Prognostic Significance of B-cell Differentiation Genes Encoding Proteins in Diffuse Large B-cell Lymphoma and Follicular Lymphoma Grade 3

Ana Borovečki[1], Petra Korać[1], Marin Nola[2], Davor Ivanković[3], Branimir Jakšić[4], Mara Dominis[1]

[1]Department of Clinical Pathology and Cytology, Merkur University Hospital, Zagreb, Croatia
[2]Department of Pathology, Zagreb University Hospital Center, Zagreb, Croatia
[3]Andrija Štampar School of Public Health, Zagreb, Croatia
[4]University Department of Internal Medicine, Merkur University Hospital, Zagreb, Croatia

Aim To define prognostic significance of B-cell differentiation genes encoding proteins and *BCL2* and *BCL6* gene abnormalities in diffuse large B-cell lymphoma and follicular lymphoma grade 3 with >75% follicular growth pattern.

Methods In 53 patients with diffuse large B-cell lymphoma and 20 patients with follicular lymphoma grade 3 with >75% follicular growth pattern the following was performed: 1) determination of protein expression of BCL6, CD10, MUM1/IRF4, CD138, and BCL2 by immunohistochemistry; 2) subclassification into germinal center B-cell-like (GCB) and activated B-cell-like (ABC) groups according to the results of protein expression; 3) detection of t(14;18)(q32;q21)/*IgH-BCL2* and *BCL6* abnormalities by fluorescent in situ hybridization in diffuse large B-cell lymphoma and follicular lymphoma grade 3 with >75% follicular growth pattern as well as in GCB and ABC groups; and 4) assessment of the influence of the analyzed characteristics and clinical prognostic factors on overall survival.

Results Isolated BCL6 expression was more frequently found in follicular lymphoma grade 3 with >75% follicular growth pattern than in diffuse large B-cell lymphoma ($P = 0.030$). There were no differences in *BCL2* and *BCL6* gene abnormalities between diffuse large B-cell lymphoma and follicular lymphoma grade 3 with >75% follicular growth pattern. Diffuse large B-cell lymphoma and follicular lymphoma grade 3 with >75% follicular growth pattern patients were equally distributed in GCB and ABC groups. t(14;18)(q32;q21) was more frequently recorded in GCB group, and t(14;18)(q32;q21) with *BCL2* additional signals or only *BCL2* and *IgH* additional signals in ABC group ($P = 0.004$). The GCB and ABC groups showed no difference in *BCL6* gene abnormalities. There was no overall survival difference between the patients with diffuse large B-cell lymphoma or follicular lymphoma grade 3 with >75% follicular growth pattern; however, GCB group had longer overall survival than ABC group ($P = 0.047$). Multivariate analysis showed that BCL6, CD10, and BCL2 expression, *BCL2* and *BCL6* abnormalities, and International Prognostic Index were not significantly related to overall survival.

Conclusion Diffuse large B-cell lymphoma and follicular lymphoma grade 3 with >75% follicular growth pattern patients have very similar characteristics and their prognosis is more influenced by protein expression of B-cell differentiation stage genes than by tumor cells growth pattern, *BCL2* and *BCL6* abnormalities, and International Prognostic Index.

> **Correspondence to:**
Ana Borovečki
Department of Clinical Pathology and Cytology
Merkur University Hospital
Zajčeva 19
10000 Zagreb, Croatia
anaborov@yahoo.com

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The World Health Organization (WHO) classification defines diffuse large B-cell lymphoma as a specific type of mature B-cell neoplasm (1). However, diffuse large B-cell lymphoma types show clinical, morphological, immunophenotypic, and cytogenetic heterogeneity (2-7). Clinical heterogeneity of diffuse large B-cell lymphoma is a consequence of the expression of B-cell differentiation stage genes. The gene expression analysis has identified three prognostically significant molecular subtypes of diffuse large B-cell lymphoma as follows: germinal center B-cell-like (GCB), activated B-cell-like (ABC), and type 3 diffuse large B-cell lymphoma, which express neither genes of normal GCB-cells nor genes that are normally induced during in vitro activation of peripheral blood B-cells (8-10).

Immunohistochemical subclassification of diffuse large B-cell lymphoma showed that GCB and ABC groups greatly differ (11-19). Immunohistochemical GCB and ABC groups are not always considered to be prognostically significant predictors (16-19).

Cytogenetic analysis shows t(14;18)(q32;q21)/*IgH-BCL2* to be more common in GCB than in ABC (20-24).

Follicular lymphoma is the second most common non-Hodgkin B-cell lymphoma (B NHL) (1,25). Follicular lymphoma grade 3, in contrast to indolent follicular lymphoma grade 1 and follicular lymphoma grade 2, is clinically aggressive and has more in common with diffuse large B-cell lymphoma (5).

The morphological subtypes of follicular lymphoma grade 3, follicular lymphoma grade 3A, and follicular lymphoma grade 3B show morphological, immunohistochemical, and cytogenetic characteristics of variable clinical importance (1). Clinicopathologic evaluation revealed no differences in survival between follicular lymphoma grade 3A and follicular lymphoma grade 3B patients, yet follicular lymphoma grade 3 cases with a predominant diffuse component (>50%) had a significantly worse survival (25-27).

Follicular lymphoma grade 3 is characterized by variable CD10 and BCL2 protein expression and cytogenetic abnormalities, ie, rearrangements of *BCL6* gene, *BCL2* gene amplification, and less common t(14;18)(q32;q21) (28). Both t(14;18)(q32;q21) and BCL2 protein expression are more common in follicular lymphoma grade 3A, while *BCL6* gene rearrangements are more common in follicular lymphoma grade 3B (29-32).

Therapy and prognosis of patients with diffuse large B-cell lymphoma and follicular lymphoma are defined according to their morphological characteristics, clinical stage, and clinical prognostic factors included in the International Prognostic Index (IPI) (33,34).

The aim of this study was to determine whether follicular lymphoma grade 3 with >75% follicular growth pattern is part of the morphological, immunophenotypic, and cytogenetic spectrum of diffuse large B-cell lymphoma. For that purpose, we determined the following parameters: 1) protein expression of B-cell differentiation stage genes BCL6, CD10, MUM1/IRF4, CD138, and BCL2 expression in patients with diffuse large B-cell lymphoma and lymphoma grade 3; 2) subclassification into GCB and ABC groups according to the results of protein expression profile; 3) t(14;18)(q32;q21)/*IgH-BCL2* and abnormalities of *BCL6* gene in diffuse large B-cell lymphoma and follicular lymphoma grade 3 with >75% follicular growth pattern, as well as in GCB and ABC groups; and 4) the influence of protein expression, cytogenetic abnormalities, and clinical prognostic factors of age, sex, Ann Arbor clinical stage, and IPI risk group on overall survival (35,36).

Patients and methods

The study included 73 patients with lymph node enlargement who were diagnosed with primary diffuse large B-cell lymphoma (n = 53) or follicular lymphoma grade 3 with >75% follicular growth pattern (n = 20) according to the WHO classification during the 2000-2004 period. Patients' data were obtained from pathology files of the Merkur University Hospital and Zagreb University Hospital Center, Zagreb. The morphological features of all cases were examined on paraffin-embedded lymph node sections (by MD and A.B.). The patients were selected on the basis of adequate lymph node morphology and availability of histologic material for immunohistochemical and fluorescent in situ hybridization (FISH) analysis at diagnosis. The lymph node morphology criteria were based on the WHO classification diagnostic criteria for diffuse large B-cell lymphoma as follows: diffuse growth pattern of large transformed lymphoid cells, predominantly centroblastic and immunoblastic morphology, immunohistochemicaly phenotype $CD20^+CD3^-$, and follicular lymphoma grade 3 with >75% follicular growth pattern with >15 centroblast per high-power field and present centrocytes, immunohistochemically $CD20^+CD3^-$.

Patients were followed-up at three institutions (Merkur University Hospital, Dubrava University Hospital, and Zagreb University Hospital Center). The clinical data were available for 64 of 73 patients. The median follow-up was 19 months (1-1843 days). Data on therapy were not available for one patient. Of the remaining 63 patients, 39 received chemotherapy as first-line therapy according to the CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) regimen and 3 of these patients received additional radiotherapy (37). Three of 63 patients received CHOP plus rituximab regimen and 4 of 63 patients received COP (cyclophosphamide, vincristine, prednisone) regimen (37). Nine of 63 patients were initially treated with ProMACE, M-BACOD, BACOP, or EPOCH regimens, and six of 63 patients underwent additional peripheral blood stem cell transplantation (37). One patient died before treatment was started and one patient refused any therapy.

The study was approved by ethics committees of the Merkur University Hospital and Zagreb University School of Medicine.

Paraffin-embedded lymph node sections were analyzed with immunohistochemistry and FISH method; in cases where adequate material was available, tissue microarray was used. For tissue microarray, two representative 3-mm cores were obtained by use of biopsy needle (11G, Somatex, Teltow, Germany) and manually inserted into a paraffin block in a gird pattern.

Immunohistochemistry

Formalin-fixed, 4-μm thick paraffin sections were deparaffinized, rehydrated, and stained with antibodies to CD20 (clone L26, DAKO, Glostrup, Denmark; dilution 1:200), CD3 (polyclonal, DAKO; dilution 1:50), BCL6 (clone P1F6, Novocastra, Newcastle, UK; dilution 1:20), CD10 (clone 56C6, Novocastra; dilution 1:20), MUM1/IRF4 (polyclonal, Santa Cruz Biotechnology, Santa Cruz, CA, USA; dilution 1:100), CD138 (clone 5F7, Novocastra; dilution 1:50), and BCL2 (clone 124, DAKO; dilution 1:10). Heat-induced antigen retrieval by pressure cooking treatment PASCAL (DAKO) was performed for CD10, BCL6, MUM1/IRF4, and CD138 in 1 mmol EDTA solution, pH 8.0, at 125°C for 30 seconds, and for BCL2 in microwave oven in Target Retrieval solution, pH 6.0, (DAKO) at 95°C for 15 minutes. The EnVision method (DAKO) was used for immunostaining according to the manufacturer's instructions. Sections were stained in an autostainer (DAKO) and counterstained with hematoxy-

lin. Cases were considered positive if 30% of tumor cells were stained with an antibody.

Patients were clustered into GCB and ABC groups according to the immunophenotypic profile criteria described by Hans et al (11). Cases were assigned to GCB group if CD10 or both CD10 and BCL6 were positive. If CD10 and BCL6 were negative, the cases were assigned to ABC group. The cases with negative CD10 and positive BCL6 expression were classified according to MUM1/IRF4 expression: the GCB group was MUM1/IRF4 negative and the ABC group was MUM1/IRF4 positive.

Fluorescent in situ hybridization

Tissue sections were analyzed with dual color, dual fusion translocation probe t(14;18)(q32;q21)/*IgH-BCL2* (Vysis Inc., Downers Grove, IL, USA), and dual color break-apart rearrangement probe *BCL6* (Vysis Inc.) (38).

To analyze hybridization, a total of 200 nuclei per case were scored for the presence of gene signals. A cut-off of 7% was used to define a positive case.

Statistical analysis

The χ^2 test was used to compare the differences in proportions. The *t* test was used to evaluate the difference in age between the patient groups. Kaplan-Meier survival analysis was performed and the curves were compared by Cox-Mantel U-test. Overall survival time was calculated from the date of diagnosis until the last follow-up visit or death. Alive patients were censored in the analysis. Multivariate analysis was performed with the Cox stepwise proportional hazards model. Variables considered in the analysis were BCL6, CD10, and BCL2 protein expression, IPI risk group (group 1 – IPI score 0, 1, and 2; group 2 – IPI score 3, 4, and 5), *BCL2* gene abnormality groups (1 – no abnormalities; 2 – (14,18)(q32;q21)/*IgH-BCL2*; 3 – *BCL2* additional signals; 4 – t(14;18)(q32;q21)/*IgH-BCL2* and *BCL2* additional signals; 5 – *IgH* additional signals), and *BCL6* gene abnormality groups (1 – no abnormalities; 2 – *BCL6* translocation; 3 – *BCL6* additional signals; 4 - *BCL6* translocation and *BCL6* additional signals). Statistical analysis was performed with the STATISTICA software, version 7.0 (StatSoft Inc. Tulsa, OK, USA). The level of significance was set at $P < 0.05$.

Results

Patients with diffuse large B-cell lymphoma were significantly older and had higher IPI

Table 1. Clinical characteristics of patients with diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma grade 3 (FL-3) with >75% follicular growth pattern (FGP) and immunohistochemical stain results at diagnosis*

Characteristics	No. of patients: diffuse large B-cell lymphoma (n = 53)	No. of patients: follicular lymphoma grade 3 with >75% follicular growth pattern (n = 20)	*P*
Age (years, median [range])	66 (23-91)	58 (25-79)	0.027†
Sex:			0.551‡
male	28	9	
female	25	11	
Ann Arbor stage (n = 64):§			0.099‡
I	10	5	
II	13	2	
III	10	8	
IV	14	2	
IPI risk group (n = 64):‖			0.048‡
1	26	14	
2	21	3	
CD10:			0.146‡
-	43	13	
+	10	7	
BCL6:			0.030‡
-	36	8	
+	17	12	
MUM1/IRF4:			0.513‡
-	3	2	
+	50	18	
CD138:			0.536‡
-	52	20	
+	1	0	
BCL2:			0.717‡
-	21	7	
+	32	13	
GCB	10	7	0.146‡
ABC	43	13	

*Abbreviations: GCB – germinal center B-cell-like; ABC – activated B-cell-like;+ – positive expression; - – negative expression; IPI – International Prognostic Index.
†t test = 2.26, df = 71.
‡χ2 test.
§Ann Arbor stage (35).
‖International Prognostic Index (36) score group 1 – low risk and low intermediate risk; score group 2 – high intermediate risk and high risk.

score than did patients with follicular lymphoma grade 3 with >75% follicular growth pattern (Table 1). However, there were no differences in sex distribution and Ann Arbor clinical stage between the two patient groups. The BCL6 protein expression was more frequently found in patients with follicular lymphoma grade 3 with >75% follicular growth pattern, whereas no differences between the two patient groups were recorded in CD10, MUM1/IRF4, CD138, and BCL2 expression.

The protein expression profiles of BCL6, CD10, and MUM1/IRF4 were analyzed according to the criteria described by Hans et al (11). In 73 patients, the following 5 patterns of protein expression were found. The $CD10^+$ $BCL6^+MUM1/IRF4^-$ pattern was found in 3 cases; the $CD10^+BCL6^-MUM1/IRF4^+$ in 6 cases; the $CD10^+BCL6^+MUM1/IRF4^+$ in 8 cases; the $CD10^-BCL6^+MUM1/IRF4^+$ in 18 cases; and the $CD10^-BCL6^-MUM1/IRF4^+$ in 38 cases. The first three patterns were classified as GCB profile and the latter two patterns were present as ABC profile. The distribution of cases according to the immunophenotypic profile criteria is shown in Table 1. The GCB profile (Figure 1) was found in 10 of 53 cases and ABC profile in 43 of 53 cases of diffuse large B-cell lymphoma. The GCB profile was present in 7 of 20 cases and ABC profile (Figure 2) in 13 of 20 cases of follicular lymphoma grade 3 with >75% follicular growth pattern (χ^2 test, $P = 0.146$).

There was no difference in BCL2 protein expression between the GCB and ABC groups. BCL2 protein expression was found in 10 of 17 GCB and 35 of 56 ABC cases.

Cytogenetic abnormalities of diffuse large B-cell lymphoma and follicular lymphoma grade 3 with >75% follicular growth pattern cases, and GCB and ABC groups are shown in Table 2. FISH analysis of t(14;18)(q32;q21)/*IgH-BCL2* detected *BCL2* gene translocation and *BCL2* or *IgH* gene additional signals. t(14;18)(q32;q21)/*IgH-BCL2* was detected in 5 of 50 cases of diffuse large B-cell lymphoma and 7 of 20 cases of follicular lymphoma grade 3 with >75% follicular growth pattern. *BCL2* gene additional signals (Figure 3) were detected in 8 of 50 cases of diffuse large B-cell lymphoma and 2 of 20 follicular lymphoma grade 3 with >75% follicular

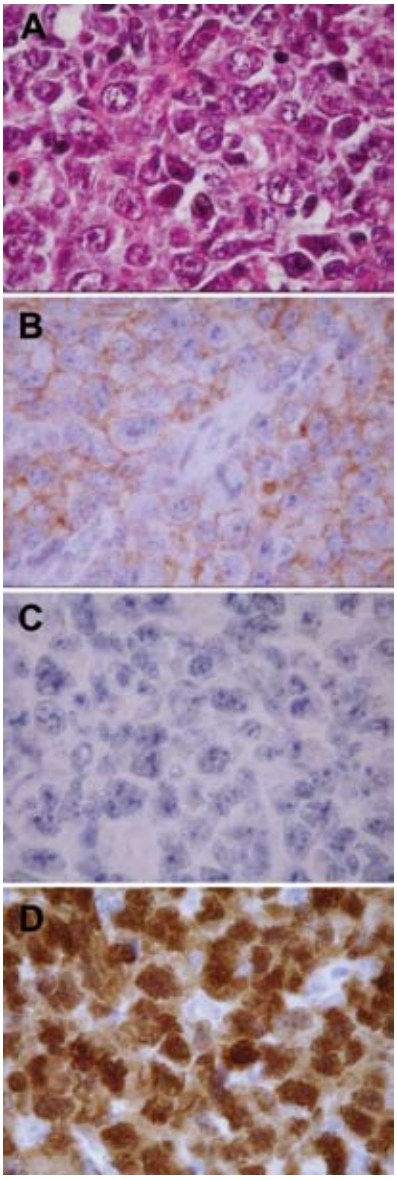

Figure 1. Diffuse large B-cell lymphoma, immunohistochemical germinal center B-cell-like patient group; (**A**) hematoxylin-eosin staining; (**B**) positive immunohistochemical staining for CD10; (**C**) negative immunohistochemical staining for BCL6; (**D**) positive immunohistochemical staining for MUM1/IRF4 (× 400 magnification).

growth pattern cases. Translocation and *BCL2* gene additional signals (Figure 4) were present in 2 of 50 cases of diffuse large B-cell lymphoma and 2 of 20 cases of follicular lymphoma grade 3 with >75% follicular growth pattern. t(14;18)(q32;q21)/*IgH-BCL2* was found in 8 of 17 GCB patients and 4 of 53 ABC patients. Also, *BCL2* gene additional signals were significantly more common in ABC group, as well as translocation and *BCL2* gene additional signals (χ^2 test, $P=0.004$).

BCL6 gene translocation was present in 7 of 51 cases of diffuse large B-cell lymphoma and 4 of 20 cases of follicular lymphoma grade 3 with >75% follicular growth pattern. *BCL6* gene additional signals were found in 14 of 51 cases of diffuse large B-cell lymphoma and 4 of 20 cases of follicular lymphoma grade 3 with >75% follicular growth pattern. Also, *BCL6* gene translocation and *BCL6* gene additional signals were found in 5 of 51 cases of diffuse large B-cell lymphomas and 1 of 20 cases of follicular lymphoma grade 3 with >75% follicular growth pattern. There were no significant differences between GCB and ABC groups in the four patterns of *BCL6* gene abnormalities.

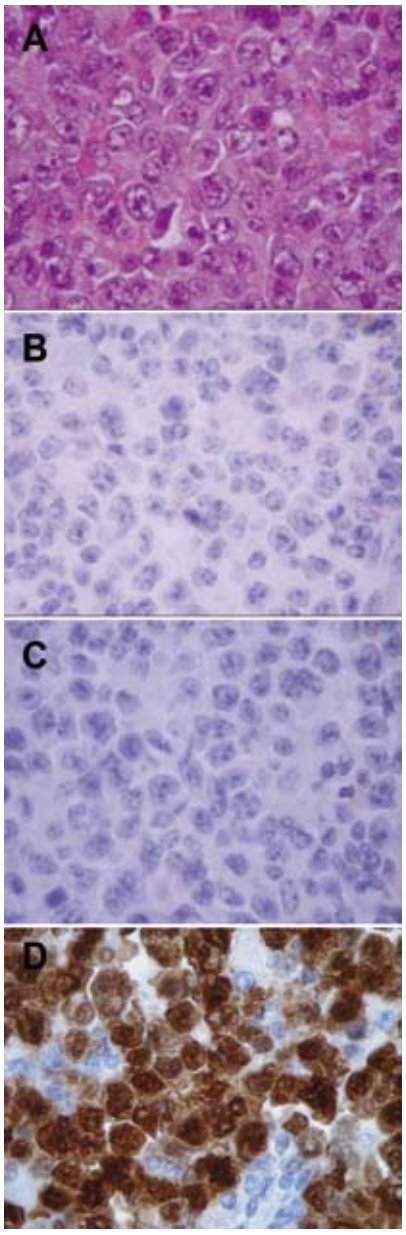

Figure 2. Follicular lymphoma grade 3, immunohistochemical activated B-cell-like patient group; (**A**) hematoxylin-eosin staining; (**B**) negative immunohistochemical staining for CD10; (**C**) negative immunohistochemical staining for BCL6; (**D**) positive immunohistochemical staining for MUM1/IRF4 (× 400 magnification).

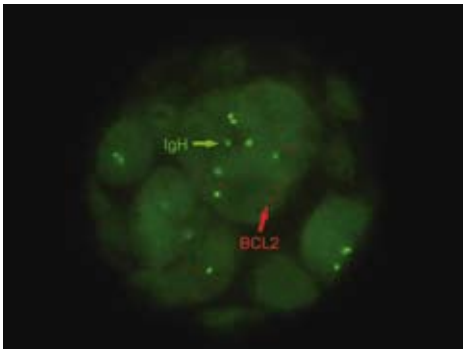

Figure 3. *BCL2* and *IgH* genes additional signals (fluorescent in situ hybridization, ×1000 magnification).

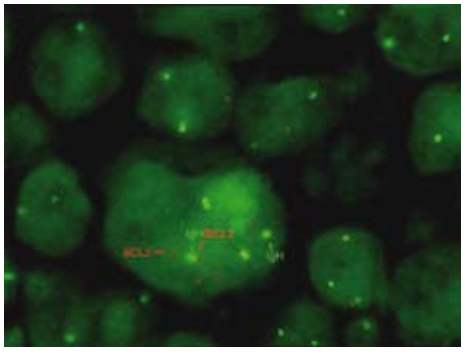

Figure 4. t(14;18)(q32;q21)/*IgH-BCL2* and additional *BCL2* and *IgH* signals (fluorescent in situ hybridization, ×1000 magnification).

Table 2. Cytogenetic abnormalities of diffuse large B-cell lymphoma and follicular lymphoma grade 3 with >75% follicular growth pattern and germinal center B-cell-like (GCB) and activated B-cell-like (ABC) patient groups

Patients group	Cytogentic abnormalities					Total	*P**
	t(14;18)(q32;q21)/*IgH-BCL2*†						0.090
	1	2	3	4	5		
Diffuse large B-cell lymphoma	29	5	8	2	6	50	
Follicular lymphoma grade 3 with >75% follicular growth pattern	8	7	2	2	1	20	
	3q27‡						0.759
	1	2	3	4			
Diffuse large B-cell lymphoma	25	7	14	5		51	
Follicular lymphoma grade 3 with >75% follicular growth pattern	11	4	4	1		20	
	t(14;18)(q32;q21)/*IgH-BCL2*						0.004
	1	2	3	4	5		
GCB	7	8	1	1	0	17	
ABC	30	4	9	3	7	53	
	3q27						0.517
	1	2	3	4			
GCB	10	3	4	0		17	
ABC	26	8	14	6		54	

*χ^2 test.
†1 – no abnormalities; 2 – t(14;18)(q32;q21)/*IgH-BCL2*; 3 – *BCL2* additional signals; 4 – t(14;18)(q32;q21)/*IgH-BCL2* and *BCL2* additional signals; 5 – *IgH* additional signals.
‡1 – no abnormalities; 2 – *BCL6* translocation; 3 – *BCL6* additional signals; 4 – *BCL6* translocation and *BCL6* additional signals.

Table 3. Clinical characteristics of immunohistochemical germinal center B-cell-like (GCB) and activated B-cell-like (ABC) patient groups

	GCB*	ABC	*P*
n	17	56	
Age (years, median [range])	63 (25-85)	64 (23-91)	0.962*
Sex (men/women)	9/8	28/28	0.832†
Ann Arbor stage (total 64):‡			0.025†
I	2	13	
II	2	13	
III	8	10	
IV	1	15	
IPI risk group (total 64):§			0.470†
1	7	33	
2	6	18	

*t test = 0.048, df = 71.
†χ2 test risk.
‡Ann Arbor stage (35).
§International Prognostic Index (36) score group 1 – low risk and low intermediate risk; score group 2 – high intermediate and high.

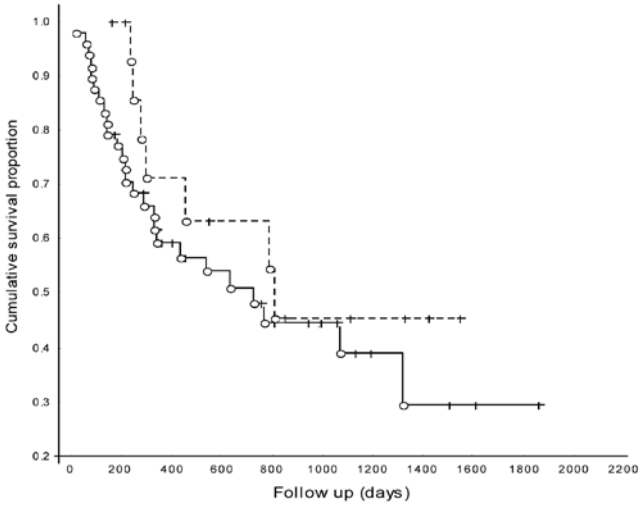

Figure 5. Overall survival distribution (Kaplan-Meier) of patients with diffuse large B-cell lymphoma (DLBCL) or follicular lymphoma grade 3 (FL-3) with >75% follicular growth pattern (FGP). Circle represents complete follow-up; plus represents censored follow-up (Cox-Mantel U-test = 2.463; $P = 0.341$); full line represents DLBCL; broken line represents FL-3 > 75% FGP.

There was no difference in the overall survival between the patients with diffuse large B-cell lymphoma and patients with follicular lymphoma grade 3 with >75% follicular growth pattern (Figure 5). Multivariate analysis included data on 59 patients with complete information for all variables. None of the variables was a significant predictor of overall survival (Wald χ^2_5 test = 8.260, $P = 0.143$).

However, overall survival was significantly better in GCB group (Figure 6). Clinical characteristics analysis of the GCB and ABC patient groups yielded a significant difference only according to the Ann Arbor clinical stage at diagnosis (Table 3). ABC patients were more often in an advanced clinical stage at diagnosis than GCB patients, without any difference according to age, sex, and IPI risk.

Discussion

Our study showed that there were no differences in protein expression between patients with diffuse large B-cell lymphoma and those

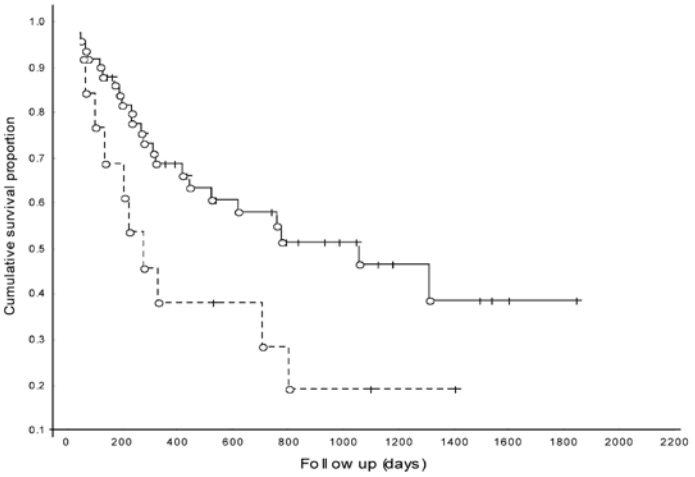

Figure 6. Overall survival distribution (Kaplan-Meier) in immunohistochemical germinal center B-cell-like (GCB) and activated B-cell-like (ABC) patient group; circle represents complete follow-up; plus represents censored follow-up (Cox-Mantel U-test = 4.309; *P* = 0.047); full line represents GCB; broken line represents ABC.

with follicular lymphoma grade 3 with >75% follicular growth pattern, but there were differences in the presence of BCL6, *BCL2,* and *BCL6* cytogenetic abnormalities. Although patients with diffuse large B-cell lymphoma and follicular lymphoma grade 3 with >75% follicular growth pattern had a significantly different IPI risk at diagnosis, they showed similar overall survival. Also, diffuse large B-cell lymphoma and follicular lymphoma grade 3 with >75% follicular growth pattern were equally distributed between GCB and ABC groups. However, GCB had longer overall survival than ABC group. t(14;18)(q32;q21) was more frequently recorded in GCB group, whereas t(14;18)(q32;q21) with *BCL2* additional signals or only *BCL2* and *IgH* additional signals were more frequent in ABC group; however, no differences in *BCL6* gene abnormalities were observed. Also, multivariate analysis showed that BCL6, CD10, and BCL2 expression, *BCL2* and *BCL6* abnormalities, and IPI were not significantly related to overall survival.

In the WHO classification, diffuse large B-cell lymphoma and follicular lymphoma grade 3 are two morphologically and clinically separate types of B NHL (1). However, the clinical course of these two types of B NHL has very similar clinical outcomes (39). Hans et al (11) showed, by correlating subclassification according to gene expression with subclassification according to protein expression in diffuse large B-cell lymphoma, that the protein expression profile of GCB and ABC groups was significantly predictive of overall survival. However, it was not uniformly confirmed as a significant predictor (11-19).

Different overall survival in GCB and ABC groups may depend on the therapy regimen. According to Nyman et al (40), in patients treated with immunochemotherapy GCB and ABC groups seem to lose their prognostic value. In the present study, only three of 63 patients received CHOP plus rituximab therapy regimen and the prognostic significance of GCB protein expression profile could not be abolished by rituximab treatment.

CD10 and BCL6 are considered germinal center differentiation stage-specific markers. However, CD10 is characterized by decreased expression in follicular lymphoma grade 3 in contrast to indolent follicular lymphoma grade 1 and follicular lymphoma grade 2 (41). In the present study, CD10 expression was found in only 35% of cases of follicular lymphoma grade 3 with >75% follicular growth pattern and 19% of cases of diffuse large B-cell lymphoma. Also the more common BCL6 expression in follicular lymphoma grade 3 with >75% follicular growth pattern than in diffuse large B-cell lymphoma indicates that the BCL6 protooncogene controls reactive germinal centers formation as well as formation of neoplastic follicles in FL-3 (42).

MUM1/IRF4 expression is present in non-neoplastic lymph node in a small number of late centrocytes and mostly in postgerminative B-cells, so it is considered as postgerminal differentiation stage-specific marker (43,44).

According to Shaffer et al (3), the high percentage of MUM1/IRF4 protein-positive diffuse large B-cell lymphoma cases, which was found in the present study, relates neoplastic B-cells in diffuse large B-cell lymphoma to postgerminal center differentiation block. Also, a large number of MUM1/IRF4 positive follicular lymphoma grade 3 cases indicate the probable late centrocyte origin of neoplastic B-cells or oncogenic translocation at an early stage of B-cell differentiation and consequently the transformed B-cell arrest at a later stage of differentiation (3). Zu et al (45) showed that interpretation of immunohistochemistry staining and scoring of MUM1/IRF4 varied greatly, with an average of only 30-37% complete agreement among observers. Also, in the present study the number of diffuse large B-cell lymphoma cases with ABC protein expression profile was higher than the number of cases recorded in literature (3,9,10,12). Therefore, we agree with de Jong et al (46) that standardization of immunohistochemistry techniques and centralized consensus review when using immunohistochemical markers are mandatory.

Negative expression of CD138 in our follicular lymphoma grade 3 with >75% follicular growth pattern cases and very low expression in diffuse large B-cell lymphoma cases, mostly with centroblast and immunoblast morphology, confirmed CD138 as a protein specific for terminal B-cell differentiation stage.

t(14;18)(q32;q21) represented an initial mechanism in the pathogenesis of GCB group, while *BCL2* gene additional signals were more frequently present in ABC group. *BCL2* and *BCL6* gene additional signals observed in the present study could indicate either additional chromosomes 18 and 3 or gene amplification (20). t(14;18)(q32;q21) was more frequently found in GCB group and *BCL2* additional signals in ABC group (19-24). Our study revealed that t(14;18)(q32;q21) and *BCL2* gene additional signals were not mutually exclusively present in the GCB and ABC groups.

In our study, there were no between-group differences in *BCL6* gene abnormalities. Barrans et al (47) found *BCL6* gene rearrangement and BCL2 expression in ABC group of patients with shorter overall survival. Bea et al (48) found additional *BCL6* gene signals only in ABC group. In the present study, additional *BCL6* signals were also detected in the GCB group.

In conclusion, the similarity between diffuse large B-cell lymphoma and follicular lymphoma grade 3 could be examined by tumor cells growth pattern, protein expression of B-cell differentiation stage genes, common cytogenetic abnormalities, or clinical prognostic factors. The present study analyzed, for the first time, only follicular lymphoma grade 3 with >75% follicular growth pattern to exclude the influence of diffuse growth pattern component for inferior survival in grade 3 follicular lymphoma. Also, our results showed very similar characteristics of diffuse large B-cell lymphoma and follicular lymphoma grade 3 cases and implied that protein expression of the B-cell differentiation stage genes could be a superior prognostic factor than tumor cells growth pattern, cytogenetic abnormalities of *BCL2* and *BCL6* genes, and clinical prognostic factors.

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