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Associations between BCL-2 expression and different histopathological prognostic factors in different molecular subtypes of invasive breast carcinoma of no special type

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Summary. Background. Breast cancer is heterogeneous and the existing prognostic classifiers are limited in accuracy, leading to the unnecessary treatment of numerous women. B-cell lymphoma 2 (BCL-2), an anti-apoptotic protein, has been proposed as a marker of poor prognosis, associated with resistance to therapy in most tumor types expressing BCL-2. In breast cancer, however, BCL-2 expression has been reported to be a favorable prognostic factor. This study aimed to describe the association between BCL-2 and other well-known pathological prognostic markers among different molecular sub-types of invasive breast carcinoma of no special type (IBC; NST).

Methods. BCL-2 expression, as well as that of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), were immunohistochemically (IHC) evaluated and compared with other pathological factors, including tumor size, grade, tumor-infiltrating lymphocytes (TILs), lymph-vascular invasion (LVI), and lymph node (LNd) metastasis, in 128 breast cancer cases diagnosed with IBC; NST. Moreover, we analyzed the correlation between BCL-2 expression and relapse-free survival (RFS) in all patients over a two-year period.

Results. We found that BCL-2 expression had different pathological prognostic factor associations with different molecular subtypes of breast carcinoma. In the luminal A (i.e., hormonal receptor-positive and HER2-negative) and triple-negative subtypes, the expression of BCL-2 in tumor cells was significantly associated with tumor size, tumor grade, and TILs. BCL2-positive expression in luminal IBC; NST patients resulted in a significantly favorable two-year survival.

Conclusion. BCL-2 expression in IBC; NST has different prognostic effects depending on the molecular subtype of the cancer. In cancers with a HER2-enriched phenotype, BCL-2 expression was a marker of poor prognosis, while in cancers with a hormone receptor-positive phenotype, BCL-2 expression had a better prognostic impact.

Key words: Bcl-2, Invasive breast carcinoma of no special type, Molecular subtypes, Pathological prognostic factors

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Introduction

Breast cancer remains a significant global health concern with a profound impact on women's lives, which necessitates the identification of specific therapeutic targets to select the most appropriate anti-cancer drugs. Invasive breast carcinoma of no special type (IBC; NST) represents the most common histological subtype of

breast cancer, constituting a heterogeneous group of tumors with varying clinical behaviors (Makki, 2015). Determining the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) has been well-established for classifying breast carcinoma into different molecular subtypes with different prognostic and therapeutic impacts: luminal (ER-positive and/or PR-positive and HER2-negative), HER2-enriched (HER2-positive and ER and/or PR-positive or negative), and triple-negative (ER, PR, and HER2-negative) (Eliyatkın et al., 2015). New therapeutic targets are needed to increase the treatment options for breast cancer patients, especially with tumors lacking conventional therapeutic targets.

BCL2, located on chromosome 18q21.33, encodes a protein of the same name, BCL-2, which plays a pivotal role in regulating cell apoptosis. The BCL-2 protein family consists of both pro-apoptotic and anti-apoptotic members, with BCL-2 itself being an anti-apoptotic member. It exerts its influence by preventing mitochondrial outer membrane permeabilization, preventing the release of cytochrome c and other pro-apoptotic factors, thus ultimately blocking cell death (Tsujimoto et al., 1984; Vaux et al., 1988).

BCL-2 is overexpressed in many cancers and has been shown to promote tumor initiation, progression, and resistance to therapy (Tsujimoto et al., 1985; Kirkin et al., 2004; Letai et al., 2004), which could suggest that BCL-2 targeting therapy may be an effective treatment for many cancers. Regarding breast carcinoma, BCL-2 expression, despite its anti-apoptotic nature, has been reported to positively correlate with favorable prognostic factors, such as ER/PR expression, HER2 negativity, slow proliferation, small tumor size, and favorable clinical outcome (Silvestrini et al., 1994; Hellemans et al., 1995; Lipponen et al., 1995; van Slooten et al., 1996; Veronese et al., 1998). However, other studies have reported that BCL-2 expression promotes tumor cell survival and resistance to treatment, contributing to the persistence and progression of breast carcinoma (Pietenpol et al., 1994; Callagy et al., 2008; Dawson et al., 2010). This study aimed to examine the dual role of BCL-2 in IBC; NST according to the different molecular subtypes of breast carcinoma.

Materials and methods

Patients and specimens

The study was performed on 128 patients who were pathologically diagnosed with invasive breast carcinoma of no special type (IBC; NST) by a core biopsy and then underwent modified radical mastectomy (MRM) at Al Nasr and Tadamon hospitals in Port Said, Egypt, from March 2020 to March 2022. Patients who received neo-adjuvant therapy were excluded from the study so as not to affect the immunohistochemical (IHC) expression of the examined proteins. Other exclusion criteria were: the presence of noninvasive carcinoma (e.g., ductal carcinoma *in situ*), other invasive breast carcinoma types (e.g., lobular carcinoma), presence of distant metastasis at diagnosis, and any other malignancy. Clinical and pathological data, including age, gender, tumor size (maximum diameter of the tumor), lymph-vascular invasion (LVI), stromal tumor-infiltrating lymphocytes (TILs), pathological grades, and pTNM stages were recorded. Moreover, the follow-up data of the 128 patients were retrieved from their medical records for a two-year period to record recurrence-free survival (RFS); which was defined as the time from the first diagnosis of breast cancer to first recurrence, including locoregional recurrence and distant recurrence. We ensured, from the medical records, that all our enrolled patients received their standard protocol treatment regimens.

Histopathological evaluation

Specimens were received at the pathology lab, sliced, and fixed with 10% formalin overnight. Tissues were then handled according to the College of American Pathologists (CAP) guidelines (Torous et al., 2021). The submitted tissues were processed and embedded in paraffin. From each block, histological sections of 3 µm thickness were submitted, mounted on a glass slide, stained with hematoxylin and eosin (H&E), and reviewed by two independent pathologists. Tumor size (T) and number of positive lymph nodes (LNds) (N) were recorded according to the AJCC 8th edition staging system (Zhu and Doğan, 2021).

Immunohistochemical (IHC) staining

Sections from the selected paraffin blocks were cut into 4-µm-thick sections for IHC staining. The following primary antibodies were purchased from Genemed (San Francisco, USA): estrogen receptor (ER 1D5, 61-0031), progesterone receptor (PR Y85, 61-0001), HER2 (c-erbB-2 GR011, 61-0154), and BCL2 (Bcl2-100, 61-0005) (Table 1). This was followed by incubation with the appropriate secondary antibody purchased from Sigma-Aldrich (St. Louis, USA): Anti-Mouse IgG (A9044) and HRP-conjugated Anti-Rabbit IgG (RABHRP1). All slides were lightly counterstained with hematoxylin for 30 seconds before dehydration and mounting.

Table 1. Antibodies for immunohistochemistry. Working dilutions of antibodies are indicated.

Primary antibodies	Working dilution
ER	1:100
PR	1:100
HER2	1:50
BCL-2	1:100

Histopathological and immunohistochemical scoring

The following pathological findings were recorded for each case: size (T), grade (according to the Nottingham modification of the Bloom-Richardson system) (Zhang et al., 2010), and presence or absence of LVI and TILs. LVI was identified as the presence of tumor cells within a definite endothelial-lined space (lymphatic or blood vessels) in the breast tissue surrounding the invasive carcinoma (Ryu et al., 2018). Stromal TILs were identified according to the recommendations of the International TIL Working Group (Pujani et al., 2020) as the percentage of the area of stromal tissue occupied by mononuclear inflammatory cells (lymphocytes and plasma cells) within the borders of the invasive tumor, and was considered positive when it represented more than 20% of the tumor area. Care was taken not to count such inflammatory cells in areas of ulceration or erosion. For IHC markers, cytoplasmic BCL-2 expression in tumor cells was considered positive when more than 10% of tumor cells showed moderate to strong cytoplasmic staining. For ER and PR, their nuclear expression in tumor cells was evaluated using the Allred scoring method (Badve et al., 2008), and only scores 3-8 was interpreted as positive. Membranous HER2 expression was evaluated based on CAP recommendations for HER2 testing (Wolff et al., 2018), and only cases that scored 3+ were considered positive. A suitable set of positive and negative controls was run with the IHC slides.

Statistical analysis and data interpretation

Data were fed into the computer and analyzed using IBM (IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp). Qualitative data were described using numbers and percentages. Quantitative data were described using the median (minimum and maximum) & inter-quartile range for non-parametric data and standard deviation for parametric data after testing normality using the Kolmogrov-Smirnov test. The significance of the obtained results was judged at the (0.05) level. The chi-Square test was used for comparison of two or more groups. The Monte Carlo test was used as a correction for the Chi-Square test when more than 25% of cells had a count less than 5 in tables (>2*2). Fischer's Exact test was used as a correction for the Chi-Square test when more than 25% of cells had a count of less than 5. Mean and standard deviation were used in numeric data. Analysis of RFS was performed

Table 2. Association between BCL-2 and pathological parameters in Group 1 patients.

			BCL-2 expression		Total	*p**
			Negative	Positive		
T	1	Count	2	0	2	0.005
		% within BCL-2	10.0%	0.0%	3.8%	
	2	Count	0	32	32	
		% within BCL-2	0.0%	100.0%	61.5%	
	3	Count	18	0	18	
		% within BCL-2	90.0%	0.0%	34.6%	
Grade	1	Count	4	16	20	0.005
		% within BCL-2	20.0%	50.0%	38.5%	
	2	Count	2	16	18	
		% within BCL-2	10.0%	50.0%	34.6%	
	3	Count	14	0	14	
		% within BCL-2	70.0%	0.0%	26.9%	
LVI	Absent	Count	2	32	34	0.005
		% within BCL-2	10.0%	100.0%	65.4%	
	Present	Count	18	0	18	
		% within BCL-2	90.0%	0.0%	34.6%	
N	1	Count	2	32	34	0.005
		% within BCL-2	10.0%	100.0%	65.4%	
	2	Count	12	0	12	
		% within BCL-2	60.0%	0.0%	23.1%	
	3	Count	6	0	6	
		% within BCL-2	30.0%	0.0%	11.5%	
TILs	Negative	Count	8	0	8	0.005
		% within BCL-2	40.0%	0.0%	15.4%	
	Positive	Count	12	32	44	
		% within BCL-2	60.0%	100.0%	84.6%	

T, tumor size; LVI, lymph-vascular invasion; N, lymph node metastasis; TILs, tumor-infiltrating lymphocytes. *Chi-square test.

for each molecular subtype of breast cancer using the Kaplan-Meire method and displayed as Kaplan-Meire curves using GraphPad Prism version 8.0 for Windows (GraphPad Software, San Diego, California USA). Comparisons of survival curves were tested for statistical significance using the Log-rank test. The hazard ratio (HR) of RFS and its 95% confidence interval (CI) were computed for BCL-2 expression in each molecular subtype.

Results

The study included 128 patients, whose mean age was 54.25 years with a standard deviation of 10 years. The patients were divided into three groups according to the molecular classification of breast carcinoma: group 1 (luminal subtype); estrogen receptor-positive expression (ER+ve); progesterone receptor-positive expression (PR+ve); and human epidermal growth factor 2 receptor-negative expression (HER2-ve) (52 patients, 40.6 %); group 2 (triple-negative breast cancer) (TNBC); ER-ve, PR-ve, and HER2-ve (22 patients, 17.2%); and group 3 (HER2 enriched subtype); HER2+ve (54 patients, 42.2 %).

We found that BCL-2 expression was absent in all group 2 patients (TNBC) (Fig. 2). In groups 1 (luminal subtype) (Fig. 1) and 3 (HER2-enriched subtype) (Figure 3), the expression of BCL-2 in tumor cells was significantly associated with tumor size (T) (p<0.005). The expression with the highest percentage was observed with T2 in group 1 and with T1 in group 3 patients (Tables 2, 3).

Moreover, BCL-2 expression showed a statistically significant association with tumor grade (p<0.005). We

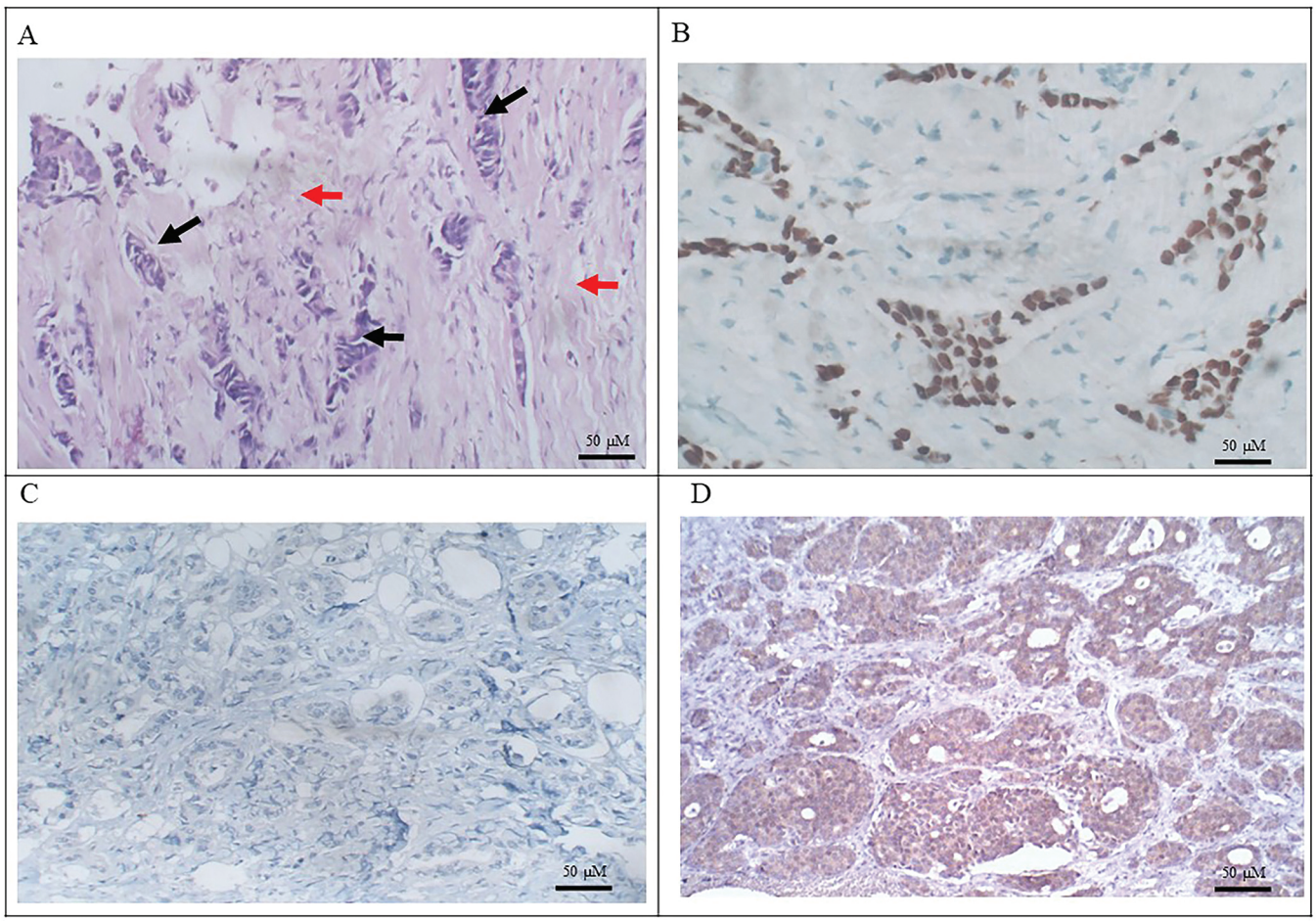

Fig. 1. Expression of BCL-2 in the luminal subtype of breast carcinoma. Representative hematoxylin and eosin (H&E) and immunohistochemical (IHC) stained figures in a case of invasive breast carcinoma of no special type (IBC; NST) from group 1 patients, and estrogen receptor (ER), human epidermal growth factor -2 (HER2) and BCL-2 marker expression. The immunoreaction (brown) for ER was detected in tumor cell nuclei, while that for Her2 was in the membrane and BCL-2 was in the cytosol. **A.** The tumor is composed of tubules and solid cords (Black arrows) formed of malignant epithelial cells, with cellular and nuclear pleomorphism and nuclear hyperchromasia. Tumor cells exhibit grade 1 differentiation. The stroma shows desmoplasia (Red arrows) (H&E). **B.** Tumor cells show a strong positive nuclear reaction to ER (IHC). **C.** Tumor cells show a negative reaction to Her2 (IHC). **D.** Tumor cells show a positive cytoplasmic reaction to BCL-2 (IHC).

found that positive expression of BCL-2 in group 1 patients was observed only in grade 1 and 2 tumors (Table 2), while most patients with positive BCL-2 expression in group 3 had grade 2 tumors (60%), followed by grade 1 tumors (33.3%), and the lowest percentage of cases had grade 3 tumors (6.7%) (Table 3).

Regarding LVI, there was a statistically significant association between the absence of BCL-2 and the presence of LVI in group 1 (Table 2). In group 3, although the presence of BCL-2 expression was associated with the presence of LVI, it was not statistically significant (Table 3).

In addition, we found that all group 1 cases with N1 showed BCL-2 expression (Table 2), while in group 3, we found that 93% of cases with N2 showed BCL-2 expression (Table 3). Regarding TILs, there was a significant association between BCL-2 expression and the presence of TILs: in group 1, all cases positive for BCL-2 expression showed TILs (Table 2), while in group 3, the presence of BCL-2 expression was observed more in the absence of TILs (Table 3).

Survival analysis illustrated a close correlation between BCL-2 expression and improved survival. From the two-year follow-up information of all patients, we found that group 1 patients (luminal subtype) showed a statistically significant difference in RFS between BCL-2-negative and positive cases, where BCL-2-positive cases had better RFS compared with BCL-2-negative cases (Log-rank χ^2 (df)= 7.24 (1), p=0.007; HR: 0.25; 95% CI: 0.07-0.93). However, in HER2-enriched group 3 patients, there were no statistically significant differences between BCL-2-negative and positive cases (Log-rank χ^2 (df)=1.81 (1), p=0.179). The HR for RFS in the HER2 subtype was not statistically significant

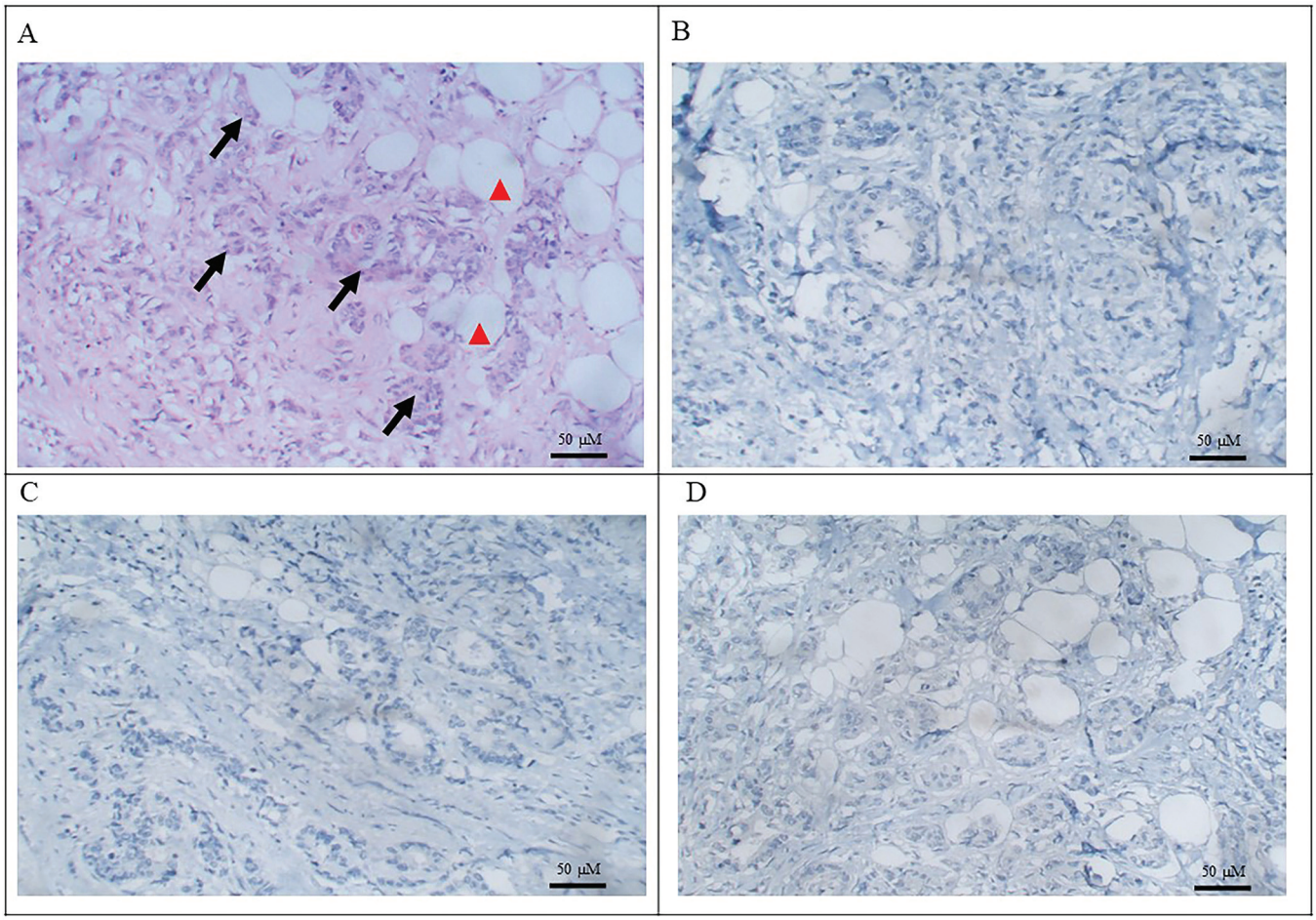

Fig. 2. Expression of BCL-2 in triple-negative breast carcinoma. Representative hematoxylin and eosin (H&E) and immunohistochemical (IHC) stained figures in a case of invasive breast carcinoma of no special type (IBC; NST) from group 2 patients, and estrogen receptor (ER), human epidermal growth factor -2 (HER2) and BCL-2 marker expression. The immunoreaction (brown) for ER was detected in tumor cell nuclei, while that for Her2 was in the membrane and Bcl-2 was in the cytosol. **A.** The tumor is composed of glandular structures and solid groups (Black arrows), formed of malignant epithelial cells, with cellular and nuclear pleomorphism and nuclear hyperchromasia. Tumor cells exhibit grade 2 differentiation and are infiltrating the surrounding fat tissue (Red arrowheads) (H&E). **B.** Tumor cells show a negative nuclear reaction to ER (IHC). **C.** Tumor cells show a negative reaction to Her2 (IHC). **D.** Tumor cells show a negative cytoplasmic reaction to BCL-2 (IHC).

(HR: 0.48; 95% CI: 0.11-1.98).

Discussion

Several biomarkers are used as prognostic and predictive factors for determining survival and selecting appropriate adjuvant therapy in breast cancer. In the present study, we tried to evaluate the expression of BCL-2 in breast carcinoma IBC; NST cases to elucidate its relation to well-known pathological prognostic factors within different molecular breast cancer subtypes.

The BCL-2 protein belongs to the BCL protein family that regulates apoptosis. Whether cells undergo apoptosis or survival depends on the relative expression of pro-apoptotic (BAX, BCL-XS, BAS, BIK/NBK, BID, and BAG-1) and anti-apoptotic (BCL-2, BCL-XL, BCL-W) proteins and their dimerization state. Increased BCL-2 shifts the balance in favor of cell survival (Callagy et al., 2006). The tumorigenic potential of inappropriate BCL-2 protein expression was first described as a result of chromosomal translocation (t (14,18)) seen in subsets of non-Hodgkin's lymphoma, where it is associated with adverse outcomes (Dawson et al., 2010).

The anti-apoptotic role of BCL-2 is well characterized, however, its function in cell cycle control has received less attention. The latter is well supported by cell line studies showing that BCL-2 expression can slow G1 progression and G1-S transition by prolonging G0, exerting growth inhibitory effects similar to p53. Whether one of these functions predominates over the other may depend on cell type and physiology, and anti-proliferative effects may suggest a tumor suppressor role in solid epithelial tumors, including breast cancer (Callagy et al., 2008).

In the present study, we found that BCL-2 expression was absent in all group 2 patients (triple-negative). It was previously reported that BCL-2 expression was detected in a minority of patients with triple-negative breast cancer and that BCL-2 negativity was associated with a two-fold increased risk of death and recurrence (Abdel-Fatah et al., 2013). On the other hand, a previous study reported a slight increase in BCL-2 expression in TNBC cases compared with non-TNBC cases (El-Hafez et al., 2013). This difference may be due to the different tumor types examined and the different methodologies used in the assessment of BCL-2-positive expression in the tissues.

In the present work, BCL-2 was expressed in both luminal breast cancer cases (ER+ve, PR+ve, and

Table 3. Association between BCL-2 and pathological parameters in Group 3 patients.

			BCL-2 expression		Total	*p**
			Negative	Positive		
T	1	Count	8	28	36	<0.005
		% within BCL-2	33.3%	93.3%	66.7%	
	3	Count	16	2	18	
		% within BCL-2	66.7%	6.7%	33.3%	
Grade	1	Count	4	10	14	<0.005
		% within BCL-2	16.7%	33.3%	25.9%	
	2	Count	6	18	24	
		% within BCL-2	25.0%	60.0%	44.4%	
	3	Count	14	2	16	
		% within BCL-2				
LVI	Absent	Count	6	2	8	0.12
		% within BCL-2	25.0%	6.7%	14.8%	
	Present	Count	18	28	46	
		% within BCL-2	75.0%	93.3%	85.2%	
N	1	Count	6	0	6	<0.005
		% within BCL-2	25.0%	0.0%	11.1%	
	2	Count	8	28	36	
		% within BCL-2	33.3%	93.3%	66.7%	
	3	Count	10	2	12	
		% within BCL-2	41.7%	6.7%	22.2%	
TILs	Negative	Count	6	18	24	0.14
		% within BCL-2	25.0%	60.0%	44.4%	
	Positive	Count	18	12	30	
		% within BCL-2	75.0%	40.0%	55.6%	

T, tumor size; LVI, lymph-vascular invasion; N, lymph node metastasis; TILs, tumor-infiltrating lymphocytes. *Chi-square test.

Her2+ve) and HER2-enriched cases (Her2+ve). This agrees with previous studies that reported a high percentage of BCL-2 expression in luminal breast carcinoma groups (Dawson et al., 2010; Ayadi et al., 2018; Sharmila and Praba, 2020), indicating an association between BCL-2 expression and the expression of ER and PR in breast cancer cells. (Nadler et al., 2008; Azmat et al., 2022) reported that BCL-2 expression was associated mostly with ER expression in breast cancer cells. Moreover, BCL-2 positivity was significantly correlated with HER2 negativity (luminal phenotype) in previous studies (Jalava et al., 2000; Honma et al., 2015).

The current study found a statistically significant association between BCL-2 expression and tumor grades 1 and 2 in luminal subtype patients (ER+ve, PR+ve, and HER2+ve). We also correlated BCL-2 expression to T2 in luminal subtype cases. This means that BCL-2 is more expressed in smaller-sized tumors, indicating its positive prognostic effect in this group of breast carcinomas, despite its anti-apoptotic nature. Moreover, we found a positive correlation between BCL-2 expression and N1 status in the luminal subtype of the breast carcinoma group. Similarly, El-Hafez et al. (2013) found a significant correlation between BCL2 expression and nodal status, with higher BCL-2 expression in the N0 status, as well as in tumors of smaller size and lower grade. Moreover, it was reported that BCL-2 expression was associated with markers of better differentiation (e.g., grade 1 lesions, which are ER-positive with low proliferative status) but did not show any significant relation with nodal status (Callagy et al., 2006; Honma

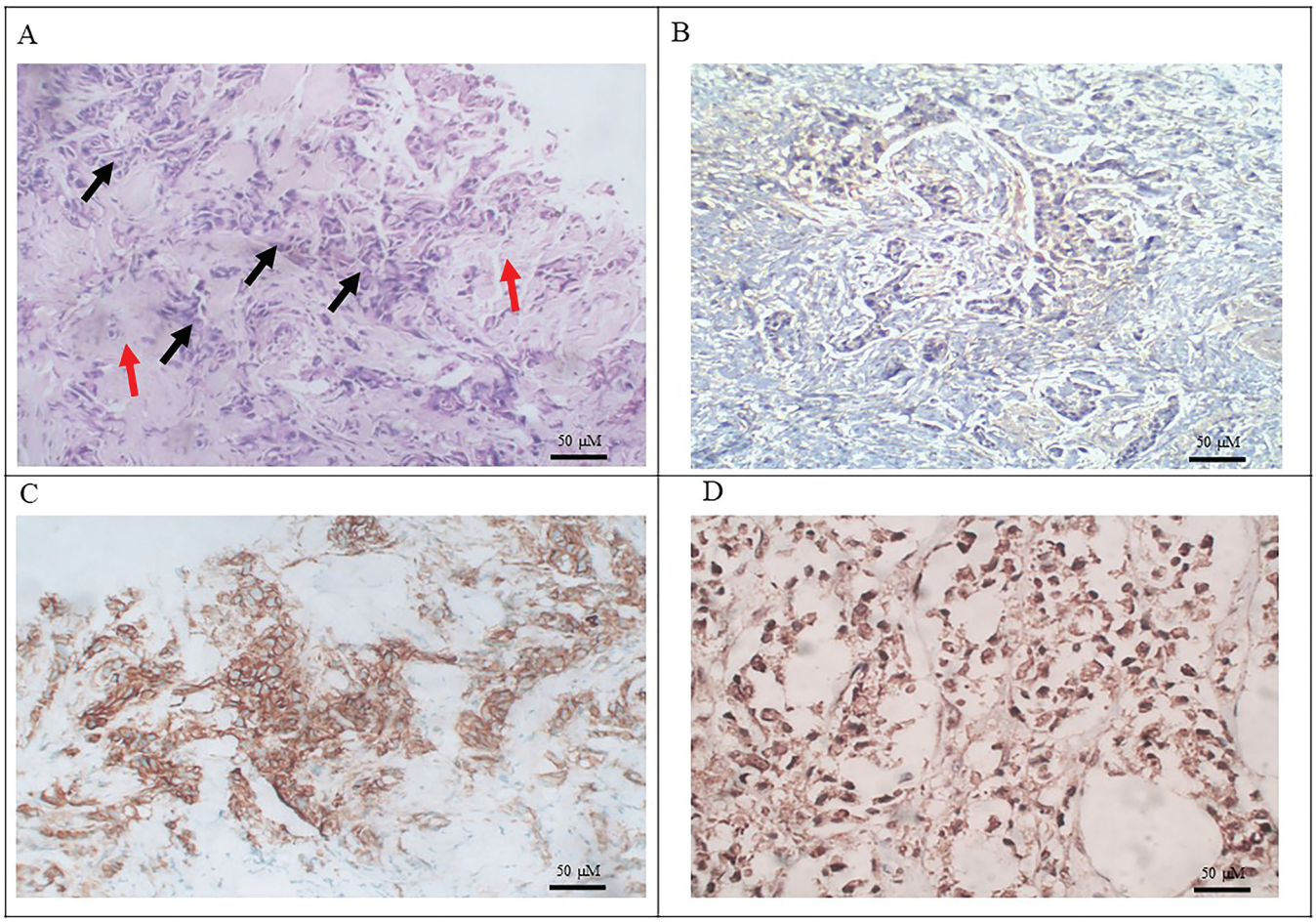

Fig. 3. Expression of BCL-2 in HER2-positive breast carcinoma. Representative hematoxylin and eosin (H&E) and immunohistochemical (IHC) stained figures in a case of invasive breast carcinoma of no special type (IBC; NST) from group 3 patients, and estrogen receptor (ER), human epidermal growth factor -2 (HER2) and BCL-2 marker expression. The immunoreaction (brown) for ER was detected in tumor cell nuclei, while that for Her2 was in the membrane and BCL-2 was in the cytosol. **A.** The tumor is composed mainly of solid cords and sheets (Black arrows) formed of malignant epithelial cells, with cellular and nuclear pleomorphism and nuclear hyperchromasia. Tumor cells exhibit grade 2 differentiation. The stroma shows desmoplasia (Red arrows) (H&E). **B.** Tumor cells show a negative nuclear reaction to ER (IHC). **C.** Tumor cells show a positive complete membranous reaction to Her2 (IHC). **D.** Tumor cells show a positive cytoplasmic reaction to BCL-2 (IHC).

et al., 2015; Eom et al., 2016).

We also reported a favorable association between BCL-2 expression in luminal A breast carcinoma patients (group 1) and RFS, which emphasizes the role of BCL-2 expression as a favorable prognostic factor in these patients, as previously reported (Eom et al., 2016; Al-Alem et al., 2023).

The current study is not without limitations. As our study was retrospective, selection bias may have been present. In addition, as we used an IHC staining method, the results may be affected by intra-tumoral and interobserver heterogeneity. However, this is the first study to track BCL-2 expression in IBC; NST with different molecular profiles. The relatively small sample size (128 patients) and the small number of patients in the triple-negative subgroup (22 patients) could limit the generalizability of the findings and thus further larger-scale studies are needed. Moreover, our study excluded patients who received neo-adjuvant therapy as it may affect the tissue expression of targeted proteins, and this could affect the observational association between BCL-2 expression and other prognostic factors in the enrolled patients. In addition, the follow-up information that we retrieved from patients' medical records was for a two-year period, which could limit the accuracy of the RFS data. We thus recommend further studies to investigate the relation between BCL-2 expression and other potential confounders, such as genetic mutations or variations in treatment protocols, and with longer follow-up periods, to clearly demonstrate the role of BCL-2 expression as a prognostic factor in patients with IBC; NST.

Conclusion

The observations of the present work indicate the changes in behavior of BCL-2 protein expression and its association with different pathological prognostic markers, depending on the molecular subtype of breast carcinoma. More studies are needed to fully elucidate the mechanisms by which BCL-2 signaling behaves and

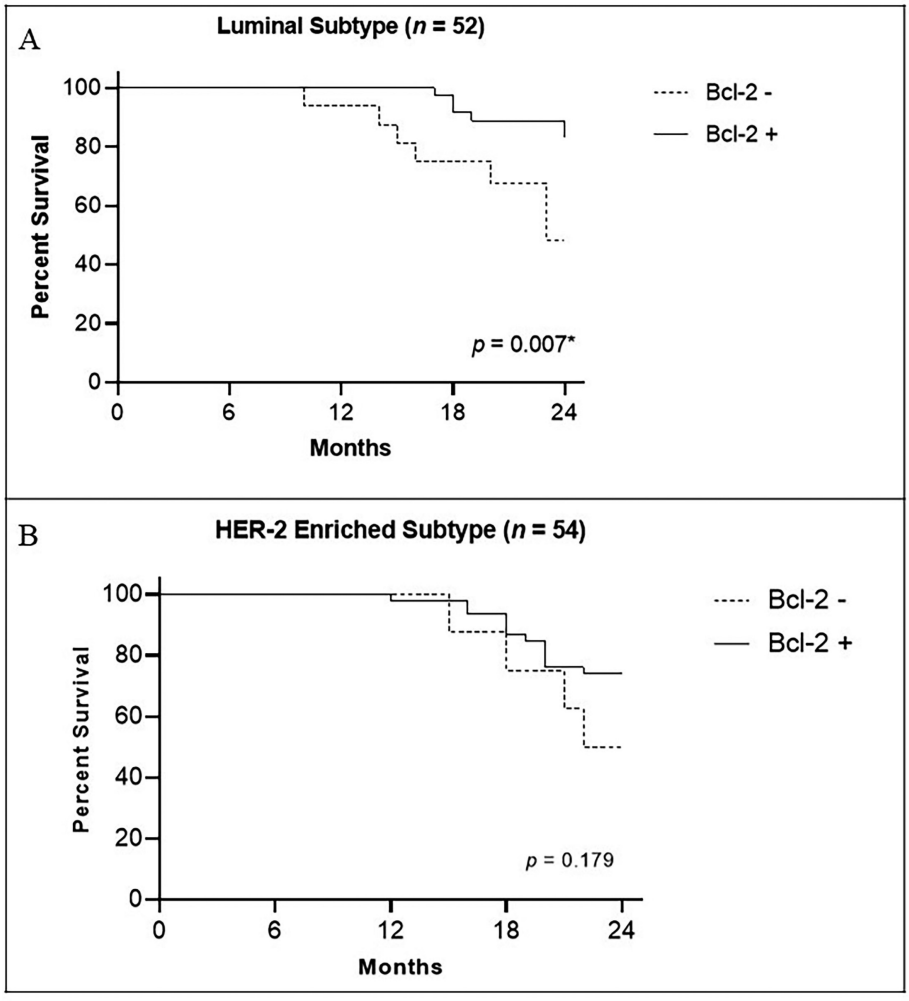

Fig. 4. Recurrence-free survival (RFS) of each molecular subtype group according to the BCL-2 expression status. **A.** Kaplan-Meire Curve of RFS for patients with luminal subtype breast cancer (group 1) (n=52) by BCL-2 expression status (negative vs. positive). Log-Rank Test for curve comparison: χ^2 (df)= 7.24 (1), *p*-value=0.007. **B.** Kaplan-Meire Curve of RFS for patients with HER-2-enriched subtype breast cancer (group 3) (n=54) by BCL-2 expression status (negative vs. positive). Log-Rank Test for curves comparison: χ^2 (df)=1.81 (1), *p*-value=0.179.

interacts with other pathways in cancer cells.

Author Contributions. Wael Abdo Hassan: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing - original draft, Writing - review & editing. Ahmed Kamal ElBanna: Formal analysis, Methodology, Writing - review & editing. Ihab Harbieh: Formal analysis, Methodology, Writing - review & editing. Noha Noufal: Data curation, Formal analysis, Methodology, Writing - review & editing. Mohamed El-Assmy: Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. Hany Lotfy: Formal analysis, Methodology, Writing - review & editing. Tarek Abdelaziz Hasan Shemais: Formal analysis, Methodology, Writing - review & editing. Ossama Ashour Haikal: Formal analysis, Methodology, Writing - original draft, Writing - review & editing. Mostafa Magdy Saber: Formal analysis, Methodology, Writing - review & editing. Rehab Ibrahim Ali: Data curation, Formal analysis, Methodology, Writing - original draft, Writing - review & editing.

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Institutional Review Board Statement. All procedures performed in the current study were approved by the Institutional Ethics Review Board of Port said University (MED s.no 117 ONC_001) in accordance with the 1964 Helsinki Declaration and its later amendments.

Informed Consent Statement. Informed consent was obtained from all individual participants included in the study. The authors will provide access to the Informed Consent Statement upon reasonable request.

Data Availability Statement. The authors will provide access to the materials used and analyzed during this study upon reasonable request.

Conflicts of Interest. The authors declare no conflict of interest.

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