Original Article

Access this article online
Quick Response Code:

Website: www.ijhonline.org
DOI: 10.4103/ijh.ijh_2_20

BAX/BCL2 ratio in chronic lymphocytic leukemia and its association with ZAP-70 expression and other clinicopathological parameters

Rihab Malik Rahid, Maysem Mouayad Alwash[1]

Abstract:

BACKGROUND: Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with a highly variable clinical course and outcome. Many clinical and biological characteristics have been used to classify patients with CLL into different subgroups of variable prognosis. Many studies have reported that in CLL, the interaction between pro- and antiapoptotic BCL2 family members influences the sensitivity to cytotoxic drugs and affects survival and overall outcome.

OBJECTIVES: The aim of the study was to assess BCL2 and BAX expression and BCL2/BAX ratio relation to other known prognostic markers (Binet stage, absolute lymphocyte count, lymphocyte percentage in bone marrow [BM], and ZAP-70 and CD38 expression).

PATIENTS AND METHODS: The study analyzed the expression of BCL2 and BAX, BCL2/BAX ratio, and ZAP-70 in the BM biopsy of 42 randomly selected CLL patients.

RESULTS: BCL2 was positively expressed in 92.9% of CLL cases, significantly associated with Binet stage of disease ($P = 0.04$), ZAP-70 ($P = 0.001$) but not with CD38, and also significantly correlated with absolute lymphocyte count ($P = 0.015$) and lymphocyte percentage in BM ($P = 0.017$). BAX was positively expressed in 64.3% of CLL cases; there was no significant association between BAX with Binet or with other assessed prognostic factors (except with ZAP-70, $P = 0.001$). BCL2 and BAX were significantly correlated with each other ($P = 0.001$). BCL2/BAX ratio was not associated with any prognostic parameters we assessed.

CONCLUSION: We may conclude that we may consider BCL2 as simple informative tool to assess disease activity while BAX and BCL2/BAX ratio alone are of no prognostic value in prediction of disease course.

Keywords:

BAX, BCL2, BCL2/BAX, chronic lymphocytic leukemia

Introduction

The most common form of adult leukemia is chronic lymphocytic leukemia (B-CLL), characterized by the accumulation of CD5+ and CD23+ B-cell lymphocytes. It is a complex disorder. It is a disease of varying clinical course; some have an indolent disease and mostly do not need treatment, while others are diagnosed with severe disease.[1]

Two major clinical staging systems (Rai and Binet staging), mainly based on tumor load, were developed to estimate prognosis in CLL. However, in the early stages of CLL, Rai and Binet staging systems cannot predict the course of the disease; therefore, all efforts focused on finding accurate markers that can help to predict the outcome and explain the clinical heterogeneity of CLL.[2]

Department of Laboratories, Al Fallujah Teaching Hospital, Al Anbar, [1]Department of Pathology, Mustansiriyah University, College of Medicine, Baghdad, Iraq

Address for correspondence:
Dr. Rihab Malik Rahid, Department of Laboratories, Al Fallujah Teaching Hospital, Al Anbar, Iraq.
E-mail: rehab.alqaissy@gmail.com

Submission: 11-01-2020
Accepted: 25-01-2020
Published: 15-04-2020

How to cite this article: Rahid RM, Alwash MM. BAX/BCL2 ratio in chronic lymphocytic leukemia and its association with ZAP-70 expression and other clinicopathological parameters. Iraqi J Hematol 2020;9:34-40.

BCL2 is an integral outer mitochondrial membrane protein. BCL2 claimed as an important protein in CLL which helps for survival prediction. It plays a role in promoting the survival of cells and inhibiting proapoptotic protein actions.[3]

BAX is a proapoptotic protein and enhances apoptosis by its competition with BCL2 proper. P53 directly activates the proapoptotic protein BAX which acts on the mitochondrial membrane to promote permeability and release of cytochrome C and Reactive oxygen species are important signals in the apoptosis cascade.[4]

By considering the functional antagonism between the BCL2 family's pro- and antiapoptotic members, it is suspected that the BCL2/BAX ratio is a vital determinant of the cells susceptibility to apoptosis, rather than the individual protein levels.[5]

Aims of the study

The aim of the study was to assess BAX and BCL2 expression and BCL2/BAX ratio by immunohistochemical study of bone marrow (BM) biopsies of patients with CLL and correlate them with Binet stage, ZAP-70, and other clinicopathological parameters.

Patients and Methods

This is a cross-sectional analysis in which 42 blocks of paraffin-embedded BM biopsy were collected during the period from September 2018 to September 2019 for patients with CLL. Patients were diagnosed by immunophenotyping and had not been treated before the biopsies of the BM. Clinical and laboratory informations regarding age, gender, presence of lymphadenopathy and organomegally, Hb, platelet count, absolute lymphocyte count, percentage of BM lymphocyte, and pattern of BM involvement in addition to CD 38 protein expression flow cytometric results were obtained from patients recording files at diagnosis. Binet system for staging was adopted for the staging of CLL patients.

Ethical approval from the Ethical Committee of Mustansiriyah University/College of Medicine was obtained to conduct this study.

Four sections of 4-μm thickness were taken from each paraffin block. One section was stained with routine hematoxylin and eosin stain to review the histopathological diagnosis and determine the BM infiltration pattern. The other three sections were stained immunohistochemically for BCL2, BAX, and ZAP-70 protein with polymeric horseradish peroxidase (HRP) method. The procedure of immunohistochemistry (IHC) was according to the instructions of the manufacturer. We used the primary antibody anti-BCL2 (PathnSitu, Livermore, CA, USA; Rabbit monoclonal, Clone EP36, dilution: 1:100), anti-BAX antibody (NovusBio, Littleton, CO., USA, Rabbit monoclonal; dilution: 1:2000), and anti-ZAP-70 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA, Clone IE7.2, dilution 1:200). Incubation of the specimen with H_2O_2 for any endogenous peroxidase activity was performed for 5–10 min. The specimen was then incubated with the respective diluted primary mouse or rabbit antibody, followed by 10 min of incubation with the PolyExcel Target Binder, followed by a polymer labeled PolyExcel HRP using recommended 10 min of incubation. Staining was completed by an incubation of 5–10 min with 3, 3'-diaminobenzidine chromogenic substrate that results in a brown precipitate at the antigen site.

Positive control for each antibody was included with the samples; follicular lymphoma was used as a positive control for BCL2, breast carcinoma for BAX, and lymph node for ZAP-70. Negative control slides were obtained by adding buffer saline instead of primary antibody.

Statistical analysis

Statistical analysis was performed with the SPSS 23 statistical software program (IBM Company, New York, USA). Univariate data were summarized using standard descriptive statistics, tabulation of categorical variables and histograms of numerical variables. Associations between categorical variables was assessed via cross tabulation and chi-square. Spearman correlation was used to measure the association between two continuous variables or when at least one variable was ordered. Exact tests were used to calculate the P value. In all statistical analyses, a P value < 0.05 was considered significant.

Results

In this study, there were 42 patients with CLL and age range between 36 and 75 years, with a mean age of 59.5 ± 11.37 years and median of 62 years with a male-to-female ratio of 2.2:1. Mean±SD of absolute lymphocyte count was 63.56 ± 68.07, and of lymphocyte percent in BM was 75.93 ± 16.426.

By application of Binet system, 15/42 (35.7%) of patients were in early stage (a), 11/42 (26.2%) were in intermediate stage (b), and 16/42 (38.1%) were in advanced stage (c).

Regarding the ZAP-70 immunohistochemical expression, positive results were indicated when ≥20% of cells showed distinct nuclear precipitation with or without cytoplasmic precipitation [Figure 1].[6] Accordingly, patients were subdivided into ZAP-70 positive in 13/42 patients (31%) and ZAP-70 negative in 29/42 (69%).

By reviewing the CD38 immunophenotyping result which was available for 27/42 patients, patients were subdivided into CD38 positive in 13/27 patients (48.1%) and CD38 negative in 14/27 (51.9%).

For BCL2 and BAX immunohistochemical expression, cytoplasmic staining was interpreted as positive for BCL2 and BAX.[5]

According to the Lunenburg Lymphoma Biomarker Consortium, the percentage of positive cells for BCL2 and BAX was scored as follows: 0 (no staining), score 1 when 1%–5% positive, score 2 when 6%–25% positive, score 3 when 26%–50% positive, score 4 when 51–75 positive, and score 5 when more than 75% of cells showed positive staining.[7]

The staining strength was graded as follows: 0 (without staining), 1 (weak), 2 (moderate) and 3 (strong).[7]

Regarding the BCL2 expression, patients were subdivided into two groups: (1) BCL2 positive in 39 patients (92.9%) [Figure 2] and (2) BCL2 negative in 3 patients (7.1%).

In the BCL2-positive cases, six patients (14.3%) have a score of 6%–25%, 7/42 patients (16.7%) have a score of 51%–75%, while 9/42 patients (21.4%) have a score of 51%–75%, and 17/42 patients (40.5%) have a score more than 75%.

The staining intensity of positive BCL2 cases was moderate in 10/42 patients (23.8%) and strong in 29/42 (69%).

Regarding the BAX protein, based on the IHC staining, patients were subdivided into two groups: (1) BAX positive in 27 patients (64.3%) [Figure 3] and (2) BAX negative in 15 patients (35.7%).

In the BAX-positive cases, six patients (14.3%) have a score of 6%–25%, 4/42 patients (9.5%) have a score of 51%-75%, while 7/42 patients (16.7%) have a score of 51%-75%, and 10/42 patients (23.8) have a score more than 75%.

Staining intensity was moderate in 11/42 patients (26.2%) and strong in 16/42 patients (38.1%).

The association between BCL2 and BAX with Binet stage is shown in Tables 1-4.

The index value was calculated by multiplying the percentage of the positive cells by predominant intensity resulting in possible scores from 0 to 15.[8] Index value was used to assess correlations between BCL2 and BAX with other established parameters [Tables 5-9].

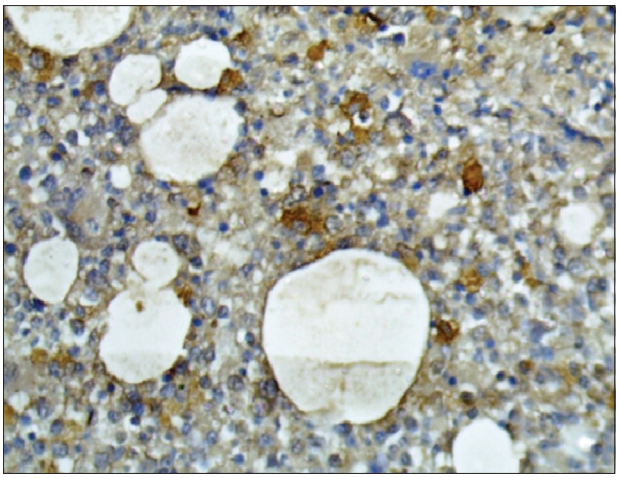

Figure 1: Immunohistochemistry expression of ZAP-70 in bone marrow tissue section of chronic lymphocytic leukemia. Leukemic lymphocytes show cytoplasmic and nuclear immunostain (×40)

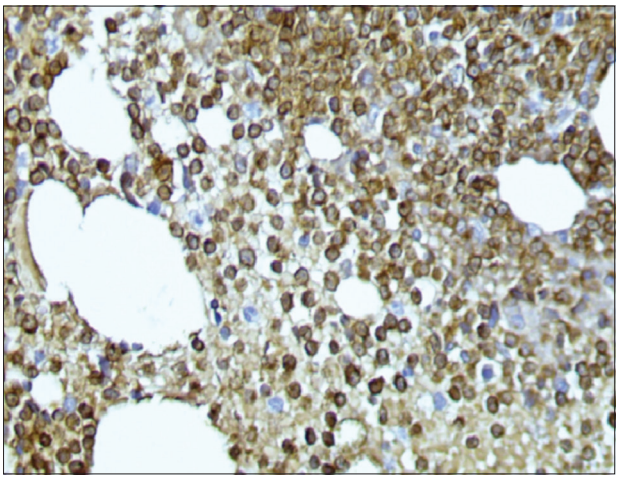

Figure 2: Immunohistochemistry expression of BCL2 in bone marrow tissue section of chronic lymphocytic leukemia. Leukemic lymphocytes show cytoplasmic brown immunostain (×40)

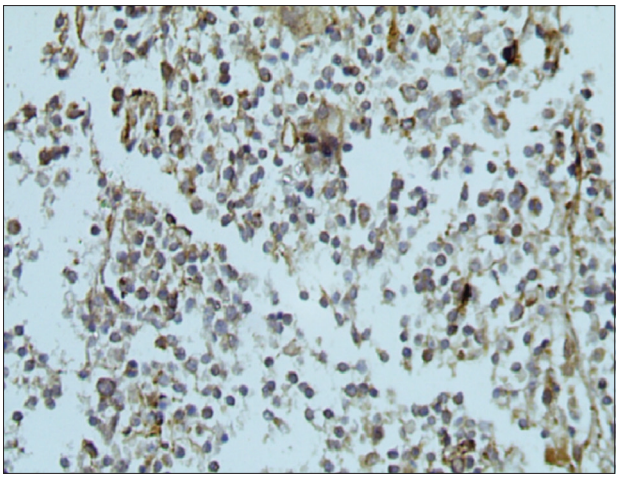

Figure 3: Immunohistochemistry expression of BAX in bone marrow tissue section of chronic lymphocytic leukemia. Leukemic lymphocytes show cytoplasmic immunostain (×40)

Table 1: Association between BCL2 expression and Binet stage

Binet stage	BCL2 expression (%)		Total (%)	*P*
	Positive	Negative		
Stage A	12 (80.0)	3 (20.0)	15 (100.0)	0.04
Stage B and Stage C	27 (100.0)	0 (0.0)	27 (100.0)	
Total	39 (92.9)	3 (7.1)	42 (100.0)	

Table 2: Association between BCL2 score and Binet stage

Binet stage	BCL2 score (%)					Total (%)	*P*
	Negative	6%-25%	26%-50%	51%-75%	>75%		
Stage A	3 (20.0)	4 (26.7)	3 (20.0)	2 (13.3)	3 (20.0)	15 (100.0)	0.022
Stage B and Stage C	0 (0.0)	2 (7.4)	4 (14.8)	7 (25.9)	14 (51.9)	27 (100.0)	
Total	3 (7.1)	6 (14.3)	7 (16.7)	9 (21.4)	17 (40.5)	42 (100.0)	

Table 3: Association between BAX expression and Binet stage

Binet stage	BAX expression (%)		Total (%)	*P*
	Positive	Negative		
Stage A	7 (46.7)	8 (53.3)	15 (100.0)	0.1 (NS)
Stage B and Stage C	20 (74.1)	7 (25.9)	27 (100.0)	
Total	27 (64.3)	15 (35.7)	42 (100.0)	

NS=Not significant

Table 4: Association between BAX score and Binet stage

Binet stage	BAX score					Total (%)	*P*
	Negative	6%-25%	26%-50%	51%-75%	>75%		
Stage A	8 (53.3)	2 (13.3)	0 (0.0)	4 (26.7)	1 (6.7)	15 (100.0)	0.07 (NS)
Stage B and Stage C	7 (25.9)	4 (14.8)	4 (14.8)	3 (11.1)	9 (33.3)	27 (100.0)	
Total	15 (35.7)	6 (14.3)	4 (9.5)	7 (16.7)	10 (23.8)	42 (100.0)	

NS=Not significant

Table 5: Correlation between BCL2 index and BAX index

BAX index	BCL2 index	
	r	*P*
	0.342	0.001

Table 6: Correlation between BCL2 index and BAX index with absolute lymphocyte count

Index	Absolute lymphocytes count	
	r	*P*
BCL2 index	0.372	0.015
BAX index	0.274	0.079

Table 7: Correlation between BCL2 index and BAX index with lymphocyte percent in bone marrow

Index	Lymphocytes percentage in BM	
	r	*P*
BCL2 index	0.367	0.017
BAX index	0.079	0.617

BM=Bone marrow

BCL2/BAX ratio

Depending on positive BCL2 and BAX expression, BCL2/BAX ratio could be calculated in 25/42 patients.

The mean was 1.593 ± 1.337, the range was 0.267–5.333, and the median was 1.0313.

Many studies including ours used the median value of BCL2/BAX as cutoff point,[5,9] and accordingly, patients were subdivided into two groups: (1) patients with high BCL2/BAX ratio (≥1.0313) (n = 12/25, [48%]) and (2) patients with low BCL2/BAX ratio (<1.0313) (n = 13/25 [52%]) [Tables 10-14].

Discussion

BCL2 was positively expressed in CLL cases in 92.9% of CLL cases. It was higher than reported in many other studies, Kitada *et al*. reported 60%, and Lazaridou *et al*. reported 76.3%, and this difference could be due to our patients' delayed diagnosis and higher clinical stages;[10,11] however, Hanada *et al*. reported 95% BCL2 expression.[12]

It was reported that CLL cells are a constant feature of strong expression of BCL2, and high BCL2 expression in CLL was hypothesized to reflect intrinsic abnormality, and it was suggested that the mechanism behind BCL2 upregulation in a large proportion of patients is hypomethylation of its promoter region. Furthermore, by deleting 13q14, (the most common genetic abnormality in CLL), miR-15a and miR-16-1 (inhibit BCL2 expression) are frequently downregulated or lost.[13]

Table 8: Association between BCL2 index and BAX index with ZAP-70 expression

Variable	ZAP-70 result, mean±SD		P
	Positive	Negative	
BCL2 index	9.96±5.1	3.58±5.4	0.001
BAX index	7.65±5.5	1.94±4.2	0.001

SD=Standard deviation

Table 9: Association between BCL2 index and BAX index with CD38 expression

Variable	CD38 result, mean±SD		P
	Positive	Negative	
BCL2 index	12.15±3.4	10.0±5.1	0.207
BAX index	9.32±5.8	7.5±6.2	0.46

SD=Standard deviation

Table 10: Correlation between BCL2/BAX ratio and Binet stage

	Binet stage	
	r	P
BCL2/BAX	0.280	0.185

Table 11: Correlation between BCL2/BAX ratio and absolute lymphocyte count

	Absolute lymphocyte count	
	r	P
BCL2/BAX	0.301	0.152

Table 12: Correlation between BCL2/BAX ratio and lymphocyte percent in bone marrow

	Lymphocyte percent in BM	
	r	P
BCL2/BAX	0.087	0.685

BM=Bone marrow

Table 13: Association between BCL2/BAX ratio and ZAP-70 expression

ZAP-70 expression	BCL2/BAX ratio (%)		Total (%)	P
	≥1.0313	<1.0313		
Positive	4 (36.4)	7 (63.6)	11 (100.0)	0.43
Negative	8 (57.1)	6 (42.9)	14 (100.0)	(NS)
Total	12 (48.0)	13 (52.0)	25 (100.0)	

NS=Not significant

Table 14: Association between BCL2/BAX ratio and CD 38 expression

CD38 expression	BCL2/BAX ratio (%)		Total (%)	P
	≥1.03125000	<1.03125000		
Positive	5 (45.5)	6 (54.5)	11 (100.0)	1.0
Negative	4 (44.4)	5 (55.6)	9 (100.0)	(NS)
Total	9 (45.0)	11 (55.0)	20 (100.0)	

NS=Not significant

Nevertheless, some studies indicated that the expression of BCL2 in CLL is not autonomous and may be affected by external stimuli, microenvironment, or in reaction to certain medications, and may not play a major role in disease pathogenesis. It was concluded that the expression of BCL2 for CLL was associated with poor response to cytotoxic therapy, but it does not seem to be a major determinant of clinical progression.[6]

In this study, we found significant association between Binet staging with BCL2 expression levels (P = 0.04) and score (P = 0.022). Marschitz *et al*., Saxena *et al*., and Jaafar AM *et al.* revealed that BCL2 expression levels were not correlated with disease stages;[14-16] this may be due to different techniques such as immune-cytochemical, Western blot technique, and *in situ* hybridization, respectively.

A significant positive correlation was found between BCL2 index with absolute lymphocyte count (P = 0.015) and lymphocyte percentage in BM (P = 0.017) and significantly associated with ZAP-70 expression (P = 0.001). There was no significant association with CD38 (P = 0.207).

Regarding the BAX marker, it was expressed in 64.3% of CLL patients, and there was a statistically significant positive correlation between BAX index and BCL2 index (P = 0.001). Vucicevic *et al*. mentioned that although the overexpression of BAX in CLL may seem paradoxical, an overexpression of both BAX and BCL2 proteins has been observed, and the overexpression of BAX in CLL cells is considered as a compensatory mechanism used by cells to resume balance between pro- and antiapoptotic proteins, which is necessary for apoptosis regulation.[5]

Pepper *et al*. 1998 revealed that high expression of BCL2 and low expression of BAX were linked with chemoresistance in chlorambucil-treated CLL cells, and a remarkable elevation of BAX protein has been observed in CLL cells undergoing apoptosis.[17]

There was no significant association between BAX expression and Binet stage, and this was consistent with many other studies.[15,18,19]

In contrast, Del Principe *et al*. and others found an association between BAX expression and clinical stages of CLL patients.[20]

Although BAX index was positively correlated with BCL2 index (P = 0.001) and associated with ZAP-70 expression (0.001), there was no association with clinical staging of the disease nor with all other bad prognostic factors (absolute lymphocyte count, lymphocyte percent in BM, and CD38).

Regarding the BCL2/BAX ratio, it was suggested that in CLL, the balance between the pro- and antiapoptotic members of the BCL2 family determines

the responsiveness to cytotoxic drugs and affects the patient's survival.[21]

In this study by analyzing BCL2/BAX ratio, the BCL2/BAX ratio was not significantly related to Binet stage ($P = 0.185$), absolute lymphocyte count ($P = 0.152$), lymphocyte percentage in BM (0.685), CD38 ($P = 1.0$), and ZAP-70 (0.43); the only positive relation was with pattern of BM involvement ($r = 0.516$, $P = 0.01$).

A study identified the prognostic power of the BAX/BCL2 ratio as determined by flow cytometric method, highlighting a significant relation with the disease stage, CD38 and CD49d, some cytogenetic profile, peripheral lymphocyte count, lymphocyte doubling time, β2 M, and soluble CD23.[20]

Furthermore, other findings from some previous studies revealed that an elevated BCL2/BAX ratio, measured at both mRNA and protein levels, is associated with poor prognostic parameters in CLL and is more important in assessing disease activity than the individual expression of BCL2 and BAX.[21,22]

In contrast, a study analyzed BCL2/BAX mRNA ratio by the quantitative PCR and evaluate it with other predictive markers in CLL; BCL2/BAX ratio was not found to be significantly associated with age, gender, Binet stage, LDH, CD38, and cytogenetic profile.[5]

Conclusion

We may conclude from this study that BCL2/BAX ratio is of no prognostic significance since we did not find relation with Binet staging or any of the prognostic parameters we studied. BCL2 can be consider as a simple informative tool to predict disease aggressiveness while BAX although it was positively correlated with BCL2 in CLL cases, it was not related to the Binet stage or any of assessed prognostic parameters (except with ZAP70).

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Darwiche W, Gubler B, Marolleau JP, Ghamlouch H. Chronic lymphocytic leukemia B-cell normal cellular counterpart: Clues from a functional perspective. Front Immunol 2018;9:683.
2. Baraka A, El Shorbagy S, Elfarargy OM, Haggag R, Abdelaziz LA, Elsayed SF, *et al*. Prognostic significance of apo-ptosis regulators in B-cell chronic lym-phocytic leukemia. Artic J Cancer Ther 2017;8:360-85. Available from: http://creativecommons.org/licenses/by/4.0/. [Last accessed on 2019 Dec 27].
3. Scarfò L, Ghia P. Chronic Lymphocytic Leukemia: Who, How, and Where? Cham: In Springer; 2019. p. 3-17. Available from: http://link.springer.com/10.1007/978-3-030-11392-6_1. [Last accessed on 2019 Jul 13].
4. Redza-Dutordoir M, Averill-Bates DA. Activation of apoptosis signalling pathways by reactive oxygen species. Biochim Biophys Acta 2016;1863:2977-92.
5. Vucicevic K, Jakovljevic V, Colovic N, Tosic N, Kostic T, Glumac I, *et al*. Association of Bax expression and Bcl2/Bax ratio with clinical and molecular prognostic markers in chronic lymphocytic leukemia. J Med Biochem 2016;35:150-7.
6. Admirand JH, Rassidakis GZ, Abruzzo LV, Valbuena JR, Jones D, Medeiros LJ. Immunohistochemical detection of ZAP-70 in 341 cases of non-Hodgkin and Hodgkin lymphoma. Mod Pathol 2004;17:954-61.1.
7. Salles G, de Jong D, Xie W, Rosenwald A, Chhanabhai M, Gaulard P, *et al*. Prognostic significance of immunohistochemical biomarkers in diffuse large B-cell lymphoma: A study from the Lunenburg Lymphoma Biomarker Consortium. Blood 2011;117:7070-8.
8. Ozer H, Yenicesu G, Arici S, Cetin M, Tuncer E, Cetin A. Immunohistochemistry with apoptotic-antiapoptotic proteins (p53, p21, bax, bcl-2), c-kit, telomerase, and metallothionein as a diagnostic aid in benign, borderline, and malignant serous and mucinous ovarian tumors. Diagn Pathol 2012;7:124.
9. Matsumoto H, Wada T, Fukunaga K, Yoshihiro S, Matsuyama H, Naito K. Bax to Bcl-2 ratio and Ki-67 index are useful predictors of neoadjuvant chemoradiation therapy in bladder cancer. Jpn J Clin Oncol 2004;34:124-30.
10. Kitada S, Andersen J, Akar S, Zapata JM, Takayama S, Krajewski S, *et al*. Expression of apoptosis-regulating proteins in chronic lymphocytic leukemia: Correlations with *in vitro* and *in vivo* chemoresponses. Blood 1998;91:3379-89.
11. Lazaridou A, Miraxtsi C, Tokmaktsis A, Hatjiharissi E, Christakis J. Detection of genetic markers on different populations of hematopoietic progenitor cells in B-cell chronic lymphocytic leukemia. Blood 2000;95:3634-5.
12. Hanada M, Delia D, Aiello A, Stadtmauer E, Reed JC. Bcl-2 gene hypomethylation and high-level expression in B-cell chronic lymphocytic leukemia. Blood 1993;82:1820-8.
13. Pekarsky Y, Balatti V, Croce CM. BCL2 and miR-15/16: From gene discovery to treatment. Cell Death Differ 2018;25:21-6.
14. Marschitz I, Tinhofer I, Hittmair A, Egle A, Kos M, Greil R. Analysis of Bcl-2 protein expression in chronic lymphocytic leukemia. A comparison of three semiquantitation techniques. Am J Clin Pathol 2000;113:219-29.
15. Saxena A, Viswanathan S, Moshynska O, Tandon P, Sankaran K, Sheridan DP. Mcl-1 and Bcl-2/Bax ratio are associated with treatment response but not with Rai stage in B-cell chronic lymphocytic leukemia. Am J Hematol 2004;75:22-33.
16. Jaafar AM, Al-Rubaie HA, Mustafa SA, Majeed BA. mRNA *in situ* hybridization analysis of p-53 cancer suppression gene and Bcl-2 oncogene in chronic lymphocytic leukemia. J Fac Med Baghda 2010;52:175-9.
17. Pepper C, Hoy T, Bentley P. Elevated Bcl-2/Bax are a consistent feature of apoptosis resistance in B-cell chronic lymphocytic leukaemia and are correlated with *in vivo* chemoresistance. Leuk Lymphoma 1998;28:355-61.
18. Faderl S, Keating MJ, Do KA, Liang SY, Kantarjian HM, O'Brien S, *et al*. Expression profile of 11 proteins and their prognostic significance in patients with chronic lymphocytic leukemia (CLL). Leukemia 2002;16:1045-52.
19. Aviram A, Rabizadeh E, Zimra Y, Yeshoron M, Marmelstein M, Shaklai M, *et al*. Expression of BCL-2 and BAX in cells isolated from B-chronic lymphocytic leukemia patients at different stages of the disease. Eur J Haematol 2000;64:80-4.
20. Del Principe MI, Dal Bo M, Bittolo T, Buccisano F, Rossi FM, Zucchetto A, *et al*. Clinical significance of BAX/BCL-2

ratio in chronic lymphocytic leukemia. Haematologica 2016;101:77-85.

21. Aguilar-Santelises M, Rottenberg ME, Lewin N, Mellstedt H, Jondal M. Bcl-2, Bax and p53 expression in B-CLL in relation to *in vitro* survival and clinical progression. Int J Cancer 1996;69:114-9.
22. Molica S, Dattilo A, Giulino C, Levato D, Levato L. Increased BCL-2/BAX ratio in B-cell chronic lymphocytic leukemia is associated with a progressive pattern of disease. Haematologica 1998;83:1122-4.