Molecular characteristics and prognostic significance of Bcl-2/IgH gene rearrangement in Serbian follicular lymphoma patients

M. RADOJKOVIC[1*], S. RISTIC[1], M. COLOVIC[2], V. CEMERIKIC-MARTINOVIC[2], D. RADOJKOVIC[3], K. KRTOLICA[4]

[1]Clinic of Internal medicine, Clinical Center Dr. Dragisa Misovic, Belgrade, e-mail:radojkov@Eunet.yu, [2]Institute of Hematology, University Clinical Center of Serbia, Belgrade [3]Institute of Molecular Genetics and Genetic Engineering, Belgrade, [4]Institute of Nuclear Sciences Vinca, Belgrade

Received January 7, 2008.

Follicular lymphoma (FL) is characterized by the presence of a t(14;18) chromosomal translocation that results in overexpression of bcl-2 protein. Bcl-2/IgH gene rearrangement is detected in 80-90% of follicular lymphomas in Western countries. The aim of this study was to analyze the bcl-2/IgH rearrangement in FL lymphoma patients in Serbia, by PCR technique, correlate molecular findings with clinical characteristics and outcome and assess the prognostic significance of these rearrangements. One hundred-seven patients (median age, 54 years; male/female ratio:60/47) diagnosed with FL were included in the study. DNA samples were obtained from paraffin embedded lymphoid tissue of patients. Bcl-2/IgH rearrangement was assessed for the major breakpoint region (MBR), 5´MBR and the minor cluster region (mcr) breakpoints by PCR technique. We detected a t(14;18) in 81.3% (87/107) of patients. The distribution of bcl-2-IgH rearrangement was as follows: 88,5% (77/87) in MBR breakpoint, 10,35% (9/87) in 5´MBR, whereas mcr bcl-2-IgH rearrangement was observed in one patient (1.15%). No rearrangements were detected in remaining 20 patients (18.7%). This is the first analyses of the frequency of the bcl-2/IgH gene rearrangement in Serbian FL patients, as well as in Eastern European countries. There was no correlation between presence of bcl-2/IgH gene rearrangement and clinical outcome of disease. Incidence of bcl-2/IgH gene rearrangement in Serbian FL patients is relatively high, and similar to frequency in Western countries. Presence of this rearrangement in tumor tissue is not of prognostic significance.

Key words: follicular lymphoma; bcl-2/IgH gene rearrangement, major breakpoint region (MBR), 5'MBR, minor cluster region (mcr)

Follicular lymphoma (FL) is the most common of the indolent lymphomas. It is characterized with follicular architecture of the tumor, B cell imunophenotype and translocation t(14;18)(q32;q21) which is the genetic hallmark of follicular lymphoma found in more than 80% of the cases at diagnosis [1]. During the translocation process the bcl-2 oncogene is fused to active immunoglobulin heavy chain gene region-IgH gene locus, forming the bcl-2/IgH rearrangement [2]. This leads to overproduction of bcl-2 protein, which blocks apoptosis and protects cells from programmed cell death [3]. The translocation plays an important role in pathogenesis of FL, but is not sufficient for malignant transformation, and additional genetic alterations are necessary.

The chromosomal breakpoints in bcl-2 gene are clustered mainly in two regions: major breakpoint region (MBR) and minor cluster region (mcr) [4, 5]. In approximately 60-70% of follicular lymphoma breakpoints are located within the 3' untranslated part of third bcl-2 exon called major breakpoint region (MBR), which spans 150 bases. In 10% to 20% of cases the breakpoints are clustered in minor cluster region (mcr), located more than 20kb downstream from MBR, and involves a region of 500bp. Other breakpoints are located between MBR and mcr, in the intermediate cluster region (icr) [6], and rarely in 3' and 5' region of bcl-2 gene [7].

The frequency of bcl-2/IgH gene rearrangement in FL differs in the investigated populations, being high in Western countries and low in Asians [8, 9, 10].

The aim of this study was to analyze the bcl-2/IgH rearrangement in FL lymphoma patients in Serbia, by PCR technique, correlate molecular findings with clinical charac-

* Corresponding author

teristics and outcome and assess the prognostic significance of these rearrangements.

Materials and methods

Patients: Hundred seven patients (60 male, 47 female; median age 54 years, range 19-81 years) with FL included in this study were diagnosed and treated in the Institute of Hematology, Clinical Center of Serbia and Clinical Center Dr.Dragisa Misovic in Belgrade, Serbia. Staging of patients was performed according to the Ann Arbor Classification. Performance status was evaluated by the Eastern Cooperative Oncology Group (ECOG) scale. Bulky disease was defined as a tumor mass>10cm. Prognostic indices: International Prognostic Index (IPI), Italian Lymphoma Intergroup (ILI) index, Follicular Lymphoma adapted International Prognostic Index (FLIPI) were calculated according to the original papers [11, 12, 13]. Treatment consisted of: monotherapy with alkylating agents, COP and COP-like chemotherapy (cyclophosphamide, vicristine and prednisone), adriamycin-containing regiments (CHOP and CHOP like regiments). Some patients received adjuvant radiotherapy, interpheron alpha maintenance therapy and 8 patients were treated with antiCD20 monoclonal antibody. Complete remission (CR) was defined as the disappearance of all clinical evidence of the disease and the normalization of all laboratory values and radiographs that had been abnormal before starting treatment. Partial remission (PR) was considered as measurable lesions reduction by at least 50%. No response (NR) was defined as less than 50% regression or progressive disease [14]. Overall survival (OS) was calculated from diagnosis until death or last follow-up. Failure-free survival (FFS) was defined as the time from the start of treatment to one of the following events: disease progression or relapse, death from any cause, or date of last observation.

*Samples and DNA extraction:*Biopsy samples of lymph node, bone marrow or other involved organs from 107 patients with FL were collected at diagnosis. Sixty eight samples (63,8%) were obtained from lymph node biopsy, 30 (28%) from bone marrow and 9 (8,4%) from other lymphoid tumor tissues (spleen, colon, tonsilla -two samples each, and 3 gaster samples). The histopathological diagnosis and grading of FL was performed according to the REAL/WHO classification [15, 16]. Sixty (56,1%) patients had grade I, 30 (28%) grade II and 17 patients (15,9%) grade IIIA subtype of FL.

Table 1. Sequences of oligonucleotides used for the PCR amplifications

	Sequence of oligonucleotides	Chromosome location
Oligo 1	5'- TAG AGA GTT GCT TTA CGT G- 3'	chromosome 18
Oligo 2	5'- TAT GGT GGT TTG ACC TTT AG-3'	chromosome 18
Oligo 3	5'- TTG TGA GCA AAG GTG ATC GT- 3'	chromosome 18
Oligo 4	5'- ACC TGA GGA GAC GGT GAC C- 3'	chromosome 14
Oligo 5	5'- ACC AGG GTC CCT TGG CCC CA-3'	chromosome 14
MC10	5'- TGC CTG GCA TAG AGC AAG CG-3'	chromosome 18
MC12	5'- GAT GGC TTT GCT GAG AGG TAT-3'	chromosome 18

The human B cell lymphoma lines which carries a t(14;18) translocation with a breakpoint in the MBR (SUDHL-6), and 5'MBR (SUDHL-4) line which served as a positive control was kindly provided by Dr. Epstein (University of Southern California, USA). DNA from lymphoma tissue of a patient positive for a rearrangement in the mcr was used as a positive control (kindly provided by Dr.Michel Stula, Department Menselijke Erfelijkheid, Katholieke Universitet Leuven). The negative control DNA samples for t(14;18) translocation were isolated from peripheral blood of 10 healthy individuals and paraffin-embedded tumor samples of 10 patients with Hodgkin's lymphoma.

High-molecular weight DNA was isolated from formalin and BP-fixed paraffin-embedded tissue and peripheral blood specimens in accordance with standard extraction procedures (17, 18).

PCR: The presence of the bcl-2/IgH translocation was estimated using a nested and seminested polymerase chain reaction (PCR), according to the previously described method [19, 20], with slight modifications using primers for the bcl-2 major breakpoint region (MBR), 5' bcl-2 major breakpoint region the (5´MBR) and bcl-2 minor cluster region (mcr), and the conserved immunoglobulin heavy-chain joining region (JH) (Table 1) in automated thermal cycler (Eppendorf Mastercycler gradient). PCR was carried out in a final volume of 50µl, with 20pmol of each oligonucleotide primer, 200µM of each of dNTP, 1,25U Taq polymerase (Ampli Taq Gold, Perkin Elmer), 2µl 25mM $MgCl_2$ in PCR buffer (100mM TrisCl, pH 8,3, 500mM KCl, 15mM $MgCl_2$, 0,01% gelatin). The first round of amplification was performed with 1µg DNA, for 39 cycles, with "touchdown" modification, using outer primers for the JH region (oligo 4), oligo 2 for MBR Bcl-2, and oligo 3 for 5'MBR Bcl-2 gene (Table 1). After initial denaturation of 10 minutes, 9 cycles consist of denaturation (45 sec, 95°C), annealing (2min, 57°C), and extension (2min, 72°C), followed with 30 cycles of denaturation (45 sec, 95°C), annealing (2min, 53°C), and extension (2min, 72°C), with final extension step of 10min. at 72°C. The second round was performed using 1µl 10x diluted PCR product from first round of amplification as a template for second DNA amplification. Reaction was performed for 30 cycles (45sec, 95°C; 1min, 57°C; 1,5min, 72°C, with final extension step 10 min, 72°C) using inner primers oligo 5 for the JH region, oligo 1 for MBR Bcl-2, and oligo 3 for 5'MBR Bcl-2 (Table 1). The presence of the Bcl-2/IgH gene rearrangement in the minor breakpoint region was performed using nested PCR. In the first round of amplification outer primers for the JH region (oligo 4) and for mcr (MC12) were used. Ten times diluted PCR product from the first round was used as a template for the second round with primers oligo 5 for JH region and MC10 for mcr (Table 1). The amplification consisted of the initial denaturation step of 10min at 95°C, followed by 35 cycles for 30 seconds at 95°C, 60°C (first round) or 64°C (second round) for 30 seconds, and 1 minute at 72°C, with final exten-

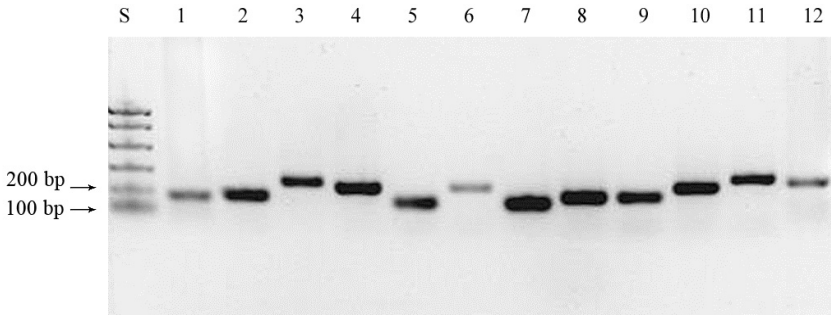

Figure 1. Analysis of the amplification products of a two-step PCR with nested primers for the detection of MBR bcl-2/IgH gene rearrangement S-size markers-100bp size ladder, lines 1-11 patients, 12-positive control (SUDHL-6 cell line)

sion step of 10min at 72°C. A volume of 10μl of each amplified product was applied to a 2% agarose gel electrophoresis and visualized by staining with ethidium bromide.

As a control of DNA quality, a 425bp fragment of CFTR (Cystic Fibrosis Transmembrane Regulator) gene was amplified using the following primers: CAA CTG TGG TTA AAG CAA TAG TGT and GCA CAG ATT CTG AGT AAC CAT AAT.

The sensitivity of the PCR assay was tested by various DNA dilutions (10^{-2}- 10^{-6}) of SUDHL4 cell line in normal mononuclear blood cells. Routinely, the 10^{-5} dilution gave band visible after ethidium bromide staining.

Nucleotide Sequence Analysis (Direct Sequencing of PCR products): To confirm the specificity of obtained bcl-2 (MBR)/JH region PCR products were sequenced in five cases. For direct sequencing the amplified products were obtained by nested PCR amplification and purified using QIA quick PCR purification Kit, according to QIAGEN protocol. Both strands of the PCR product were sequenced with primers used in the second round of amplification. The resulting sequences were compared with published germ line sequence.

Statistical analysis: Differences in patients characteristics and response were tested with Pearson's χ^2-test. Overall survival (OS) and failure-free survival (FFS) curves were calculated for each prognostic group according to Kaplan-Meier method, and differences were evaluated by log-rank test.

Results

Incidence of bcl-2/IgH gene rearrangement in FL: We analyzed bcl-2/IgH gene rearrangement using two-step PCR assay with DNA from paraffin-embedded tumor samples obtained at time of diagnosis in 107 patients with FL. Bcl-2/IgH gene rearrangement was detected in 87 of 107 patients (81,3%), while in 20 cases (18,7%) no bcl-2/IgH fusion gene was detected. In control DNA specimens isolated from peripheral blood of healthy individuals and paraffin-embedded tumor samples of patients with Hodgkin lymphoma, bcl-2/IgH gene rearrangement was not detected.

Bcl-2/IgH gene rearrangement occurred in 80% (48/60) FL patients with grade I histological subtype, in 83,3% (25/30) grade II, and in 82,3% (14/17) cases with grade IIIA FL. The relation between a morphologic subtype and presence of bcl-2/IgH gene rearrangement was examined by Pearson's χ^2 test, and no significant difference in frequency was detected in grade I, II and IIIA follicular lymphomas.

Distribution of bcl-2 rearrangement sites: The bcl-2/IgH gene rearrangement was detected by PCR using two specific primer sets for MBR, 5'MBR and mcr breakpoints.

The distribution within MBR, 5'MBR or mcr site in 87 patients with bcl-2 breakpoint sites was 88,5%, 10,35% and 1,15%, respectively. The amplified DNA was separated by 2% agarose gel electrophoresis and visualized by staining with ethidium bromide (Figures 1).

In our experiments amplified PCR products ranged from 110-250bp for bcl-2/IgH gene rearrangement for MBR**,** 107bp for 5'MBR, and 150bp for mcr. The size of amplified DNA fragments within the MBR region varied between different patients indicated different breakpoints either on BCl-2 gene or on IgH 4 to 6 cluster region of the gene in analyzed cases.

Bcl-2/IgH gene rearrangement in circulation, lymph node and bone marrow biopsy: PCR products from diagnostic lymph node biopsy, bone marrow (BM) and peripheral blood (PB) in 8 patients were compared. We found identical size of amplified DNA fragments from different tissues, and the pattern was characteristic for each patient.

Direct sequencing of PCR products: Nucleotide sequence data analysis and comparison with corresponding germline sequences revealed that each patient have unique gene rearrangement and DNA sequence as a result of the different breakpoints on 14 and 18 chromosomes, and the insertion of N segment with different length and structure due to the insertion of *de novo* nucleotide synthesis (Table 2).

Correlation between bcl-2/IgH gene rearrangement and clinical characteristics and laboratory findings: Complete clinical status was known for 99 patients. A large majority of patients (88,9%) were in advanced clinical stage (Ann Arbor III-IV). Almost a half of the patients (48,5%) had presence of

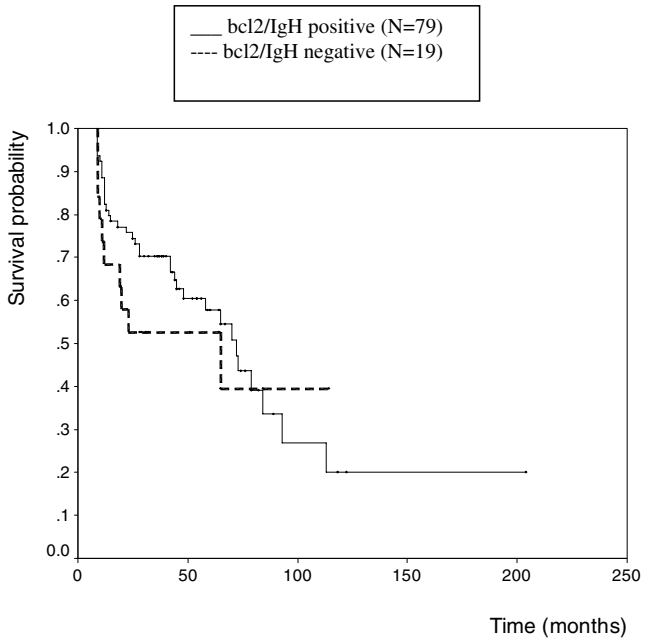

Figure 2. Overall survival curves according to the presence of bcl-2/IgH gene rearrangement
log rank=0,61, p=0,433

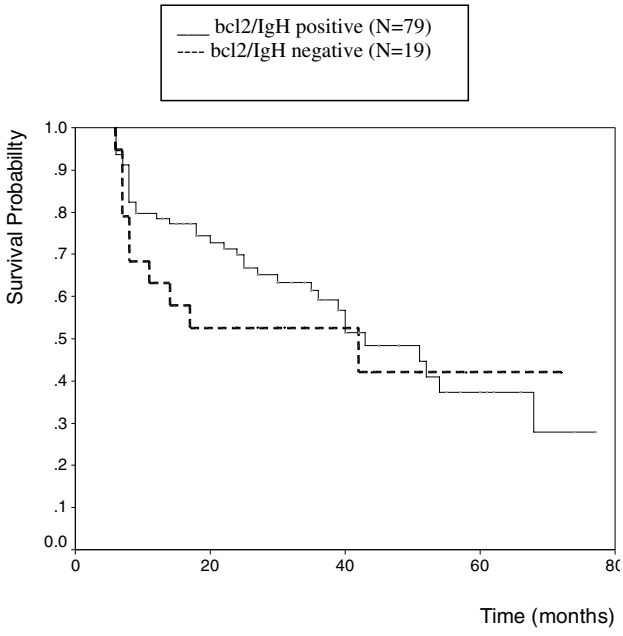

Figure 3. Failure free survival curves according to the presence of bcl-2/IgH gene rearrangement
log rank=0,44, p=0,508

B symptoms, while 30,3% of patients had poor performance status. More than 70% of patients had bone marrow involvement, and 22,2% at presentation were with bulky disease. High percentage of patients (45,5%) had anaemia, 46,5% elevated erythrocyte sedimentation rate (ESR) ≥ 30 mm/h, and elevated serum LDH was present in 29,3% of cases.

Clinical characteristics and laboratory findings were compared with presence of bcl-2/IgH gene rearrangement (Tables 3). No significant differences were found among bcl-2/IgH positive and negative group according to clinical and laboratory features, except to anaemia (Hb<100g/l) and thrombocytopenia (Plt.< 100 $x10^9$/l) which were present in significant higher percent in patients with bcl-2/IgH gene rearrangement.

Upon the correlation of prognostic indices (IPI, FLIPI and ILI) and presence of bcl-2/IgH gene rearrangement, no significant difference was observed in distribution of low, intermediate and high IPI, FLIPI and ILI prognostic scores between bcl-2/IgH positive and negative group. Anyway the trend of poor prognostic indices (IPI, FLIPI, ILI 3 3) was detected in the patients without translocation in comparison with patients bearing bcl-2/IgH gene rearrangement, as follows: for IPI 57,9% *vs* 31,2%; for FLIPI 52,6% *vs* 36,3% and ILI 52,6% *vs* 35,0%.

Correlation between bcl-2/IgH gene rearrangement and treatment response: Among 98 patients, after initial treatment, 53 patients (54,1%) achieved complete response (CR), 23 (21,4%) partial response (PR), and treatment failure was present in 24 (24,5%). We didn't observed a significant relationship between treatment response and bcl-2/IgH gene rearrangement (χ^2=1,945; p>0,05). Patients without bcl-2/IgH gene rearrangement had treatment failure in higher percent (36,8%), than patients with translocation (21,5%), but that difference was not statistically significant.

Correlation between bcl-2/IgH gene rearrangement and survival: Five year overall survival (OS) rate of patient with bcl-2/IgH gene rearrangement was 60%, comparing to 53% in patients without translocation t(14;18). No significant difference in OS and FFS was found between these two groups (Figures 2, 3).

We analyzed influence of bcl-2 breakpoint site on clinical outcome. Overall survival of patients with MBR bcl-2/IgH and 5'MBR bcl-2/IgH gene rearrangement was not significantly different. Mcr rearrangement was excluded because it was detected in only one patient.

Table 2. Sequences of bcl-2/IgH MBR rearrangement by nested PCR in five patient with FL

cases	bcl-2 MBR	N segment position	JH segment	PCR product
1	4562	ttgactacaggctaccatg	JH6	203bp
2	4483	aacttctcgaaggcccaaa	JH5	123bp
3	4482	gatcacggat	JH4	112bp
4	4533	gcgtgcttctggggcgtaaag	JH5	166bp
5	4562	gaccagataccctgacttactacgggccaga	JH6	189bp

Table 3. Clinical characteristics and laboratory findings of FL patients according to the presence of bcl-2/IgH rearrangement

Clinical characteristics and laboratory findings	Bcl-2/IgH positive N=80 (%)	Bcl-2/IgH negative N=19 (%)	p value
Median age	53,8 ± 14,2 year	55,4 ± 14,2 year	NS
Male sex	45 (56,2%)	8 (42,1%)	NS
Ann Arbor stage III – IV	70 (87,5%)	18 (94,7%)	NS
B symptoms	37 (46,3%)	11 (57,9%)	NS
Performance status ECOG 3 2	21 (26,3%)	9 (47,4%)	NS
Hb <100g/l	8 (10,0%)	6 (31,6%)	P=0,015
Plt. < 100 x10^9/l	4 (5,0%)	4 (21,1%)	P=0,021
Serum LDH> 320 U/L	21 (26,3%)	8 (42,1%)	NS
ESR 3 30mm/h	34 (42,5%)	12 (63,2%)	NS

N-number of patients; NS- not significant (p> 0,05)

Discussion

Different studies have demonstrated a marked geographic variation in the incidence rates of FL between Asian and Western countries, as well as geographic variation in the frequency of bcl-2 rearrangements [9, 10, 21, 22]. In addition, molecular epidemiological studies showed a geographic variance in pattern of chromosomal breakpoint locations, suggesting the existence of pathogenetically distinct subtypes in different world regions [23].

In this study we analyzed the incidence and significance of the bcl-2/IgH gene rearrangement in Serbian patients with FL. We found bcl-2/IgH gene rearrangement in 81,3% of FL patients, that is broadly consistent with the previously published European and American frequencies [8, 24]. The breakpoint site in the MBR of bcl-2 gene was located in 77 patients, within 5'MBR in 9 cases, and within mcr in only one patient. We found similar distribution of translocation in all three histological grades of FL, although some authors observed lower incidence of rearrangement in grade III FL [25].

The detection rate of bcl-2/IgH gene rearrangement depends on the applied method [26, 27]. The main advantages of PCR in comparison with cytogenetic analysis and Southern blot are that it requires small quantities of DNA and can be performed on archived material. The FISH is considered as a gold standard for the detection of bcl-2/IgH rearrangement, but its relative cost per test would favor the use of PCR. PCR could be used as a screen for the presence of bcl-2/IgH gene rearrangement, followed by FISH performed on PCR-negative cases [28]. Unfortunately, the proposed strategy could not have been applied to our PCR-negative FL patients, because FISH is still not available as a test for translocation t(14;18) in Serbia.

Several PCR based methods are in use in detection of bcl-2/IgH gene rearrangement, and nested PCR strategy has been shown to have an increased sensitivity up to 1000-fold higher than conventional PCR [29], which was the reason for standardization semi-nested and nested PCR protocol in our study. To avoid false positive results which are more frequent in nested PCR, each sample was analyzed in two independent amplification experiments.

The majority of follicular lymphomas express bcl-2 protein, but presence of translocation t(14; 18) does not correlate completely with expression of bcl-2 protein. In our study expression of bcl-2 protein was found in all analyzed cases (46 patients), but 8 patients expressed bcl-2 protein in the absence of bcl-2/IgH gene rearrangement (data not shown). This indicates that translocation is not the only mechanism that causes increased expression of bcl-2 protein. It is possible that patients without detectable translocation may have breakpoint regions which can not be found by primers we used, or other mechanisms independent of the translocation are responsible for bcl-2 expression [30, 31].

Finding of bcl-2/IgH gene rearrangement positive B cells in peripheral blood of healthy individuals [32] indicates importance of detecting translocation in tumor tissue. Nucleotide sequence of bcl-2/IgH rearrangement in lymphoma cells is specific tumor marker. In all patients in whom we examined the bcl-2/IgH gene rearrangement in lymph node specimens, bone marrow biopsy, and peripheral blood we amplified DNA fragments of identical size. The sequence of rearrangement is specific to the individual FL, and each patient had unique gene rearrangement.

In about 15% of patients translocation t(14;18) can not be detected by using cytogenetic, Southern blot analysis or PCR. Follicular lymphoma lacking the translocation t(14;18) may be a different subtype of follicular lymphoma with distinctive molecular pathogenesis and biological features [33, 34]. Further studies are needed to distinguish heterogenity of this malignancy, and to identify patients who may require different treatment.

The prognostic significance of bcl-2/IgH gene rearrangement is still controversial issue. Some studies [8, 24] suggested that patients without t (14; 18) had a worse prognosis than patients with bcl-2 rearrangement, but subsequent studies failed to demonstrate prognostic significance of bcl-2 gene rearrangement [35, 36]. In our investigation we found no correlation between the presence of bcl-2/IgH gene rearrangement and survival. Patient without bcl-2/IgH gene rearrangement

had worse clinical and laboratory findings, but apart of anemia and thrombocytopenia these differences were not statistically significant. Also, the complete response rate was lower in patients without bcl-2/IgH gene rearrangement, and even a trend toward decreased survival (p=0,052) was observed. Although Lopez-Guillermo (24) found the association of mcr translocation with better FFS, we observed very low incidence of this rearrangement similarly to the studies in Turkey and Spain [36, 37]. It is possible that some other gene and environmental factors might have influence on the type of rearrangement.

Alternative breakpoints might explain geographic variation in incidence of translocation in FL among diverse populations and geographic areas [38]. Additional cluster of breakpoints in intermediate cluster region (icr), which spread between MBR and mcr occurs more frequently in Swiss and Japanese FL patients than mcr rearrangement [6, 39]. Screening for icr rearrangement may yield more PCR positive cases with bcl-2/IgH gene rearrangement in FL, as already shown in some studies [40, 41]

This study represents the first report on the distribution of bcl-2/IgH gene rearrangement in FL patients of Serbian ethnicity. Our results confirm that a presence of bcl-2/IgH gene rearrangement is useful in diagnosis of FL, but not of importance in prognostic evaluation. Quantitative monitoring of bcl-2/IgH positive cells might be of clinical value in prognosis and quantitative PCR screening could be used for monitoring of minimal residual disease and predicting relapse.

This work is sponsored by research projects of Serbian Ministry of Science, number 145061 and 143010.

References

[1] TSUJIMOTO Y, COSSMAN J, JAFFE E et al. Involvement of the bcl-2 gene in human follicular lymphoma. Science 1985; 228: 1440–1443

[2] TSUJIMOTO Y, CROCE CM. Analysis of the structure, transcripts, and protein products of bcl-2, the gene involved in human follicular lymphoma. Proc. Natl. Acad. Sci. USA 1986; 83: 5214–5218

[3] KORSMEYER SJ. Bcl-2 initiates a new category of oncogenes: Regulators of cell death. Blood 1992; 80: 879–886

[4] BAKHSHI A, WRIGHT J, GRANINGER W et al. Mechanism of the t(14;18) chromosomal translocation: Structural analysis of both derivative 14 and 18 reciprocal partners. Proc. Natl. Acad. Sci USA 1987; 84: 2396–2400

[5] CLEARY ML, GALILI N, SKLAR J. Detection of a second t(14;18) breakpoint cluster region in human follicular lymphomas. J Exp. Med. 1986; 164: 315–320

[6] ALBINGER-HEGYI A, HOCHREUTENER B, ABDOU M-T et al. High frequency of t(14;18)-translocation breakpoints outside of major breakpoint and minor cluster regions in follicular lymphomas: improved polymerase chain reaction protocols for their detection. Am J Pathol 2002; 160: 823–832

[7] BUCHONNET G, LENAIN P, RUMINY P et al. Characterisation of Bcl-2-JH rearrangements in follicular lymphoma: PCR detection of 3' Bcl-2 breakpoints and evidence of a new cluster. Leukemia 2000; 14: 1563–1569

[8] JOHNSON A, BRUN A, DICTOR M et al. Incidence and prognostic significance of t(14;18) translocation in follicle center cell lymphoma of low and high grade: a report from southem Sweden. Ann Oncol. 1995; 6: 789–794

[9] LOKE SL, PITTALUGA S, SRIVASTAVA G et al. Translocation of bcl-2 gene in non-Hodgkincs lymphomas in Hong Kong Chinese. Br.J.Haematol. 1990; 76: 65–69

[10] MITANI S, AOKI N, MUZUTANI S et al. Bcl-2 gene rearrangement analysis of Japanese follicular lymphomas by polymerase chain reaction in formalin-fixed, paraffin embedded tissue specimens. Jpn J Cancer Res. 1993; 84: 37–41

[11] FEDERICO M, VITOLO U, ZINZANI PL et al. Prognosis of follicular lymphoma: a predictive model based on a retrospective analysis of 987 cases. Blood 2000; 95: 783–789

[12] SOLAL-CELIGNY P, ROY P, COLOMBAT P et al. Follicular lymphoma international prognostic index. Blood 2004; 104: 1258–1265

[13] The international Non-Hodgkin's Lymphoma Prognostic Factors Project. A predictive model for aggressive non-Hodgkin's lymphoma. N.Engl.J.Med. 1993; 329: 987–994

[14] CHESON BD, HORNING SJ, COIFFIER B et al. Report of an International Workshop to standardize response criteria for non-Hodgkincs lymphomas. J Clin Oncol 1999; 17: 1244–1253

[15] HARRIS NL, JAFFE ES, DIEBOLD J et al. World Health Organization Classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee Meeting- Airlie House, Virginia, November 1997. J Clin Oncol 1999; 17: 3835–3849

[16] The non-Hodgkincs Lymphoma Pathologic Classification Project. National Cancer Institute sponsored study of classification of non-Hodgkincs lymphomas: summary and description of a working formulation for clinical usage. Cancer 1982; 49: 2112–2135

[17] WRIGHT DK, MANOS MM Sample preparation from paraffin-embedded tissues. In Innis MA et al, editors. A guide to methods application. Academic Press, Berkley, California, 1990: 153–158

[18] DEL SAL G, MANFIOLETTI G, SCHNEIDER C. The CTAB-DNA precipitation method: A common mini-scale preparation of template DNA from phagemids, phages or plasmids suitable for sequencing. Bio Techniques 1989; 7: 514–519

[19] HOSLER GA, BASH RO, BAI X et al. Development and validation of quantitive polymerase chain reaction assay to evaluate minimal residual disease for T cell acute lymphoblastic leukemia and folicular lymhoma. Am.J.Pathol. 1999; 154: 1023–1035

[20] LAMBRECHTS AC, HUPKES PE, DORSSERS LCJ et al. Translocation (14;18)-positive cells are present in the circulation of the majority of patients with localized (stage I and II) follicular non Hodgkincs lymphoma. Blood 1993; 82: 2510–2516

[21] ESSOP MF, MANUEL YE, CLOSE PM. Detection of the t (14;18) translocation in follicle centre cell lymphomas in South African ethnic groups usining PCR. Leukemia and Lymphoma 1999; 34: 609–613

[22] ASTER JC, LONGTINE JA. Detection of BCL2 rearrangements in follicular lymphoma Am J Pathol 2002; 160: 759–763

[23] BIAGI JJ, SEYMOUR JF. Insights into the molecular pathogenesis of follicular lymphoma arising from analysis of geographic variation. Blood 2002; 99: 4265–4275

[24] LOPEZ-GUILLERMO A, CABANILLAS F, TIMOTHY I et al. Correlation of Bcl-2 rearrangement with clinical characteristics and outcome in indolent follicular lymphoma. Blood 1999; 93: 3081–3087

[25] WEISENBURGER DD, GASCOYNE RD, BIERMAN PJ et al. Clinical significance of the t(14;18) and BCL-2 overexpression in follicular large cell lymphoma. Leuk Lymphoma 2000; 36: 513–523

[26] TURNER GE, ROSS FM, KRAJEWSKI AS. Detection of t(14;18) in British follicular lymphomas using cytogenetics, Southern blotting and polymerase chain reaction. Br J Haematol 1995; 89: 223–225

[27] VAANDRAGER JW, SCHUURING E, RAAP T et al. Interphase FISH detection of Bcl2 rearrangement in follicular lymphoma using breakpoint-flanking probes. Genes Chromosomes Cancer 2000; 27: 85–94

[28] SHAMINIE J, PEH SC, TAN MJ. Improvement in the detection rate of t(14;18) translocation on paraffin-embedded tissue: a combination approach using PCR and FISH. Pathology 2003; 35: 414–421

[29] JOHNSON PWM, SWINBANK K, MACLENNAN S et al. Variability of polymerase chain reaction detection of the bcl-2-IgH translocation in an international multicentre study. Annals of Oncology 1999; 10: 1349–1354

[30] KURLAND JF, KODYM R, STORY MD et al. NF-kB1 (p50) homodimers contribute to transcription of the bcl-2 oncogene. Journal of Biologica Chemistry 2001; 276: 45380–45386

[31] PAPAKONSTANTINOU G, VERBEKE C, HASTKA J et al. Bcl-2 expresion in non-Hodgkincs lymphomas is not associated with bcl-2 gene rearrangements. Br J Haematol 2001; 113: 383–390

[32] SCHULER F, HIRT C, DOLKEN G. Chromosomal translocation t(14;18) in healthy individuals. Semin Cancer Biol 2003; 13: 203–209

[33] HORSMAN DE, OKAMOTO I, LUDKOVSKI O et al. Follicular lymphoma lacking the t(14;18)(q32;21): identification of two disease subtypes Br J Haematol. 2003; 120: 424–433

[34] KARUBE K, GUO Y, SUZUMIYA J et al. CD10(-)MUM1(+) follicular lymphoma lacks BCL2 translocation and shows characteristic biological and clinical features. Blood 2007; 109: 3076–3079

[35] IQBAL S, JENNER MJ, SUMMERS KE et al. Reliable detection of clonal IgH/Bcl-2 MBR rearrangement in follicular lymphoma: methodology and clinical significance. Br J Haematol 2004; 124: 325–328

[36] MONTOTO S, LOPEZ-GUILLERMO A, COLOMER D et al. Incidence and clinical significance of bcl-2/IgH rearrangements in follicular lymphoma. Leuk Lymphoma 2003; 44. 71–76

[37] SAYHAN N, TUZUNER N, AKI H et al. Detection of t(14;18) in Turkish follicular lymphomas using the polymerase chain reaction. Leuk Res 2000; 24: 475–479

[38] NORIEGA MF, DE BRASI C, NARBAITZ M et al. Incidence of bcl-2 gene rearrangements in Argentinean non-Hodgkin lymphoma patients: increased frequency of breakpoints outside of MBR and MCR. Blood, Cell, Molecul and Diseases 2004; 32: 232–239

[39] AKASAKA T, AKASAKA H, YONETANI N et al. Refinement of the BCL-2/immunoglobulin heavy chain fusion gene in t(14;18)(q32;q21) by polymerase chain reaction amplification for long targets. Genes, chromosomes and cancer 1998; 21: 17–29

[40] BATSTONE PJ, GOODLAD JR. Efficasy of screening the intermediate cluster region of the bcl2 gene in follicular lymphomas by PCR. J Clin Pathol. 2005; 58: 81–82

[41] WEINBERG OK, AI WZ, MARIAPPAN MR et al. "Minor" BCL2 breakpoints in follicular lymphoma: frequency and correlation with grade and disease presentation in 236 cases. J Mol Diagn. 2007; 9: 530–537