggest that Libyan breast cancer patients with positive expression of bcl-2 had a favorable prognosis, and with negative expression of bcl-2, had a worse prognosis.

This study shows that the expression of bcl-2 and the expression of ER and PR were highly associated. This is in agreement with previously published data [16, 17, 19]. These observations support the hypothesis that bcl-2 expression in breast carcinoma may be an estrogen receptor-regulated phenomenon and PR is under estrogen regulation via ER [31–33]. The bcl-2 oncoprotein has been shown to be expressed in 45–93 % of breast cancer; whereas, 70 % of breast cancer tumors are ER positive, and most ER positive is also PR positive [27, 28, 34]. Binding of estrogen with ER induces phosphorylation and dimerization followed by transcription of a variety of genes, including growth and angiogenic factors as well as PR and bcl-2 [35]. Linke et al. [35] found that the DFS and overall survival for patients with ER+/PR− tumor and who had low bcl-2 expression were not significantly different from ER-negative tumor. However, if either PR was present or the bcl-2 expression was high, the ER-positive patients had a better prognosis [35].

On other hand, high expression of bcl-2 may be indicative of an intact ER pathway that is driving tumor growth and might be responsible for more response to hormonal therapy [35]. The benefit of tamoxifen is high in the patients with ER+/bcl-2+ tumors than those who had ER+/bcl-2− [36]. The strong association between bcl-2, ER, and PR may suggest co-targeting of these molecules in hormone receptor-positive breast cancer and might play a role as beneficial planning response of these tumors to chemotherapy [26].

High bcl-2 expression is associated with small tumor size [12–14, 37]. Bcl-2 expression may create a restrictive environment for the expansion of genetically unstable and potentially malignant cells, causing a delay in tumor progression [37, 38]. The bcl-2 positivity in the present study is high in the small tumor size (93.3 % in T1) and decrease with the increasing size of tumors (36.1 % in T4).

A significant association between expression of bcl-2 and differentiation was reported in this study. The same findings were reported by Hun Lee et al. [37] and others [12, 16–20]. The expression of bcl-2 changes from high expression in low-grade tumors with low apoptotic indices to low expression in high-grade tumors with high apoptotic indices [16].

Apoptosis is controlled by the ratio of various bcl-2 family members [39]. When levels of apoptosis promoters (bax and bcl-$X_S$) increase, apoptosis is accelerated; whereas when the inhibitors of apoptosis (bcl-2 and bcl-$X_L$) increase, the cells are inclined to be resistant to apoptosis in response to external stimuli. Bcl-2 plays an important role in the inhibition of apoptosis stimulated by different factors, such as irradiation, chemotherapeutic agents, and withdrawal of growth factors [40]. In cell culture, high concentrations of estrogen could induce apoptosis directly through a FAS/FASL pathway [41]. However, physiological concentrations of estradiol could induce apoptosis in both cell culture and animal models [42]. Furthermore, in MCF-7 cells, estrogen has been shown to inhibit cytotoxic drug-induced apoptosis through up-regulating bcl-2 levels [43].

In breast carcinoma, bcl-2 expression has been shown to be a favorable prognostic factor, strong associate with ER status, and predictor of response to endocrine therapy [44].

This may explain the response to endocrine therapy in patients with bcl-2-positive tumors.

Bukholm et al. [45] found a significant inverse correlation between bcl-2 expression determined by methods of IHC and nodal invasion. Our finding confirms this result and others [46]. We observed that the expression of bcl-2 was associated with tumors without lymph nodes metastasis.

In addition, we observed that the bcl-2 expression was associated with older age (>50 years). This result is in agreement with results from the studies of Daidone et al. [47] and others [46, 48]. In Africa, the premenopausal breast cancer is more common and more aggressive than the postmenopausal breast cancer [5].

Our findings confirm this result. Libyan young women with breast cancer tended to have tumors with higher S-phase fraction than older women with breast cancer [49]. Breast tumors of elderly patients were associated with more favorable pathological phenotype than those of younger age group [47].

One of the important observation in this study revealed that patients who had tumors with positive bcl-2 were associated with lower incidence of disease recurrence, lower rate of death, and longer survival time. Patients who had tumors with negative bcl-2 were associated with higher incidence of disease recurrence, higher rate of death, and shorter survival time.

On other hand, patients who had tumors with high bcl-2 staining intensity (from moderate to strong) were associated with the best survival. These data also suggest that Libyan patients with a higher bcl-2 expression had a favorable prognosis and patients with negative expression of bcl-2 had a worse prognosis.

Cox regression analysis showed that Bcl-2 and clinical stage were independent predicators of disease-specific survival. For DFS, only Bcl-2 proved to be an independent predictor. Others [9, 12, 20], also in Cox analysis confirmed that the bcl-2 is an independent and powerful prognostic marker in breast cancer.

Study of the bcl-2 expression in Libyan patients showed that the bcl-2 is a powerful prognosticator in both univariate and multivariate statistical analysis, and it can be added to traditional prognostic factors in evaluation of the prognosis.

Furthermore, the traditional variables, such as lymph node status, tumor size, and histological grade show powerful prognosticators in this study. In comparing expression of bcl-2 and these variables, the results suggest that the bcl-2 appears also as a powerful prognosticator and has value that is equivalent to the three above-mentioned traditional variables.

Comparison between our study from Libya and another from Europe (Jalava et al.) [50] is interesting in light of the differences in African and European breast cancer. These differences may be associated with the differences in health care or be explained by differences in biology, genetics, or etiology. The results seem to suggest that even through bcl-2 expression is a favorable sign in both studies, the prognostic value is of a different degree.

The difference between 5-year survival rates was about 70 % between bcl-2-positive and bcl-2-negative breast cancer among the Libyan patients. Among the European patients, this difference was 20 % at the highest but can only be seen in postmenopausal lymph node-positive (N+) patients.

In Europe (Finland), bcl-2 can only be used as a prognosticator in the latter patient group.

The question arises whether the difference is caused by stages of development in medical care between Europe and Africa (Libya) or whether there are other causes (including genetic differences).

The differences found between Afro-Americans and Caucasian Americans with breast cancer seem to suggest the latter [51, 52]. However, final conclusions on the issue can only be drawn after more intensive studies done in various parts or Africa, Europe, and America.

## Conclusions

Positive expression of bcl-2 was found in 62.4 % of patients. Patients with positive expression of bcl-2 had low-grade tumors, lower incidence of disease recurrence, lower rate of death, and longer survival time. Bcl-2 is an independent predictor of breast cancer outcome, and it is useful in providing prognostic information in Libyan breast cancer. Libyan breast cancers can be classified into two groups: bcl-2-positive ones with good prognosis and bcl-2-negative tumors with poor prognosis. Thus, it could be used with classical clinicopathological factors to improve the selection of patients for different treatment approaches.

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**Conflict of interest** None

## References

1. Abussa A. Hospital cancer registry annual report. Sabratha, Libya: African Oncology Institute; 2007. p. 18–20.
2. El Mistiri M, Verdecchia A, Rashid I, El Sahli N, El Mangush M, Federico M. Cancer incidence in eastern Libya: the first report from the Benghazi Cancer Registry, 2003. Int J Cancer. 2007;120(2):392–7.
3. Abulkhair O, Saghir N, Sedky L, et al. Modification and implementation of NCCN guidelines on breast cancer in the Middle East

and North Africa region. J Natl Compr Canc Netw. 2010;8 Suppl 3:S8–S15.
4. Stapleton JM, Mullan PB, Dey S, et al. Patient-mediated factors predicting early- and late-stage presentation of breast cancer in Egypt. Psycho-Oncology. 2011;20(5):532–7.
5. Boder J, Abdalla F, Elfageih M, et al. Breast cancer patients in Libya: comparison with European and central African patients. Oncology Letters. 2011;2:323–30.
6. Galea MH, Blamey RW, Elson CE, Ellis IO. The Nottingham Promostic Index in primary breast cancer. Breast Cancer Res Treat. 1992;22:207–19.
7. Kumar R, Vadlamudi RK, Adam L. Apoptosis in mammary glande and cancer. Endocrine-Related Cancer. 2000;7:257–69.
8. Krajewski S, Krajewski M, Turner BC, et al. Prognostic significance of apoptosis regulators in breast cancer. Endocrine-Related Cancer. 1999;6:29–40.
9. Arun B, Kilic G, Yen C, et al. Correlation Bcl-2 and p53 expression in primary breast tumors and corresponding metastatic lymph nodes. Cancer. 2003;98(12):2554–9.
10. Adms JM, Cory S. The Bcl-2 protein family: arbiters of cell survival. Science. 1998;28:1322–6.
11. Hermine O, Hajoun C, Lepage E, et al. Prognostic significance of bcl-2 protein expression in aggressive non-Hodgkin's lymphoma. Group d Etude des Lymphomes de l Adulte (GELA). Blood. 1996;87:265–72.
12. Callagy GM, Pharoah PD, Pinder SE, et al. Bcl-2 is a prognostic marker in breast cancer independently of the Nottingham Prognostic Index. Cancer Res. 2006;12:2468–75.
13. Milano A, Lago LD, Sotiriou C, et al. What clinicians need to know about antioestrogen resistance in breast cancer therapy? Eur J Cancer. 2006;42:2696–705.
14. Lipponen P, Pietilainen T, Kosma VM, Aaltomaa S, Eskelinen M, Syrjanen K. Apoptosis suppressing protein bcl-2 is expressed in well-differentiated breast carcinomas with favourable prognosis. J Pathol. 1995;177:49–55.
15. Jager JJ, Jansen RLH, Arends JW. Clinical relevance of apoptosis markers in breat cancer not yet clear. Apoptosis. 2002;7:361–5.
16. Daidone MG, Luisi A, Veneroni S, et al. Clinical studies of bcl-2 and treatment benefit in breast cancer patients. Endocr Relat Cancer. 1999;6:61–8.
17. Le MG, Mathieu M-C, Douc-Rasy S, et al. c-myc, p53 and bcl-2, apoptosis-related genes in infiltrating breast carcinomas: evidence of a link between bcl-2 protein over-expression and a lower risk of metastasis and death in operable patients. Int J Cancer. 1999;84:562–7.
18. Silvestrini R, Benini E, Veneroni S, et al. P53 and bcl-2 expression correlated with clinical outcome in series of node-positive breast cancer patients. J Clin Oncol. 1996;14(5):1604–10.
19. Callagy GM, Webber MJ, Pharoah PD, Caldas C. Met-analysis confirm BCL2 is an independent prognostic marker in breast cancer. BMC Cancer. 2008;8:153.
20. Dawson S-J, Makretsov N, Blows FM, et al. BCL2 in breast cancer: a favourable prognostic marker across molecular subtypes and independent of adjuvant therapy received. Br J Cancer. 2010;103:668–75.
21. AJCC: 5th Edition AJCC Cancer Staging Manual. Philadelphia: Lippincott-Raven; 1997.
22. Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol. 1998;11:155–68.
23. Zhang G-J, Kimijima I, Abe R, et al. Correlation between the expression of apoptosis-related bcl-2 and p53 oncoprotein and the carcinogenesis and progression of breast cancer. Clin Cancer Res. 1997;3:2329–35.
24. Sorlie T, Perou CM, Tibshirani R, Eystein Lonning P, Borresen-Dale AL, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implication. Proc Natl Acad Sci USA. 2001;98:10869–74.
25. Trere D, Montanaro L, Ceccarelli C, et al. Prognostic relevance of a novel semiquantitative classification of Bcl3 immunohistochemical expression in human infiltrating ductal carcinomas of the breast. Annals Oncolog. 2007;18(6):1004–14.
26. Nadler Y, Camp RL, Giltnane JM, et al. Expression patterns and prognostic value of Bag-1 and Bcl-2 in breast cancer. Breast Cancer Res. 2008;10(35):1186.
27. Van Slooten HJ, Clahsen PC, van Dierendonck JH, et al. Expression of bcl-2 in node-negative breast cancer is associated various prognostic factors, but does not predict response to onecc course of perioperative. Br J Cancer. 1996;74:78–85.
28. Bottini A, Berruit A, Bersiga A, et al. p53 but not bcl-2 immunostaining is predictive of poor clinical complete response to primary chemotherapy in breast cancer. Clin Cancer Res. 2000;6:2751–8.
29. Tang S-C, Jessalyn B, Murphy S, et al. BAG-1 expression correlates with Bcl-2, p53, differentiation, estrogen and progesterogen receptors in invasive breast carcinoma. Breast Cancer Res Treat. 2004;84:203–13.
30. Kroger N, Milde-Langosch K, Iethof S, et al. Prognostic and predictive effects of immunohistochemical factors in high-risk primary breast cancer patients. Clin Cancer Res. 2006;12(1):159–68.
31. Malamou-Mitsi V, Gogas H, Dafni U, et al. Evaluation of the prognostic and predictive value of p53 andBcl-2 in breast cancer patients participating in a randomized study with dose-dense sequential adjuvant chemotherapy. Annals of Oncol. 2006;17(10):1504–11.
32. Zinkel S, Gross A, Yang E. Bcl-2 family in DNA damage and cell cycle control. Cell Death Differ. 2006;13:1351–9.
33. Gee JM, Robertson JRR, Ellis IO. Immunocytochemical localization of Bcl-2 protien in human breast cancers and its relationship to a series of prognostic markers and response to endocrine therapy. Int J Cancer. 2006;5:619–28.
34. Freedman R, Winer E. Adjuvant therapy for menopusal women with endocrine-sensetive breast cancer. Breast. 2010;19:69–75.
35. Linke SP, Bremer TM, Herold CD, et al. A multimarker model to predict outcome in tamoxifen-treated breast cancer patients. Clin Canc Res. 2006;15:1175–83.
36. Kymionis GD, Konstadoulakis MM, Dimitrakakis CE, et al. Can expression of apptosis genes, bcl-2 and bax, predict survival and responsiveness to chemotherapy in node-negative breast cancer patients? Surg Res. 2001;99(2):161–8. Abstract.
37. Hun Lee K, Ah lm S, Youn Oh D, et al. Progmostic significance of Bcl-2 expression in stage lll breast cancer patients who had receivef doxorubicin and cyclophosphamide followed by paclitaxel as adjuvant chemotherapy. BMC Cancer. 2007;7:63–70.
38. Koman IE, Gurova KV, Kwek SS, et al. Apoptosis inhibitor as a suppressor of tumor progression: expression of Bcl-2 eliminates selective advantages for p53-deficient cells in the tumor. Canc Biol Ther. 2002;1(1):39–44.
39. Reed JC, Miyashita T, Takayama S, et al. Bcl-2 family proteins: regulators of cell death involved in the pathogenesis of cancer and resistance to therapy. J Cell Biochem. 1996;60:23–32.
40. Jaattela M, Benedict M, Tewari M, Shayman JA, Dixit VM. Bcl-x and Bcl-2 inhibit TNF and Fas-induced apoptosis and activation of phospholipase A2 in breast cancer cells. Oncogene. 1995;10:2297–305.
41. Song RX, Mor G, Naftolin F, et al. Effect of long-term estrogen deprivation on apoptotic response of breast cancer cells to 17 beta-estradiol. J Natl Cancer Inst. 2001;93:1714–23.
42. Jordan VC, Liu H, Dardes R. Re: Effect of long-term estrogen deprivation on apoptotic response of breast cancer cells to 17 beta-estradiol and the two faces of Janus: sex steroids as mediators of both cell proliferation and cell death. J Natl Cancer Inst. 2002;94:1173–5.

43. Teixeira C, Reed JC, Pratt MA. Estrogen promotes chemotherapeutic drug resistance by a mechanism involving Bcl-2 proto-oncogene expression in human breast cancer cells. Cancer Res. 1995;55:3902–7.
44. Zhang GJ, Tsuda H, Adachi I, Fukutomi T, Yammamoto H, Hirohashi S. Prognostic indicators for breast cancer patients with one to three regional lymph node metastase, with special reference to alteration in expression levels of bcl-2, p53 and c-erb B-2 protiens. Jpn. J Clin. 1997;27:371–7.
45. Bukhoim IR, Bukholm G, Nesland JM. Reduced expression both Bax and Bcl-2 is idependently associated with lymph node metastasis in human breast carcinomas. APMIS. 2002;110:214–20.
46. Alis Dema, Simona Dragan, Elena Lazar, Danina Munteanu, Sorina Taban, Codruta Lzureanu, T. Nicola, S. Dema. Bcl-2 expression in primary breast carcinomas: correlation with other prognostic factors. Nr. 1-2/2008; pp. 65–71.
47. Daidone MG, Coradini D, Martelli G, et al. Primary breast cancer in elderly women: biological profile and relation with clinical outcome. Crit Rev Oncol Hemat. 2003;45:313–25.
48. Yu B, Sun X, Hong-yan S, et al. J Exp Clin Res. 2010;29:107.
49. Ermiah E, Abdala F, Buhmeida A, et al. Prognostic significance of DNA image cytometry in Libyan breast cancer. Oncology. 2012;83:165–76.
50. Jalava P, Collan Y, Kuopio T, Juntti-patinen L, Kronqvist P. Bcl-2 immunostaining: a way to finding unresponsive postmenopausal N+breast cancer patients. Anti Cancer Res. 2000;20:1213–20.
51. Olopade OI, Fakenthal JD, Dunston G, et al. Breast cancer genetics in African Americans. Cancer. 2003;97:236–45.
52. Loo LW, Wang Y, Flynn EM, et al. Genome-wide copy number alteration in subtypes of invasive breast cancers in young white and African Americans women. Breast Canc Res Treat. 2011;127:297–308.