The Potential Role of Bcl-2 Expression, Apoptosis and Cell Proliferation (Ki-67 Expression) in Cases of Gastric Carcinoma and Correlation with Classic Prognostic Factors and Patient Outcome

ATHANASSIOS C. TSAMANDAS[1], DIMITRIOS KARDAMAKIS[2], PANTELIS TSIAMALOS[3], ANNA LIAVA[1], VASSILIKI TZELEPI[1], VASSILIOS VASSILIOU[2], THEODORE PETSAS[4], KONSTANTINOS VAGENAS[3], VASSILIKI ZOLOTA[1] and CHRISOULA D. SCOPA[1]

Departments of [1]Pathology, [2]Radiotherapy/Oncology, [3]Surgery, and [4]Radiology, University of Patras, School of Medicine, Patras, Greece

Abstract. *Background: This study investigated the presence of apoptosis and proliferation in gastric cancer and assesses their possible correlation with classic prognostic markers and patients' survival. Patients and Methods: The study comprised 110 patients with gastric carcinoma who underwent gastrectomy for therapeutic reasons, and did not receive any pre- or postoperative treatment. Patients were followed up for 3.5-140 months. Thick paraffin sections (4 μm) were subjected to immunohistochemistry using anti-Bcl-2 and anti-Ki-67 antibodies and to in situ hybridization [TUNEL method-apoptotic body index (ABI)]. Morphological and immunohistochemical results were correlated with clinicopathologic parameters. Results: Bcl-2 protein was detected in 67% of adenocarcinomas with increased expression in low-grade and early-stage tumors. Bcl-2 expression did not correlate with Ki-67 index, ABI or patients' survival. Ki-67 expression was correlated with a poorer survival rate. Apoptosis was more frequently observed in advanced stage and high-grade tumors. Cox analysis revealed that tumor stage and grade, as well as Ki-67 index, constituted independent prognostic factors. Conclusion: This study included patients with gastric cancer none of whom received any additional pre- or post-operative treatment. Thus the prognostic value of each marker studied was not affected by additional treatments. Bcl-2 expression in advanced-stage and high-grade gastric carcinomas, indicate that Bcl-2 is involved in early stage of tumor development. Ki-67 expression constitutes an independent prognostic factor regarding the outcome of patients with gastric cancer. The positive association between apoptosis and proliferation suggests that apoptosis might reflect not only cell loss but also the proliferative activity. However, further research is required in order to determine if these markers may provide useful information for the prediction of prognosis in patients with colorectal carcinoma.*

Correspondence to: Athanassios C. Tsamandas, MD, Department of Pathology, University of Patras School of Medicine, Patras University Hospital, Department of Pathology, Room F1-35, Rion, Patras, 26504, Greece. Tel: +30 2610999650, Fax: +30 2610991810, e-mail: tsamanda@otenet.gr

Key Words: Gastric carcinoma, Bcl-2, Ki-67, apoptosis, prognostic factors.

Gastric carcinoma is one of the most devastating cancers in humans for which the molecular pathogenesis has been under intense investigation as a part of the effort to develop more effective therapeutic strategies for this tumor. Traditionally, the tumor stage, histological type and grade of differentiation constitute the main parameters for prognosis as well as for the optimization of therapeutic approaches. In addition, other variables such as primary tumor size, location within the stomach, tumor margins, surgical margins, degree of peritumoral lymphocytic infiltration, angioinvasive growth, number and location of lymph node metastases, oncogene expression, DNA ploidy, and cell proliferation have also been used to pursue prognostic information (1).

In the absence of extensive disease, aggressive surgical therapy with *en bloc* resection of lymph nodes remains the only hope for curative treatment of gastric carcinoma (2). The type of resection depends on the location and stage of the tumor, while controversy still exists about the extent of lymph node dissection between Western and Japanese surgeons. The role of adjuvant and neoadjuvant therapy in gastric cancer has been tested in several studies (3). Postoperative chemotherapy or radiotherapy is considered by many as an important factor for improving overall and disease-free survival, but larger randomized trials after overcoming regional disparities are needed for verification (4).

Today it is widely accepted that abnormal regulation of cell growth resulting in malignancy occurs due to increased cell proliferation and decreased apoptosis.

Apoptosis (programmed cell death) is characterized by cytoplasmic fragmentation and nuclear condensation, and it contributes to both physiological and pathological processes (5-8). More specifically, in gastric cancer, apoptosis has been found to be higher in advanced-stage tumors (9). Apoptosis is regulated by a variety of genes. The *Bcl-2* gene, located at chromosome 18q21, was first identified at the breakpoint of translocation occurring in follicular lymphomas (14, 18), but was also later detected in tumors of epithelial origin (10, 11). The *Bcl-2* gene encodes a 26-kDa protein localized mainly in the mitochondrial membrane, but also found in the nuclear envelope, perinuclear membrane, and endoplasmic reticulum, and blocks programmed cell death without affecting cellular proliferation (5, 6, 8, 12). This protein suppresses apoptosis and subsequently enhances cell survival. Interestingly, Bcl-2 has emerged as a key regulator of apoptosis because it can protect cells from death induced by a number of agents including radiation, chemotherapy or growth factor deprivation (13, 14). In some studies, Bcl-2 protein expression has been linked to a favorable prognosis in several malignant tumors (15, 16), while in others, its expression has been related to a worse prognosis (17-19). Other investigators have reported no correlation between Bcl-2 expression and prognosis (9, 20). Regarding the Bcl-2 expression in gastric cancer, previous studies have shown that Bcl-2 is expressed more frequently in low-grade and early-stage tumors but had no significant impact on the outcome of patients (21-24).

The degree of cell proliferation in a tumor can be evaluated by measuring the tumor growth fraction, by immunohistochemically identifying specific cell cycle-related antigens. A marker reflecting cell proliferation is the Ki-67 antigen, which is expressed in nuclei during all phases of the cell cycle except G0, whilst its presence has been related to tumor recurrence, stage and grade (25). The proportion of Ki-67-labeled cells in a given cell population (Ki-67 index) provides a measure of the growth fraction (26).

The present retrospective study was performed in order to investigate two hypotheses: (i) the evaluation Bcl-2 and Ki-67 expression and the presence of apoptosis in gastric carcinoma, and (ii) assessment of a possible correlation of proliferative activity and Bcl-2 immunoreactivity with important clinicopathological parameters such as tumor differentiation, staging and patients' survival.

Patients and Methods

Patients. The study included 110 consecutive surgical specimens of primary gastric carcinomas resected from an equal number of patients at Patras University Hospital, Greece, during a 10-year period (1996 to 2005). Archival tissues and data derived from the pathology records and clinical follow-up were readily available for all patients. The study comprised 71 men and 39 women aged 25 to 82 years (median 66 years), all with resectable gastric adenocarcinoma, who did not receive any prior systemic cytotoxic therapy or radiotherapy before surgery. During this 10-year period in the University of Patras, the surgical interventions in all 110 patients was similar, consisting of total or subtotal gastrectomy, depending on tumor location. The extent of resection was classified as follows: R0 resection, macroscopic tumor resected during surgery providing no sign of microscopic disease was revealed during histopathological examination of the specimen; R1 resection, resection of any macroscopically evident disease but with evident microscopic residual tumor during histopathological examination; R2 resection (palliative), both macroscopic and microscopic disease evident during surgery and histopathological examination.

Routine pathology. Slides and pathology reports for each patient were drawn from the files of the Pathology Department and were reviewed to confirm the pathological grade and stage. The review was carried out in a blind fashion. All tumors included in the study were staged according to TNM classification of malignant tumors (27) and graded according to a modification in the grading system proposed by WHO for colorectal tumors (28). Thus, the adenocarcinomas of intestinal type (according to Lauren classification), which were well or moderately differentiated, were recorded as low-grade tumors, whereas the poorly differentiated intestinal type adenocarcinomas and the diffuse type adenocarcinomas were recorded as high-grade tumors. Patients were followed up for a period ranging from 3.5-140 months by both clinical and radiological evaluations.

Immunohistochemistry. The detection of cells expressing Bcl-2 and Ki-67 antigens relied on immunohistochemistry based on a streptavidin-biotin-peroxidase method (Biogenex, San Ramon, CA, USA) as described elsewhere (20, 29, 30). Thick paraffin sections (4 μm) were used and primary antibodies included the following: monoclonal antibody to Bcl-2 (DAKO, CA, USA; at a dilution of 1:40) and monoclonal antibody to Ki-67 (DAKO; at a dilution of 1:40). All incubations were performed for 30 minutes at room temperature. Diaminobenzidine (Sigma Fast 3,3'-diaminobenzidine tablets, D-4293; Sigma, St. Louis, MO, USA) was used as a chromogen. Cytoplasmic staining for Bcl-2 and nuclear staining for Ki-67 was considered as positive. For positive-control purposes, the same staining procedure (for Bcl-2 or Ki-67) was performed on paraffin sections from human tonsils. For negative-control purposes, the aforementioned streptavidin-biotin technique was used on tissue sections in which 1% BSA in PBS substituted the primary antibody.

In situ labelling of fragmented DNA for the detection of apoptotic cells (TUNEL method). A standard TUNEL method was employed on 4 μm-thick paraffin sections to detect the fragmented nuclear DNA associated with apoptosis. For this purpose, the *in situ* cell death detection Kit, POD (Roche) was used as previously described (20, 29, 30). After standard deparaffinization, hydration, incubation with proteinase K and blocking of endogenous peroxidase, tissue sections were incubated as follows: (i) with terminal deoxynucleotidyl transferase (TdT) and digoxigenin-dUTP (TUNEL reaction mixture) at 37˚C for 60 minutes, and (ii) with peroxidase converter anti-fluorescein antibody at 37˚C for 30 minutes. Diaminobenzidine (Sigma Fast 3,3'-diaminobenzidine tablets, D-4293; Sigma) was used as the chromogen. For physiological positive controls, sections of rat small intestine were subjected to the same procedure. For negative controls, some slides were incubated with labelling solution that did not contain TdT.

Table I. *Bcl-2 and Ki-67 expression and ABI presence in relation to various pathological parameters.*

		Positive cases [N (median of positive cells, %)]					
Pathological factor	Cases (N)	Bcl-2		Ki-67		ABI	
Tumor location							
Cardia	13	4 (29)		13 (32)		13 (32)	
Fundus	15	5 (24)	$p>0.05$	15 (31)	$p>0.05$	15 (32)	$p>0.05$
Corpus	51	41 (22)		51 (36)		51 (35)	
Antrum/Pylorus	31	24 (30)		31 (35)		31 (30)	
Stage							
I	11	8 (45)		11 (19)		11 (21)	
II	35	28 (35)	$p=0.012$	35 (20)	$p=0.002$	35 (23)	$p=0.003$
III	44	27 (26)		44 (35)		44 (35)	
IV	20	11 (14)		20 (52)		20 (50)	
Grade							
Low	95	66 (37)	$p=0.019$	95 (22)	$p=0.004$	95 (23)	$p=0.005$
High	15	8 (20)		15 (48)		15 (48)	
Total	110	74		110		110	

Morphometric analysis. All immunohistochemical and *in situ* hybridization slides were analyzed and scored in a blind fashion without knowledge of the clinicopathological data. As has been previously reported (14, 20, 29, 30), cell counts were performed manually at ×400 magnification using a 10×10 microscope grid. Both the number of immunoreactive cells and the total number of tumor cells (at least 500 cells) in selected areas were determined by visual inspection of five different fields per section. For each field, the percentage immunoreactivity for Bcl-2 and Ki-67 and the presence of apoptotic bodies (apoptotic body index-ABI) were obtained by dividing the number of positive tumor cells by the total number of cells counted. The values in the same field did not differ by more than 10% . The average scores were then calculated.

Statistical analysis. Results are expressed as mean and median values. Intergroup comparisons, regarding correlation of pathological parameters with staining results, were performed using one-way analysis of variants (ANOVA). Whenever the equal variance test or normality tests failed, the Kruskal-Wallis nonparametric test was applied. In order to address the problem of multiple comparisons, the ANOVA and Kruskal-Wallis tests were followed by a *post hoc* Bonferroni test. Spearman's rank correlation was used to detect the relationship between Bcl-2, Ki-67 and ABI. Finally, the Cox proportional hazards model was employed to reveal the effects of other prognostic factors (stage, grade, Bcl-2 and Ki-67 expression and ABI presence) on survival. The Kaplan-Meier procedure was also used to compare the survival curves. Data were analysed using the SPSS statistical package (SPSS®, Release 10.0.1). Significance was defined as $p<0.05$.

Results

Routine pathology. A total of 11 tumors were classified as stage I, 35 as stage II, 44 as stage III, and 20 as stage IV. Of these, 95 adenocarcinomas were assigned as low-grade and 15 as high-grade malignancies. The location of the tumor varied from 13 tumors at the cardia of the stomach, 15 in the fundus, 51 in the corpus and the remaining 31 in the antrum/pylorus.

Immunohistochemistry and in situ hybridization. Table I demonstrates the correlation between immunohistochemical and *in situ* hybridization results with classic prognostic factors. Bcl-2 presence was detected in 67% (74/110) of the tumors, displaying a diffuse cytoplasmic or perinuclear envelope staining (Figures 1A-C). In the adjacent non-neoplastic gastric mucosa, Bcl-2 staining was observed within the cytoplasm of epithelial cells in the base of the crypts as well as in the infiltrating lymphocytes. The median index for Bcl-2(+) cells was 25% . In the positive cases, a certain degree of heterogeneity in Bcl-2 stain was commonly observed within the neoplastic cell population. In other words, the positively stained cells were randomly distributed throughout the tumor sections. Variations in staining intensity were noted from case to case, although the reactivity of lymphocytes was equally strong in all specimens. There was a trend for lower Bcl-2 expression in tumors of advanced stage and higher grade ($p=0.012$ and $p=0.019$, respectively). Ki-67 antigen was detected in all 110 of the tumors and displayed nuclear staining of neoplastic cells (Figures 2A-C). The mean index for Ki-67(+) cells was 25.3±47.3% and the median 30% . Ki-67 was also detected in the nuclei of the epithelial cells in the adjacent normal gastric mucosa and in occasional lymphocytes in the lamina propria. High-grade tumors exhibited higher indices for Ki-67 ($p=0.004$). In addition Ki-67 was more frequently expressed in advanced-stage tumors ($p=0.002$). Apoptotic bodies were present in the neoplastic cells of all tumors examined, in the base of the adjacent normal gastric epithelium, and in the lymphocytes. In the tumor the median ABI value was 32% . There was a strong correlation of increased ABI values with tumors of advanced stage, and tumors of high grade ($p=0.003$ and $p=0.005$, respectively).

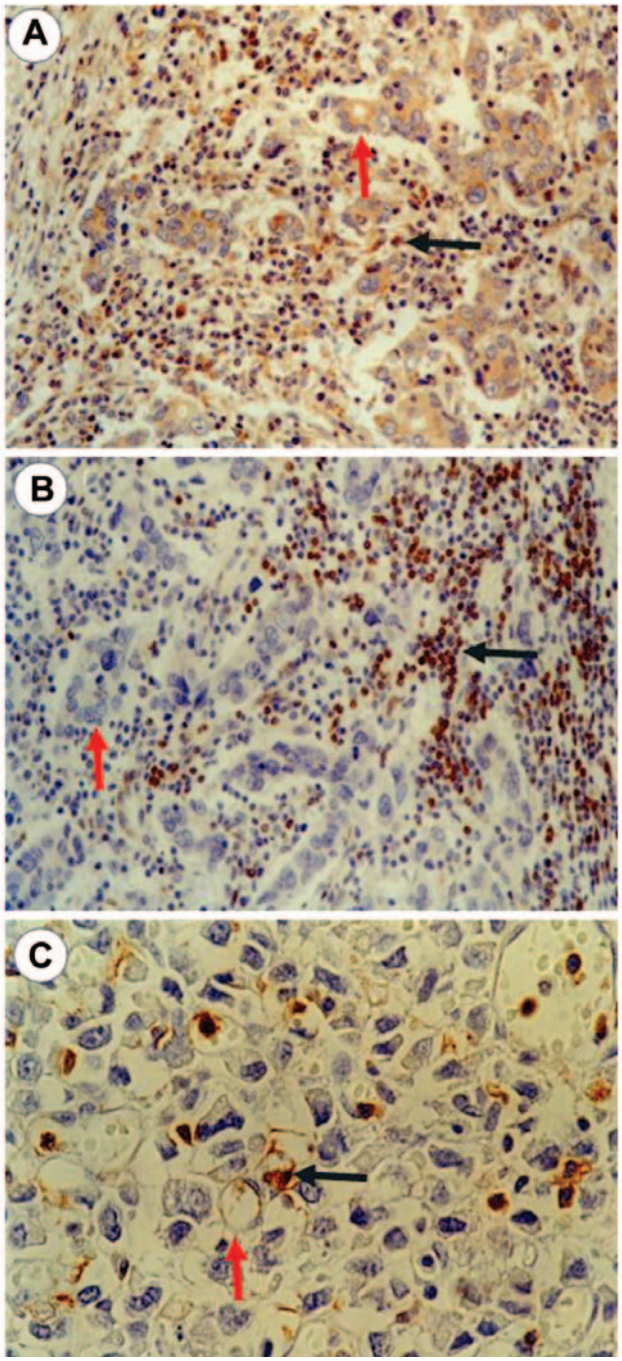

Figure 1. *A, Microphotograph showing Bcl-2 protein expression in an intestinal type gastric carcinoma. Note the positive cytoplasmic staining of tumor cells (red arrow) and of lymphocytes (positive control, black arrow) (streptavidin-biotin, peroxidase, ×200). B, Microphotograph showing the absence of Bcl-2 protein expression in an intestinal type gastric carcinoma. Note the absence of staining of tumor cells (red arrow) and the positive cytoplasmic staining of lymphocytes (positive control, green arrow) (streptavidin-biotin, peroxidase, ×200). C, Microphotograph showing the absence of Bcl-2 protein expression in a diffuse type gastric carcinoma. Note the absence of staining of tumor cells (signet ring cell, red arrow) and the positive cytoplasmic staining of lymphocytes (positive control, black arrow) (streptavidin-biotin, peroxidase, ×400).*

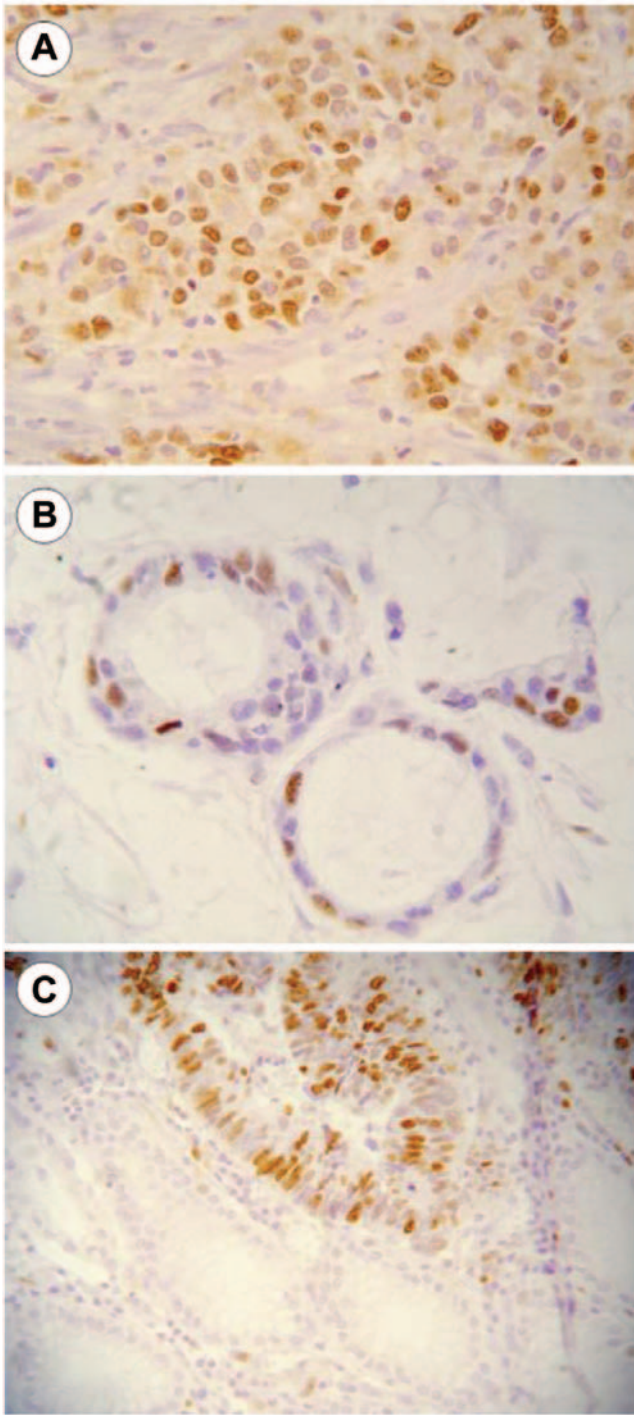

Figure 2. *Microphotographs showing Ki-67 expression in a diffuse type gastric carcinoma (A), high-grade intestinal type gastric carcinoma (B) and advanced-stage low-grade intestinal type gastric carcinoma (C). In C, note the absence of staining of normal gastric mucosa (low left) (streptavidin-biotin-peroxidase A and B ×400, C ×200).*

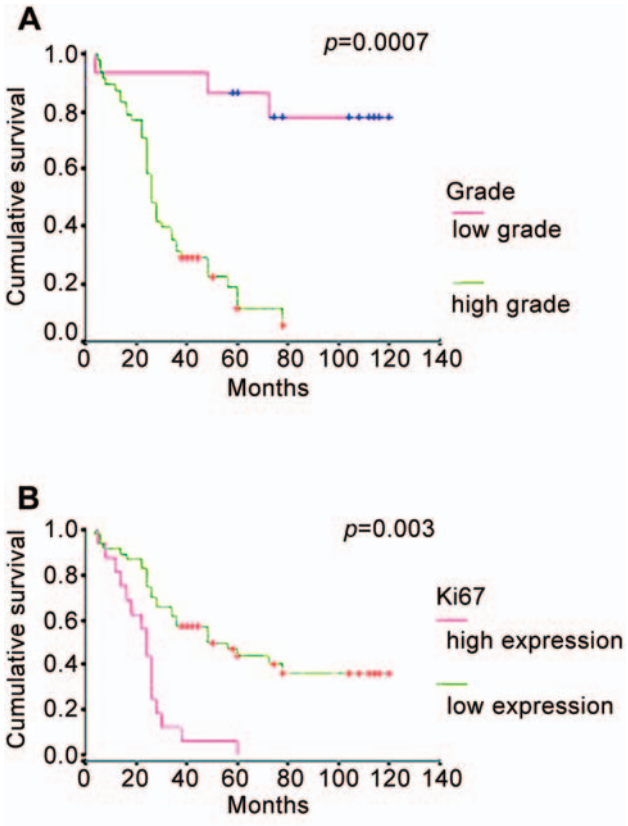

Figure 3. *A, Kaplan-Meier survival curves showing the relation between tumor grade and survival in all 110 patients (see text for grade details). B, Kaplan-Meier survival curves showing the relation between Ki-67 index (cut-off 5%) and survival in all 110 patients.*

Statistical analysis. A statistically significant lower disease-free survival was correlated with both high-grade tumors (p=0.0007) (Figure 3A) and advanced stage neoplasms (p=0.0008). In contrast, no significant correlates were found between survival and gender or tumor location. Statistical correlation between the immunohistochemical and *in situ* hybridization results for Bcl-2 oncoprotein as well as for Ki-67 antigen expression and ABI revealed a positive correlation between ABI and Ki-67 expression (p=0.014). No significant correlation was observed between Bcl-2 and Ki-67 or ABI values. Additionally, no statistically significant correlation was found between Bcl-2 expression and survival (p>0.05), whereas a statistically significant correlation was observed between higher Ki-67 index and adverse outcome (p=0.003) (Figure 3B). More specifically, this statistically significant difference was recorded when the tumors that exhibited Ki-67(+) cells less than 5% (cut-off value) were compared to those tumors which displayed a Ki-67 more than 5%. When all possible combinations between Bcl-2 expression (positive or negative) and Ki-67 (more or less than 5% expression) were examined, only Bcl-2(–)/Ki-67<5% cases displayed higher survival when compared to Bcl-2(–)/Ki-67>5% cases (p=0.014), implying that Bcl-2 expression does not have any impact on the outcome of patients with gastric cancer even when combined with Ki-67 index. Finally, Cox regression analysis revealed that tumor grade, stage and Ki-67 index were independent prognostic factors (Table II).

Discussion

The current study: a) included patients with gastric cancer of whom none received any additional pre- or postoperative treatment, thus the prognostic value of each marker studied was not affected by additional treatments; b) indicates that Bcl-2 is involved in early stages of tumor development, since its expression decreased in advanced-stage and high-grade gastric carcinomas; c) demonstrates that Ki-67 expression is correlated with poorer survival and constitutes an independent prognostic factor regarding the outcome of patients with gastric cancer. Interestingly, the positive association between apoptosis and proliferation demonstrated in this study implies that apoptosis might reflect not only cell loss but also the proliferative activity.

In the present study, Bcl-2 protein was detected in 67% of gastric adenocarcinomas examined. These results are comparable with those of two previous studies (22, 24) and higher when compared to the results of others (21, 23). These discrepancies could be attributed to the immunohistochemical methods used in each study. In this study, well controlled and reproducible immunohistochemical methods with appropriate positive controls were used.

A positive trend for increased Bcl-2 expression in low-grade and early-stage tumors was recorded. These findings are supported by other studies in which an increased Bcl-2 expression was found in tumors with favorable prognostic histopathological parameters (21-24). Although the role of the *Bcl-2* gene in the development or progression of gastric carcinomas has not been established yet, the significant loss of Bcl-2 expression in high-grade tumors of other organs suggests that Bcl-2 expression may be an early event in tumorigenesis (10). Previous studies have shown Bcl-2 protein expression in various premalignant lesions, implying that abnormal expression of Bcl-2 may lead to the accumulation of long-living cells, resulting in tumor development (11, 31). In the present study, Bcl-2 protein expression was clearly observed in the crypts of normal gastric mucosa and low-grade carcinomas, whereas its presence was less frequent in high-grade carcinomas. Similar findings to these can be corroborated elsewhere (21-24). The loss of Bcl-2 in high-grade carcinomas may reflect a deregulation of the mechanisms that control Bcl-2 expression (10, 32). A factor responsible for Bcl-2 deregulation could be the *p53* tumor suppressor gene, since previous studies have shown

Table II. *Relationship of potential prognostic factors with survival (Cox's proportional hazards regression analysis model).*

Factor	B	SE	Wald	Df	Odds ratio	95% CI for odds		Significance (*p*)
						Lower	Upper	
Gender	0.088	0.365	0.058	1	1.092	0.534	2.232	0.810
Age	0.012	0.014	0.724	1	1.012	0.985	1.039	0.395
Bcl-2	1.089	0.902	1.457	1	2.970	0.507	17.397	0.227
Ki-67	0.766	0.380	4.076	1	2.152	1.065	41.082	0.032*
ABI	0.294	0.686	1.084	1	1.341	0.350	5.142	0.668
Stage	1.390	0.947	4.157	1	6.892	1.167	5.408	0.019*
Grade	–1.792	0.694	6.659	1	0.167	0.032	0.502	0.029*

*$p<0.05$, ABI: apoptotic body index.

that p53 can down-regulate *Bcl-2* gene expression both *in vitro* and *in vivo* (8, 20, 33). In addition, Bcl-2 expression appears to play its main role in the early and not in the late stages of gastric carcinogenesis (21-24).

Previous reports on the prognostic significance of Bcl-2 protein expression in tumor pathology are contradictory (14–20). A partial explanation of the different roles of Bcl-2, in various types of neoplasia might be the fact that the Bcl-2 functional activity in different tissues is determined by tissue-specific expression of Bcl-2-binding proteins (34). Regarding gastric cancer, three previous studies (21, 23, 24) have reported similar results, whereas in another study investigating the impact of Bcl-2 and p53 expression in pT1 and pT2 gastric carcinomas found that Bcl-2(–)/p53(–) tumors displayed statistically significant longer survival when compared to Bcl-2(–)/p53(+) tumors (22). In the current study, no correlation was observed between the levels of Bcl-2 protein and survival, in spite of the fact that a significant inverse correlation of Bcl-2 expression with tumor grade and stage was demonstrated. It was further noted that Bcl-2(–)/Ki-67<5% cases showed higher survival only when compared to Bcl-2(–)/Ki-67>5% cases, a finding that further supports the notion that Bcl-2 protein expression does not have any impact on the outcome of patients with gastric cancer.

Previous studies have shown an inverse association between Bcl-2 and Ki-67 expression in tumors of various types (35, 36). This study did not demonstrate any significant correlation between Bcl-2 presence and proliferative activity. However, a positive relationship was observed between apoptotic body index and Ki-67 index. These results are in agreement with those previously reported in gastric (9) and colorectal carcinomas (29). The positive association between apoptosis and cell proliferation in the current study may suggest that apoptosis might reflect not only cell loss but also proliferative activity. This implies a rather mechanistic link between these two pathways which might be explained by the fact that apoptosis occurs primarily in the late G1 and G2 phase of cycling cells but not in the G0 and M phases (29). Previous studies have indicated that Bcl-2-positive carcinomas were more likely to have a low apoptotic index than those with low or absent Bcl-2 (9, 36). In the current study, a non-statistically significant correlation was found between Bcl-2 immunoreactivity and apoptotic body index, implying that apoptosis regulation is a complex phenomenon and other factors, including tumor heterogeneity, might also be involved (29).

Finally, statistical analysis showed that expression of Ki-67 antigen or frequency of apoptosis was correlated with a poor prognosis, whereas a nonstatistically significant correlation was found between Bcl-2 immunoreactivity and apoptotic body index and survival. However, Cox regression analysis revealed that tumor stage and grade and Ki-67 index were independent prognostic factors significantly related to patient survival.

In conclusion, it can be said that Bcl-2 expression in advanced-stage and high-grade gastric carcinomas indicates that Bcl-2 is involved in early stage of tumor development and that Ki-67 expression is correlated with poorer survival and constitutes an indepndent prognostic factor regarding the outcome of patients with gastric cancer. The positive association between apoptosis and proliferation suggests that apoptosis might reflect not only cell loss but also the proliferative activity. However, further research is warranted in order to determine if these markers may provide useful information for prognosis and for applying individualized treatments to patients with gastric carcinoma.

References

1 Rosai J: Stomach. *In*: Rosai and Ackerman's Surgical Pathology. Rosai J (ed.). Mosby, New York, p. 648, 2004.

2 Swan R and Miner TJ: Current role of surgical therapy in gastric cancer. World J Gastroenterol *12*: 372-379, 2006.

3 Kodera Y, Fujiwara M, Koike M and Nakao A: Chemotherapy as a component of multimodal therapy for gastric carcinoma.. World J Gastroenterol *12*: 2000-2005, 2006.

4 Ohtsu A, Yoshida S and Saijo N: Disparities in gastric cancer chemotherapy between the East and West. J Clin Oncol *24*: 2188-2196, 2006.
5 Bosman FT, Visser BC and van Oeveren J: Apoptosis: pathophysiology of programmed cell death. Pathol Res Pract *192*: 676-683, 1996.
6 Cummings MC, Winterford CM and Walker NI: Apoptosis. Am J Surg Pathol *21*: 88-101, 1997.
7 Staunton MJ and Gaffney EF: Apoptosis. Basic concepts and potential significance in human cancer. Arch Pathol Lab Med *122*: 310-319, 1998.
8 Geske FJ and Gerschenson LE: The biology of apoptosis. Hum Pathol *32*: 1029-1038, 2001.
9 Koshida Y, Saegusa M and Okayasu I: Apoptosis, cell proliferation and expression of Bcl-2 and bax in gastric carcinomas: immunohistochemical and clinicopathological study. Br J Cancer *75*: 367-373, 1997.
10 Cleary ML and Sklar J: Nucleotide sequence of a t(14;18) chromosomal breakpoint in follicular lymphoma and demonstration of a break-point cluster region near a transcriptionally active locus on chromosome 18. Proc Natl Acad Sci USA *82*: 7439-7443, 1985.
11 Lu QL, Abel P, Foster CS and Lalani EN: Bcl-2: role in epithelial differentiation and oncogenesis. Hum Pathol *27*: 102-110, 1996.
12 Krawejski S, Tanaka S, Takayama S, Schibler MJ, Fenton W and Reed JC: Investigations of the subcellular distribution of the Bcl-2 oncoprotein: residence in the nuclear envelope, endoplasmic reticulum and outer mitochondrial membranes. Cancer Res *53*: 4701-4714, 1993.
13 Soini Y, Paakko P and Letho V-P: Histopathological evaluation of apoptosis in cancer. Am J Pathol *153*: 1041-1053, 1998.
14 Scopa CD, Vagianos C, Kardamakis D, Kourelis TG, Kalofonos HP and Tsamandas AC: Bcl-2/bax ratio as a predictive marker for therapeutic response to radiotherapy in patients with rectal cancer. Appl Immunohistochem Mol Morphol *9*: 329-334, 2001.
15 Diebold J, Baretton G, Felchner M, Meier W, Dopfer K, Schmidt M and Lohrs U: Bcl-2 expression, p53 accumulation and apoptosis in ovarian carcinomas. Am J Clin Pathol *105*: 341-349, 1996.
16 Pezzella F, Turley H, Kuzu I, Tungekar MF, Dunnill MS, Pietce CB, Harris A, Gatter KC and Mason DY: Bcl-2 protein in non-small cell lung carcinoma. N Engl J Med *329*: 690-694, 1993.
17 Bubendorf L, Sauter G, Moch H, Jordan P, Blochlinger A, Gasser TC and Mihatsch MJ: Prognostic significance of Bcl-2 in clinically localized prostate cancer. Am J Pathol *148*: 1557-1565, 1996.
18 Saegusa M, Takano Y, Hashimura M, Okayasu I and Shiga J: The possible role of Bcl-2 expression in the progression of tumors of the uterine cervix. Cancer *76*: 2297-2303, 1995.
19 Karamitopoulou E, Perentes E, Tolnay M and Probst A: Prognostic significance of MIB-1, p53 and Bcl-2 immunoreactivity in meningiomas. Hum Pathol *29*: 140-145, 1998.
20 Tsamandas AC, Kardamakis D, Petsas T, Zolota V, Vassiliou V, Makatsoris, T, Kalofonos HP, Vagianos C and Scopa CD: Bcl-2, bax and p53 expression in rectal adenocarcinoma. Correlation with classic pathologic prognostic factors and patients' outcome. In Vivo *21*: 113-118, 2007.
21 Müller W, Schneiders A, Hommel G and Gabbert HE: Prognostic valure of Bcl-2 expression in gastric cancer. Anticancer Res *18*: 4699-4704, 1998.
22 Kopp R, Diebold J, Dreier I, Cramer C, Glas J, Baretton G and Jauch KW: Prognostic relevance of p53 and Bcl-2 immunoreactivity for early invasive pT1/pT2 gastric carcinomas. Surg Endosc *19*: 1507-1512, 2005.
23 Zafirellis K, Karameris A, Milingos N and Androulakis G: Molecular markers in gastric cancer: can p53 and Bcl-2 protein expressions be used as prognostic factors? Anticancer Res *25*: 3629-3636, 2005.
24 Lauwers GY, Scott GV and Karpeh MS: Immunohistochemical evaluation of Bcl-2 protein expression in gastric adenocarcinomas. Cancer *75*: 2209-2213, 1995.
25 Scott RJ, Hall PA, Haldane JS, van Noorden S, Price Y, Lane DP and Wright NA: A comparison of immunohistochemical markers of cell proliferation with experimentally determined growth fraction. J Pathol *165*: 173-178, 1991
26 Kruger S and Muller H: Correlation of morphometry, nucleolar organizer regions, proliferating cell nuclear antigen and Ki-67 antigen expression with grading and staging in urinary bladder carcinomas. Br J Urol *75*: 480-484, 1995.
27 Greene FL, Page DL and Fleming ID *et al*. (eds.). Stomach. AJCC Cancer Staging Handbook, TNM Classification of Malignant Tumors, Springer, New York, p. 111, 2002.
28 Jass JR and Sobin LH: Histological Typing of Intestinal Tumors: World Health Organization (2nd ed.). Springer, New York, 1989.
29 Scopa CD, Tsamandas AC, Zolota V, Kalofonos HP, Batistatou A and Vagianos C: Potential role of Bcl-2 and Ki-67 expression and apoptosis in colorectal carcinoma: a clinicopathologic study. Dig Dis Sci *48*: 1990-1997, 2003.
30 Salakou S, Kardamakis D, Tsamandas AC, Zolota V, Apostolakis E, Tzelepi V, Papathanasopoulos P, Bonikos DS, Papapetropoulos T, Petsas T and Dougenis D: Increased Bax/Bcl-2 ratio up-regulates caspase-3 and increases apoptosis in the thymus of patients with myasthenia gravis. In Vivo *21*: 123-132, 2007.
31 Nakamura T, Nomura S, Sakai T and Nariya S: Expression of Bcl-2 oncoprotein in gastrointestinal and uterine carcinomas and their premalignant lesions. Hum Pathol *28*: 309-315, 1997.
32 Stewart BW: Mechanism of apoptosis: integration of genetic, biochemical and cellular indications. J Natl Cancer Inst *86*: 1286-1298, 1994.
33 Miyashita T and Reed JC: Tumor suppressor *p53* is a regulator of *Bcl-2* and *bax* gene expression *in vitro* and *in vivo*. Oncogene *9*: 1799-1805, 1994.
34 Boise LH, Gonzales-Garcia M, Postema CE, Ding L, Lindsten T, Turka LA, Mao X, Nunez G and Thompson CB: *Bcl-x*, a Bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. Cell *74*: 597-608, 1993.
35 Doglioni C, Dei Tos AP, Laurino L, Chiarelli C, Barbareschi M and Viale C: The prevalence of Bcl-2 immunoreactivity in breast carcinomas and its clinicopathological correlates, with particular reference to estrogen receptor status. Virchows Arch *424*: 47-51, 1994.
36 Saegusa M, Takano Y and Okayasu I: Bcl-2 expression and its association with cell kinetics in human gastric carcinomas and intestinal metaplasia. J Cancer Res Clin Oncol *121*: 357-363, 1995.

Received July 29, 2008
Revised October 24, 2008
Accepted November 6, 2008