J BUON 2013; 18(1): 138-146
ISSN: 1107-0625 www.bu-on.org/jbuon
E-mail: jbuon@ath.forthnet.gr

ORIGINAL ARTICLE

Prognostic significance of biological apoptosis factors in gastric cancer

C. Sezer[1], M. Yildirim[2], M. Yildiz[3], A. Sezgin[1], U. Donem Dilli[1], S. Goktas[1], N. Bulbuller[1]

[1]Department of Pathology, [2]Department of Medical Oncology, [3]Department of General Surgery, Antalya Education and Research Hospital, Antalya, Turkey

Summary

Purpose: Gastric cancer is a biologically heterogeneous disease containing many genetic and epigenetic alterations. In our study, the expression status of apoptosis-inducing p53 and apoptosis-inhibiting Bcl-2 in gastric cancer and their relation with prognosis, if any, was investigated.

Methods: Patients that were being followed in our clinic and had histopathologically diagnosed gastric adenocarcinoma were included in this study. The p53 and bcl-2 expressions were investigated immunohistochemically and patients were grouped according to p53 and Bcl-2 expression as follows: group A: both p53 and Bcl-2 negative; group B: p53 positive and Bcl-2 negative; group C: p53 negative and Bcl-2 positive; group D: both p53 and Bcl-2 positive.

Results: In 19 (51.4%) patients positive immunostaining with p53 was observed, while negative in 18 (48.6%). A significant relationship between the metastatic ability of the tumor and p53 expression was determined (p=0.004). In 78.6% of the metastatic tumors no p53 expression was observed, while in 69.6% of the non-metastatic tumors p53 expression was positive. No significant relationship was detected between p53 expression and survival.

Positive immunostaining with Bcl-2 was observed in 9 (16.7%) patients, and negative in 45 (83.3%). No significant relationship was determined between the Bcl-2 expression and the depth of invasion, dissemination to lymph nodes and metastatic ability of the tumor. A borderline statistically significant relationship was determined between the Bcl-2 expression and survival (p=0.051).

Group B patients showed a statistically significant survival difference compared with the other groups (p=0.022).

Conclusion: The results of this study suggest that concurrent evaluation of p53 and Bcl-2 in patients with gastric adenocarcinoma may have prognostic importance.

Key words: apoptosis, bcl-2, gastric cancer, p53

Correspondence to: Mustafa Yildirim, MD.
Antalya Education and Research Hospital, Department of Medical Oncology, Varlik mahallesi, Kazim Karabenir cad., Soguksu, Antalya, Turkey. Tel: +90 5333948252, Fax: +90 242 2494402
E-mail: mustafayildirim7@yahoo.com
Received: 03/07/2012; Accepted: 31/07/2012

Introduction

Although the incidence of gastric cancer declines in many countries, it is still among the most common cancers worldwide [1]. The disease demonstrates a fast progression following diagnosis. Despite advances in diagnosis and the use of postoperative adjuvant treatment combinations in local disease, the disease may still progress [2]. Gastric cancer is a biologically heterogeneous disease containing many genetic and epigenetic alterations. Despite this heterogeneity, patients in the same disease stage are been treated with similar treatments. Considering the fact that trastuzumab produces survival advantage in patients with metastatic gastric cancer, in whom HER-2 expression was demonstrated immunohistochemically or by fluorescent *in situ* hybridization, this situation is changing [3]. Treatment options depending on the biology of the disease are being discussed.

From definite clinical and laboratory parameters determined during diagnosis, the prognosis of the disease can be predicted. Determination of the mechanisms playing a role in oncogenesis can bring forward targeted treatments. Laboratory parameters can be used for determining the intensity and type of the treatment to be applied to the patients. The tumor protein p53 (TP53) acts by stopping the cell cycle and starting induction of apoptosis. In more than 50% of malignancies mutated p53 has been shown [4]. Bcl-2, which has initially been detected in human B-cell lymphoma cells, is a member of a protein family which has important tasks in the apoptotic mechanisms. Bcl-2 inhibits apoptosis [5].

The purpose of our study was to demonstrate the expression status of the apoptosis-inducing p53 and apoptosis-inhibiting Bcl-2 in gastric cancer and to determine their relationship with prognosis, if any.

Methods

Patient selection

Included patients were followed at the Medical Oncology Clinic of Antalya Education and Research Hospital between 2008-2010; all of them had histopathologically diagnosed gastric adenocarcinoma and were staged according to the staging system of the American Joint Committee on Cancer (AJCC; 7th Edn). The patient files were studied and data about the age, gender, disease stage and the treatments applied to the patients were registered. Patients without histopathological diagnosis and those whose treatments had been initiated at another center and continued at our center were excluded from study.

Patient grouping

Patients were grouped according to p53 and Bcl-2 expression as follows: group A: both p53 and Bcl-2 negative; group B: p53 positive and Bcl-2 negative; group C: p53 negative and Bcl-2 positive; group D: both p53 and Bcl-2 positive.

Immunohistochemistry

Tumor specimens obtained during surgery or endoscopy were fixed in 10% formaldehyde and embedded in paraffin. The paraffin blocks were cut in sections of 4 μm and firstly evaluated after hematoxylin-eosin staining.

The sections were deparaffinized in an incubator at 60°C for 1 h. Then, they were immersed in xylene for 10 min and in 100% alcohol for 5 min and washed with water. The slides were kept in 10% citrate buffered solution in microwave at maximum power (800 watts) for 15 min. Then, the power was reduced by 50% and the slides were kept in the microwave for another 20 min. The slides were then removed and kept at room temperature for 20 min. Endogenous peroxidase activity was blocked by incubation with 3% H_2O_2 for 20 min. Then, the slides, washed with distilled water, were treated with phosphate buffer saline (PBS) for 5 min x 3 times and protein blocking agent (Novocastra Protein Block, Newcastle, UK). After 5 min, without washing off the blocking agent, p53 and Bcl-2 antibodies were dripped onto the slides. After keeping in the primary antibody for 30 min, the slides were transferred into PBS, washed for 5 min and treated with biotinylated secondary antibody for 20 min, washed in PBS for 5 min and incubated with peroxidase conjugate antibody (Novocastra Peroxidase Block, Newcastle, UK) for 20 min. Then, they were washed in PBS for 5 min and kept in chromogen (DAB) for 5 min. Then, they were washed with tap water, counterstained with hematoxylin, dehydrated,

Table 1. General patient characteristics

Characteristics	*Average ± SD*	*Median*
Age (years)	61.5±11	62
BUN (mg/dl)	20.6 ± 10.3	19
Cre (mg/dl)	0.59±0.33	0,6
AST (U/L)	24.8 ± 22.1	19
ALT (U/L)	22.6 ± 12.9	19
LDH (U/L)	229.7±157.6	200
ALP (U/L)	150.2±205.1	92
WBC (10^3/mm^3)	7.62±2.66	7.36
PLT (10^3/mm^3)	308.5±129.7	298.5
Hb (g/dl)	10.2 ± 3.86	11.3

BUN: blood urea nitrogen, Cre: creatinine, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, ALP: alkaline phosphatase, WBC: white blood cells, PLT: platelets, Hb: hemoglobin, SD: standard deviation

dried and covered with rapid embedding medium (Entellan, Electron Microscopy Sciences, Hatfield, USA).

For immunostaining, lyophilized mouse monoclonal antibody against p53 (Clone B P53;12, 1:100, Invitrogen, Carmennillo, Canada) and lyophilized mouse monoclonal antibody against Bcl-2 (Clone100/D5, 1:50, ThermoScientific, Fremont, USA) were used. After staining, the samples were inspected using Nikon Eclipse 80I microscope (USA, Nikon).

Immunohistochemical scoring

If samples were showing high expression, they were assessed by low power magnification. Low and negative expressions were studied by high power magnification. The expression rates of the positive tumor cells were evaluated by two different pathologists who were unaware of the clinical patient characteristics p53 and Bcl-2 expressions were assessed according to the nuclear and cytoplasmic staining of the cells. Staining > 10% in the samples was considered positive and ≤ 10% was considered negative.

Statistics

The statistical analyses were performed by employing the SPSS 13.0 software. Differences between groups were studied using Chi-square and Mann-Whitney U tests. The relation between each of the positive and negative immunohistochemical results and survival were investigated by Kaplan-Meier survival analysis. Statistical differences were confirmed by the log-rank test. A p value <0.05 was accepted as statistically significant.

Results

A total of 60 patients (40 males; 66.7% and 20 females; 33.3%) were included in the study. Their mean age was 61.5 ± 11 years (Table 1).

The clinical symptom most frequently encountered was abdominal pain (19 patients; 31.7%, followed by weight loss (18.3%) and dyspeptic complaints (10%). Comorbidities were detected in 16 patients (26.7%), the most common being hypertension in 10 patients (16.6%). Other frequently encountered comorbidities were diabetes mellitus and atherosclerotic cardiovascular disease with 4 (6.7%) patients each . Stage 1 disease was determined in one (1.7%) patient, stage 2 in 9 (15%), stage 3 in 16 (23.3%) and stage 4 in 34 (56.7%) patients . Local invasion and lymph node involvement were assessed pathologically in operated patients and by screening methods in patients who had only biopsy. Most frequently encountered local invasion was T3 in 32 (53.3%) patients. This was followed by T4 in 13 (21%) patients, T2 in 4 (6.7%) patients and T1 in 2 (3.3%) patients. The metastatic site most frequently encountered was

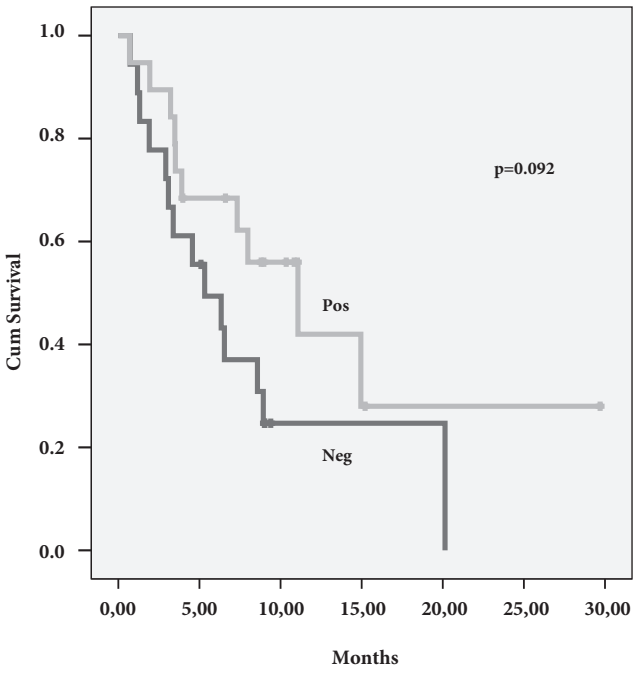

Figure 1. Kaplan-Meier survival according to p53 expression.

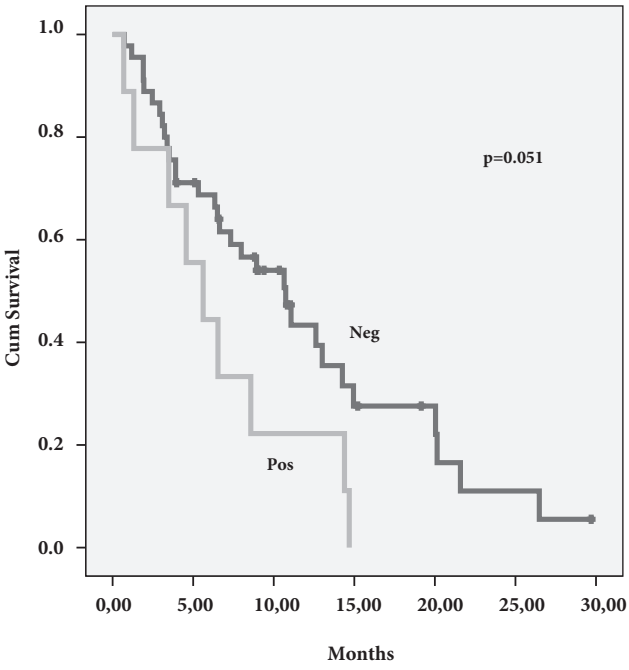

Figure 2. Kaplan-Meier survival according to Bcl-2 expression.

the liver (18 patients; 52.9%). Other metastatic sites were peritoneum-only in 8 patients, liver and peritoneum in 4 patients, lung metastasis in 3 patients and bone metastasis in 1 patient.

Due to the inadequacy of the endoscopic biopsies, p53 expression was assessed only in 37 out of 60 patients. While in 19 (51.4%) patients immunostaining was positive, immunostaining was negative in 18 (48.6%) patients. A significant relationship between the metastatic potential of the tumor and p53 expression was determined (p=0.004). While in 78.6% of the metastatic tumors p53 expression was not observed, in 69.6% of the non-metastatic tumors p53 expression was positive. No relationship was detected between p53 expression and the depth of tumor invasion and lymph node involvement (p=0.646 and p=0.336, respectively). p53 expression was detected in 2 (50%) stage 1 patients, in 5 (62.5%) stage 2 patients, in 7 (50%) stage 3 patients and in 16 (47.1%) stage 4 patients (Table 2). The median patient survival of was 8.9 months (95% confidence interval/CI 4.8-13). No significant relationship was found between the p53 expression and survival (p=0.092) (Figure 1).

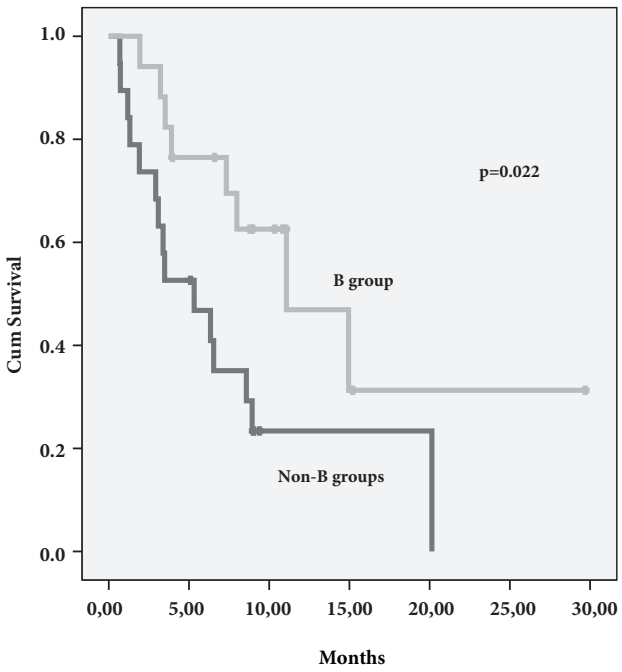

Figure 3. Kaplan-Meier survival for p53 positive, Bcl-2 negative (group B) patients.

Table 2. The relation of p53 and Bcl-2 expressions with clinicopathological parameters

Parameters		*p53*			*Bcl-2*			
	N(%)	*Negative N (%)*	*Positive N (%)*	*p-value*	*N (%)*	*Negative N (%)*	*Positive N (%)*	*p-value*
Gender								0.9
Female	16 (43.2)	6 (33.3)	10 (52.6)	0.243	19 (35.2)	16 (35.6)	3 (33.3)	
Male	21 (56.8)	12 (66.7)	9 (47.7)		35 (64.8)	29 (64.4)	6 (66.7)	
Age (years)				0.8				0.46
<60	17 (46)	8 (44.4)	9 (47.4)		24 (44.4)	21 (46.7)	3 (33.3)	
>60	20 (54)	10 (55.6)	10 (52.6)		30 (55.6)	24 (53.3)	6 (66.7)	
T stage				0.276				0.49
T1	3 (8.1)	1 (5.6)	2 (10.5)		4 (7.4)	4 (8.9)	0	
T2	2 (5.4)	1 (5.6)	1 (5.3)		3 (5.6)	2 (4.4)	1 (11.1)	
T3	19 (51.3)	8 (44.4)	11 (57.9)		28 (51.9)	22 (48.9)	6 (66.7)	
T4	13 (35.2)	8 (44.4)	5 (26.3)		19 (35.2)	17 (37.8)	2 (22.2)	
N stage				0.9				1.0
Negative	4 (10.8)	2 (11.2)	2 (10.5)		6 (11.1)	5 (11.1)	1 (11.1)	
Positive	33 (89.2)	16 (88.9)	17 (89.5)		48 (88.9)	40 (88.9)	8 (88.9)	
Metastasis				0.004				0.9
Yes	23 (62.2)	7 (38.9)	16 (84.2)		23 (42.6)	19 (42.2)	4 (44.4)	
No	14 (37.8)	11 (61.1)	3 (15.8)		31 (57.4)	26 (57.8)	5 (55.6)	
Total	37	18 (48.6)	19 (51.4)		54	45 (83.3)	9 (16.7)	

Also, due to inadequacy of the endoscopic biopsies, Bcl-2 expression was assessed in 54 out of 60 patients. No significant relationship was determined between the Bcl-2 expression and the depth of invasion, dissemination to lymph nodes and metastatic potential of the tumor (p=0.816, p=0.862, p=0.608, respectively). Bcl-2 expression was detected in one (25%) stage 1 patient; in stage 2 and 3 patients, this rate was 22.3% and 6.3%, respectively, while it was 20.6% in stage 4 patients. A borderline significant relationship was determined between Bcl-2 expression and survival (p=0.051). While median survival was 10.7 months (95% CI 7-14.4) in the Bcl-2 negative group, it was 5.6 months (95% CI 4.8-13) in the Bcl-2 positive group (p=0.051) (Figure 2).

When p53 and Bcl-2 expressions were assessed together in 37 patients, TP53 was determined as positive and Bcl-2 was determined as negative (group B) in 16 patients (Table 3). A statistically significant survival difference was determined between group B and groups A, C and D (p=0.022). In group B patients, median survival was 11.07 months (95% CI 3.9-18.2) while it was 5.3 months (95% CI 1.3-9.2) when assessed together with the other groups (Figure 3).

Table 3. Patient groups according to p53 and Bcl-2 expressions

Bcl-2	*p53*		*Total*
	Negative N	*Positive N*	
Negative	15 (Group A)	16 (Group B)	31
Positive	3 (Group C)	3 (Group D)	6

Discussion

Determination of prognostic factors in gastric cancer may help estimate patient survival and choosing therapy among different treatment options. In many studies it was demonstrated that the depth of tumor invasion (T) and the presence of lymph node metastasis (N) are important prognostic factors [6,7]. T, N

and the presence of distant metastasis (M) are used in the TNM disease staging. Studies show that patients at the same pathological stage may have different prognoses. Many biological factors have been studied to determine these different prognostic groups [8,9].

Biological factors such as oncogenes, tumor-suppressor genes, cell cycle regulators and DNA repair genes, which have role in the genesis, growth, invasion and metastasis of cancer, have been investigated for determination of prognosis and estimating the treatment response in cancer patients. Apoptosis also is a mechanism that plays an important role in carcinogenesis. Defects in the apoptotic processes change the tumor behavior. In this study, we demonstrated that the absence of apoptosis-inducing p53 expression and of anti-apoptotic Bcl-2 expression, which have central roles in the apoptotic process, provides stage-independent survival advantage in gastric cancer patients.

In DNA-damaged cells, p53 stops the cell cycle at the transition from the G1 to the S phase. This way, DNA-damaged cells cannot proliferate and are removed by apoptotic processes. On the other hand, mutated DNA-damaged cells which express p53 continue to proliferate. In normal cells, p53 is expressed at levels which cannot be easily detected by immunohistochemistry. p53 is located on the short arm of chromosome 17, and mutated p53 is formed as a result of various point mutations and deletions. The mutant p53 can be highly expressed in tumor cells [10].

In many studies, p53 expression in gastric cancer ranged between 31-76.7% [11-15]. In our study, p53 expression rate was detected in 51.4% of the cases. These differences in the p53 expression rate can be the result of many factors. Differences in the total number of patients and differences within the same disease stage can explain these discrepancies. For example, in our study we found that p53 expression decreased in stage 4 disease. This expression rate may be lower, due to the fact that most of our patients had stage 4 disease. Immunohistochemistry-related technical differences and analysis differences may also contribute to this observation.

In our study, no relationship was determined between p53 expression and clinicopathological parameters. In a similar study, which investigated factors predicting lymph node metastasis in early gastric cancer, no relationship between nodal metastasis and p53 expression has been demonstrated [16].

Many authors have investigated the prognostic role of p53 expression in gastric cancer. In some studies it has been proposed that patients with gastric cancer not expressing p53 had longer survival and that p53 was an adverse prognostic factor [17-21].

Lazar et al. [22] reported that in 61 operated gastric cancer patients 41% had positive p53 expression and demonstrated that the 5-year survival of the p53 positive patients was shorter. In our study we did not find any relationship between survival and p53 expression.

In many other studies, no relationship has been detected between p53 expression, and survival and other clinicopathological parameters, similarly to our study [23-30]. In terms of prognosis, these differences between studies may be related with the low total number of the patients included. Besides, differences in the number of patients at different disease stages may also affect survival analyses.

Bcl-2 functions as an oncogene, but unlike other oncogenes, it doesn't increase cell proliferation. It provides survival advantage to the rapidly proliferating cells due to DNA damages. Although Bcl-2 plays a role in carcinogenesis in most cancer types, it is less related to aggressive cancer progression. In non-small cell lung cancer, breast cancer, ovarian cancer, soft tissue sarcoma and colon cancer, it has been demonstrated that increased Bcl-2 expression was a good prognostic factor [31]. On the other hand, in some cancer types, it has been demonstrated to be an adverse prognostic factor. In patients with prostate cancer, increased Bcl-2 expression has been associated with resistance to hormonal treatment and recurrence, and in patients with breast cancer it has been associated with dismal prognosis [32].

The prognostic role of Bcl-2 expression in gastric cancer has been investigated in some studies [33,34]. Although there are studies suggesting its relationship with prognosis, there are other studies denying it [35,36]. In our study, similarly to these studies, no

statistical relation was determined between survival and Bcl-2 expression. Still, we determined that the Bcl-2 negative patient group displayed survival advantages.

Despite the existence of studies demonstrating a relation between p53 and Bcl-2, we did not determine any relationship between p53 expression and Bcl-2 expression.

The relation between the pro-apoptotic p53 expression and anti-apoptotic Bcl-2 expression and their prognostic role when evaluated together, has been examined by many authors. Although there are studies that demonstrate the prognostic significance when p53 expression and Bcl-2 expression are evaluated together, there are also studies demonstrating the opposite [37-44].

Liu et al. in their study with 501 stage 1-3 Chinese gastric cancer patients, who had undergone D2 resection, have examined the p53, Bcl-2 and bax expressions by immunohistochemistry. p53 expression rate was 64.9% and Bcl-2 expression 22.2%. While they did not determine any relationship between p53 expression and pathological stage and lymph node status, they showed a relationship between Bcl-2 expression and stage. In our study we noticed that p53 expression was decreased, especially in stage 4 patients. In our stage 2 and 3 patients, similar to the results of this study, we found p53 expression in 66.7% and 66.8%, respectively and we determined this rate as 21.4% in metastatic patients. The borderline statistical significance that we found between Bcl-2 expression and survival, was also demonstrated in this study [45].

The most important limitations of the present study were the low number of patients and the fact that most of them had the metastatic disease. Since histopathological diagnosis is obtained by endoscopic biopsy in most of the metastatic patients, no tissue samples of some of them could be obtained. For this reason, these patients could not be included in the immunohistochemical examination.

The development and progression of cancer is a very complex process. Apoptosis is one of the mechanisms that play an important role in this process. Induction of apoptosis or blocking the anti-apoptotic pathways is among the goals of cancer treatment [46]. When p53 and Bcl-2, which play an important role in apoptosis, are considered individually, they do not seem prognostically important. In apoptosis, many genes function together. This fact brings forward the concurrent use of the biological prognostic factors. The results of this study suggest that concurrent evaluation of p53 and Bcl-2 in patients with gastric adenocarcinoma may have prognostic importance.

References

1. Parkin D, Bray F, Ferlay J, Pisani P. Global Cancer Statistics, 2002. CA Cancer J Clin 2005;55: 74–108.
2. McDonald J, Smalley S, Benedetti J et al. Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction. N Engl J Med 2001;345:725–730.
3. Bang YJ, Van Cutsem E, Feyereislova A et al. ToGA Trial Investigators. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet 2010; 376:687-697.
4. Hollstein M, Sidransky D, Vogelstein B, Harris C. P53 mutations in human cancers. Science 1991;253: 49–53.
5. Cory, S, Huang, DC, Adams, JM. The Bcl-2 family: roles in cell survival and oncogenesis. Oncogene 2003; 22: 85-90.
6. Adachi Y, Mori M, Maehara Y, Suqimachi K. Dukes' classification: a valid prognostic indicator for gastric cancer. Gut 1994; 35: 1368–1371.
7. Maruyama K. The most important prognostic factors for gastric cancer patients: a study using univariate and multivariate analyses. Scand J Gastroenterol 1987;22: 63–68.
8. Chau I, Morman AR, Cunningham D, Waters JS, Oates J. Multivariate prognostic factor analysis in locally advanced and metastatic esophago-gastric cancer – pooled analysis from three multicenter, randomized, controlled trials using individual patient data. J Clin Oncol 2004; 22: 2359–2403.
9. Dicken BJ, Saunders LD, Jhangri GS et al. Gastric cancer: Establishing predictors of biologic behaviour with use of population-based data. Ann Surg Oncol 2004;11: 629–635.
10. Kerns BJ, Jordan PA, Moore MB et al. p53 overexpression in formalin-fixed, paraffin-embedded tissue detected by immunohistochemistry. J Histochem Cytochem 1992;40:1047-1051.
11. Zali MR, Moaven O, Asadzadeh Aghdaee H et al. Clin-

icopathological significance of E-cadherin, β-catenin and p53 expression in gastric adenocarinoma. J Res Med Sci 2009;14:239-247.
12. Kim DH. Prognostic implications of cyclin B1, p34cdc2, p27(Kip1) and p53 expression in gastric cancer. Yonsei Med J 2007;48:694-700.
13. Radovanović D, Knezević M, Canović D, Aćimović L. . Correlation between p53 expression and clinical-pathological characteristics of gastric cancer. Vojnosanit Pregl 2011;68:832-836.
14. Chen HC, Chen HJ, Khan MA et al. Genetic mutations of p53 and k-ras in gastric carcinoma patients from Hunan, China. Tumour Biol 2011;32:367-373.
15. Zhang J, He XH, Xie XY, Hu X, He C. The potential for serum p53 to predict the response to chemotherapy of patients with gastric cancer. J Int Med Res 2010;38:423-431.
16. Lim MS, Lee HW, Im H et al. Predictable factors for lymph node metastasis in early gastric cancer-analysis of single institutional experience. J Gastrointest Surg 2011 ;15:1783-1788.
17. Martin HM, Filipe MI, Morris RW, Lane DP, Silvestre F. p53 expression and prognosis in gastric carcinoma. Int J Cancer 1992;50:859-862.
18. Joypaul BV, Hopwood D, Newman EL et al. The prognostic significance of the accumulation of p53 tumour-suppressor gene protein in gastric adenocarcinoma. Br J Cancer 1994;69:943-946.
19. Starzynska T, Markiewski M, Domagala W et al. The clinical significance of p53 accumulation in gastric carcinoma. Cancer 1996;77:2005-2012.
20. Maehara Y, Tomoda M, Hasuda S et al. Prognostic value of p53 protein expression for patients with gastric cancer--a multivariate analysis. Br J Cancer 1999;79:1255-1261.
21. Tzanakis NE, Peros G, Karakitsos P et al. Prognostic significance of p53 and Ki67 proteins expression in Greek gastric cancer patients. Acta Chir Belg 2009 ;109:606-611.
22. Lazăr D, Tăban S, Sporea I et al. The immunohistochemical expression of the p53-protein in gastric carcinomas. Correlation with clinicopathological factors and survival of patients. Rom J Morphol Embryol 2010;51:249-257.
23. Motojima K, Furui J, Kohara N, Ito T, Kanematsu T. Expression of p53 protein in gastric carcinomas is not independently prognostic. Surgery 1994;116:890-895.
24. Gabbert HE, Muller W, Schneiders A, Meier S, Hommel G. The relationship of p53 expression to the prognosis of 418 patients with gastric carcinoma. Cancer 1995;76: 720-726.
25. Joo YE, Chung IJ, Park YK et al. Expression of cyclooxygenase-2, p53 and Ki-67 in gastric cancer. J Korean Med Sci 2006;21:871-876.
26. Juvan R, Hudler P, Gazvoda B, Repse S, Bracko M, Komel R. Significance of genetic abnormalities of p53 protein in Slovenian patients with gastric carcinoma. Croat Med J 2007;48:207-217.
27. Deveci MS, Deveci G. Prognostic value of p53 protein and MK-1 (a tumor-associated antigen) expression in gastric carcinoma. Gastric Cancer 2007;10:112-116.
28. Lee DY, Park CS, Kim HS, Kim JY, Kim YC, Lee S. Maspin and p53 protein expression in gastric adenocarcinoma and its clinical applications. Appl Immunohistochem Mol Morphol 2008;16:13-18.
29. Azarhoush R, Keshtkar AA, Amiriani T, Kazemi-Nejad V. Relationship between p53 expression and gastric cancers in cardia and antrum. Arch Iran Med 2008;11:502-506.
30. Ismail HM, Moneer M, El-Baradie M, Khorshid O, Touny A. Clinicopathologic and prognostic significance of overexpression of her-2/neu and p53 oncoproteins in gastric carcinoma using tissue microarray. J Egypt Natl Canc Inst 2007;19:147-157.
31. Manne U, Myers RB, Moron C et al. Prognostic significance of Bcl-2 expression and p53 nuclear accumulation in colorectal adenocarcinoma. Int J Cancer 1997;74:346–358.
32. Sahan E, Tetikkurt US, Balci C, Igdem AA, Gultekin SE, Erdogan N. Evaluation of P53 and Bcl-2 expression with Ki-67 proliferation index in prostatic aciner adenocarcinoma; Their correlation with histopathological and clinical prognostic factors. Turkish J Urol 2003; 29: 250-257.
33. Müller W, Schneiders A, Hommel G, Gabbert HE. Prognostic value of bcl-2 expression in gastric cancer. Anticancer Res 1998;18:4699-4704.
34. Tsamandas AC, Kardamakis D, Tsiamalos P et al. 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. Anticancer Res 2009;29:703-709.
35. Yildirim M, Suren D, Goktas S et al. The predictive role of Bcl-2 expression in operable, locally advanced or metastatic gastric carcinoma. J BUON 2012; 17: 106-109.
36. Zafirellis K, Karameris A, Milingos N, Androulakis G. Molecular markers in gastric cancer: can p53 and bcl-2 protein expressions be used as prognostic factors? Anticancer Res 2005;25:3629-3636.
37. Haldar S, Negrini M, Monne M, Sabbioni S, Croce CM.

Down-regulation of bcl-2 by p53 in breast cancer cells. Cancer Res 1994;54:2095-2097.
38. Saegusa M, Takano Y, Okayasu I. Bcl-2 expression and its association with cell kinetics in human gastric carcinomas and intestinal metaplasia. J Cancer Res Clin Oncol 1995;121:357-363.
39. Smith L, Berrieman HK, O'Kane SL, Campbell A, Maraveyas A, Cawkwell L. Immunohistochemical detection of apoptotic markers in gastric cancer. Oncol Res 2006;15:441-444.
40. Aizawa K, Ueki K, Suzuki S et al. Apoptosis and bcl-2 expression in gastric carcinomas: correlation with clinicopathological variables, p53 expression, cell proliferation and prognosis. Int J Oncol 1999;14:85-91.
41. Inada T, Kikuyama S, Ichikawa A, Igarashi S, Ogata Y. Bcl-2 expression as a prognostic factor of survival of gastric carcinoma. Anticancer Res 1998;18:2003-2010.
42. Triantafyllou K, Kitsanta P, Karamanolis DG, Kittas C, Ladas SD. Epithelial cell turnover, p53 and bcl-2 protein expression during oncogenesis of early and advanced gastric cancer in a Western population. Dig Liver Dis 2008;40:39-45.
43. Wiksten JP, Lundin J, Nordling S, Kokkola A, Haglund C. Comparison of the prognostic value of a panel of tissue tumor markers and established clinicopathological factors in patients with gastric cancer. Anticancer Res 2008;28:2279-2287.
44. Lee HK, Lee HS, Yang HK et al. Prognostic significance of Bcl-2 and p53 expression in gastric cancer. Int J Colorectal Dis 2003;18:518-525.
45. Liu X, Cai H, Huang H, Long Z, Shi Y, Wang Y. The prognostic significance of apoptosis-related biological markers in Chinese gastric cancer patients. PLoS One 2011;6:e29670.
46. Qiao L, Wong BC. Targeting apoptosis as an approach for gastrointestinal cancer therapy. Drug Resist Updat 2009;12:55-64.