

## Original Article

## Expression of CD10, BCL-6 and MUM-1 Markers and Their Effects on Prognosis in Diffuse Large B Cell Lymphoma

### Diffüz Büyük B Hücreli Lenfoma'da CD10, BCL-6 ve MUM-1 Markerların Ekspresyonu ve Prognoz Üzerine Etkisi

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#### ABSTRACT

**Background and Aim:** Immunohistochemistry may serve as a surrogate to define Diffuse large B-cell lymphoma (DLBCL) cases as germinal center B cell-like (GCB) or non-GCB subtypes and to provide prognostic information. In this study, we aimed to investigate the frequency and prognostic impact of CD10, B-cell lymphoma 2 and 6 (BCL2 and BCL6) and multiple myeloma oncogene 1 (MUM1) expressions in pathology sections of patients with DLBCL to determine the response of these subgroups to the rituximab including chemotherapy regimens.

**Materials and Method:** Patients were grouped into 2 regarding the chemotherapy regimens they were treated, as cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) or Rituximab-CHOP. The treatment response, follow-up periods and outcomes of patients were recorded. The immunohistochemical panel was stained in pathology sections for CD10, BCL6 and MUM1/IRF 4 biomarkers. The patients were subgrouped as GCB or ABC regarding the immunohistochemical panel.

**Results:** Totally 81 patients, (39 male, 42 female) were included in the study. At the time of diagnosis, CD10 was positive in 31 patients (38.3%); BCL-6 in 53 patients (65.4%); MUM-1 was positive in 47 patients (58%) and BCL-2 was positive in 53 patients (65.4). With these results, 36 patients (44.4%) were in the GCB group and 45 patients (55.6%) were in the ABC group. No significant difference was found between the individual markers and subgroups in survival analyses.

**Conclusion:** We did not determine any significant effect of CD10, BCL-6, MUM-1 or BCL-2 positivity or GCB and non-GCB groups in the survival of patients with DLBCL.

**Keywords:** Diffuse large B-cell lymphoma; Prognostic Impact, ABC and GCB

#### ÖZET

**Giriş ve Amaç:** İmmünohistokimya kullanılarak, Diffüz büyük B hücreli lenfoma (DBBHL) vakaları germinal merkez B hücresi benzeri (GCB) ve ABC grubu olmak üzere prognostik açıdan önemli olan alt tiplere ayrılabilir. Biz bu çalışmada DBBHL'li hastaların patoloji preparatlarında CD10, B hücreli lenfoma 2 ve 6 (BCL2 ve BCL6) ve multipl miyelom onkogen 1 (MUM1) markerlarının sıklığını ve rituksimab içeren kemoterapi gruplarındaki prognostik etkisini araştırmayı amaçladık.

**Yöntem ve Gereçler:** Hastalar, kemoterapi rejimleri açısından, siklofosfamid, doksorubisin, vinkristin ve prednizon (CHOP) veya Rituksimab-CHOP olarak 2'ye ayrıldı. Hastaların tedaviye yanıtı, takip süreleri ve tedavi sonuçları kaydedildi. Tanı anındaki patoloji preparatlarından CD10, BCL6 ve MUM1 / IRF 4 biyomarkerları için boyama yapılarak hastalar immünohistokimyasal panel açısından GCB veya ABC olarak alt gruplara ayrıldı.

**Bulgular:** Toplam 81 hasta (39 erkek, 42 kadın) çalışmaya dahil edildi. Tanı sırasında 31 hastada (% 38,3) CD10; 53 hastada BCL-6 (% 65,4); MUM-1 47 hastada (% 58) ; BCL-2 53 hastada (65,4) pozitif.

Bu sonuçlarla 36 hasta (% 44.4) GCB grubu, 45 hasta (% 55.6) ABC grubu olarak tanımlandı. Sağkalım analizinde gruplar ve biyomarkerlar arasında istatistiksel olarak anlamlı bir farklılık saptanmamıştır.

**Tartışma ve Sonuç:** DLBCL hastalarının sağkalımında CD10, BCL-6, MUM-1 veya BCL-2 pozitifliği veya GCB ve ABC gruplarının anlamlı bir etkisi saptanmamıştır.

**Anahtar Kelimeler:** Diffüz Büyük B Hücreli Lenfoma; Prognositik Etki, ABC ve GCB

## Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma type worldwide accounting for 30-40% of the cases. DLBCLs are derived from the germinal center or post-germinal center B cells. They are exceedingly heterogeneous with a wide variation in genetic features, morphology, and patient outcomes [1]. In more than half of the patients, long-term disease-free survival is expected but on the other hand, approximately 30-40% of patients do not respond properly to the current treatments [2-4]. In that aspect, some biomarkers are required to define the outcomes of DLBCL subgroups.

DLBCLs are classified as germinal center B cell-like (GCB) group, activated B cell-like (ABC) group and unclassified group by gene expression profiling. Not only the morphology but also the therapeutic responses to these subgroups are known to be different from each other [5, 6]. Immunohistochemistry may serve as a surrogate to define DLBCL cases as GCB or non-GCB subtypes and to provide similar prognostic information as complementary DNA microarray does [7] because gene expression profiling studies are often long, costly and require fresh frozen tissue samples present in very few cases [8]. In that aspect, using some antibodies such as CD10, B-cell lymphoma 2 or 6 (BCL2 or BCL6), and multiple myeloma oncogene 1 (MUM1) algorithms have been developed to identify these subtypes [9].

In this study, we aimed to investigate the frequency and prognostic impact of CD10, BCL2, BCL6, and MUM1 expressions in pathology sections of patients with DLBCL to define the GCB and ABC subgroups and to determine the prognostic impact of these subgroups to the rituximab including chemotherapy regimens.

## Material and Method

Though 104 patients included in the study, 23 of them excluded due to lack of data. Totally 81 (39 male, 42 female) patients diagnosed with DLBCL and treated in Gazi University Medical Faculty, Medical Oncology Department, between January 1997 and October 2010 were analyzed in this study. The study was approved by the local ethics committee.

The patient records were retrospectively reviewed. The stages of patients were determined regarding Ann Arbor classification and IPI scores were recorded in all patients [10].

Patients were grouped into 2 regarding the chemotherapy regimens they were treated, as cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) or Rituximab-CHOP. The treatment response, follow-up periods and outcomes of patients were recorded.

The pathology sections at the time of the first diagnosis were reached. Hematoxylin-Eosin stained and immunohistochemical sections of all patients were evaluated retrospectively. Moreover, in all patients, the immunohistochemical panel was stained in pathology sections for CD10, BCL-6, BCL-2 MUM1/IRF 4 biomarkers.

An immunohistochemical examination was performed to determine the CD 10, BCL-6, MUM-1 and BCL-2 expressions on formalin-fixed paraffin sections using the streptavidin-biotin triple indirect immunoperoxidase method. Sections were cut to 4 µm thickness, de-paraffinized in xylene and rehydrated through graded alcohol in distilled water. Antigen retrieval was done in a commercial buffer by autoclaving for 12 hours at 56 °C. After autoclaving, sections were allowed to cool at room temperature for 20 minutes,

Table 1. Antibodies used for immunohistochemical staining

Antibody	Clon	Isotype	Dilution	Brand Name
CD10	56C6	Mouse monoclonal	Ready to use	NovoCastra (UK)
BCL-6	PG-B6p	Mouse monoclonal	1:50	DakoCytomation (Denmark)
MUM1	MUM1p	Mouse monoclonal	Ready to use	DakoCytomation (Denmark)
BCL-2	124	Mouse monoclonal	Ready to use	DakoCytomation (Denmark)

rinsed thoroughly with distilled water and placed in phosphate buffer saline (PBS). Endogenous peroxidase activity was blocked with hydrogen peroxide and, subsequently, slides were washed with PBS. All primary antibodies were incubated for 2 hours at room temperature. Following primary antibody incubation, sections were washed thoroughly with PBS and incubated with secondary antibody (Ultra streptavidin detection system-HRP; Signet, Massachusetts, USA) for 20 minutes at room temperature. Subsequently, sections were washed with PBS and incubated with Streptavidin-biotin complex (Dako Cytomation, Denmark) for 30 minutes at room temperature. Following a final wash with PBS, immunoreactivity was visualized with AEC (3-Amino 9-Ethylcarbazole; Lab-Vision, Neomarkers, USA). Finally, sections were briefly counterstained with Mayer's hematoxylin and examined by light microscopy. Reactive tonsil tissues that were known to express the markers of interest were used as positive controls.

All specimens were analyzed in a semi-quantitative way after a thorough examination of the whole immunostained slides. The ratio of positively stained tissues to all tumor tissues was determined. As defined before by Hans et al, more than 30% staining in tumoral cells was defined as positive [9]. The patients were subgrouped as GCB or ABC regarding the immunohistochemical panel.

Antibodies used for immunohistochemical staining and their brand names are summarized in Table 1.

### Statistical Analysis

The Statistical Package for the Social Sciences program was used for data analysis (for Windows 17.0, SPSS Inc., USA). Variables were analyzed with the One-Sample Kolmogorov-Smirnow test for the determination of normal distribution. Normally distributing data were expressed as mean± standard deviation and non-normally distributing data were expressed as median (minimum-maximum). The Mann-Whitney test was used to compare ABC and GCB subgroups. In the comparison of more than 2 groups, Kruskal-Wallis variance analysis was performed. Pearson correlation analysis was performed to evaluate the correlation between CD10, BCL-6, MUM1, BCL-2, and other parameters.

Complete remission (CR) was defined as the total disappearance of tumors after treatment. Partial remission (PR) was defined as a more than 50% decrease in the tumor while stable disease (SD) was defined as less than 50% decrease or less than a 25% increase in the tumor. Progressive disease (PD) was defined as a more than a 25% increase in tumor or determination of new tumor foci.

The Kaplan-Meier survival curves and Log-rank test were used to represent the overall survival (OS) or progression-free survival (PFS) distributions. Overall survival was defined as the period from the time of diagnosis to death from any cause or the last contact. The significance level was set as  $p \leq 0.05\%$ .

## Results

Totally 81 patients, (39 male, 42 female) were analyzed in the study. The mean age of the patients was  $56.1 \pm 14.8$  (range: 18–82) years. The demographic and clinical features of patients regarding the immunohistochemical subgroups are summarized in Table 2. At the time of diagnosis, CD10 was positive in 31 patients (38.3%), BCL-6 in 53 patients (65.4%), MUM-1 was positive in 47 patients (58%) and BCL-2 was positive in 53 patients (65.4). According to the algorithm defined by Hans et al, 36 patients (44.4%) were in the GCB group and 45 patients (55.6%) were in the ABC group. Patients were divided into two groups as  $< 60$  years (younger),  $\geq 60$  years (elderly). While 47% of the patients in the GCB subgroup were young, 62% of the patients in the ABC subgroup were young, but the difference between these two groups was not statistically significant ( $p = 0.17$ ).

The median follow-up time was  $27 \pm 24.08$  months in study participants. No significant difference was found between the individual markers and subgroups in terms of OS and PFS in survival analysis. In younger patients than in elderly patients and low-risk patients according to IPI risk scoring, OS and PFS durations were found to be higher. Besides, OS and PFS durations were significantly longer in patients with CR (Table 3).

Twenty-five (69%) of the 36 patients in the GCB group received the R-CHOP regimen, and 11 (31%) of them received the CHOP regimen, while 25 (56%) of the 45 patients in the ABC group received R-CHOP and 20 (44%) of them received the CHOP regimen. The 5-year OS rate in the GCB group was 78% in the R-CHOP group and 49% in the CHOP group ( $p = 0.90$ ), while the 5-year OS rate in the ABC group was 73% in the R-CHOP group and it was 75% in the CHOP group ( $p = 0.54$ ) (Figures 1a and 1b).

Of the 28 BCL-6 (-) patients, 21 (75%) received the R-CHOP regimen while 7 (25%) received the CHOP regimen. Of the 53 patients with BCL-6 (+), 29 (55%) received the R-CHOP regimen and 24 (45%) received the CHOP regimen. In the BCL-6 (-) patient

group, the 5-year OS rate was 54% in the R-CHOP regimen group and 57% in the CHOP regimen group ( $p = 0.51$ ). In the BCL-6 (+) patient group, the 5-year OS rate was 84% in the R-CHOP regimen group and 72% in the CHOP regimen group ( $p = 0.60$ ).

When the CHOP and R-CHOP regimens were compared according to the 5-year OS and 3-year PFS rates, OS and PFS rates were higher in the group receiving R-CHOP regimen but did not reach a statistically significant level (5-year OS rates were 76% and 64%, respectively) ( $p = 0.39$ ).

When patients were analyzed according to their chemotherapy (CT) regimens, adding rituximab to CT increased the 5-year OS rate in the GCB group, but this did not reach a statistically significant level (Table 4).

## Discussion

In this study, we have analyzed the role of immunostaining in predicting the prognosis of patients with DLBCL who were treated with CHOP or R-CHOP. We determined that, among study participants, 44.4% were in the GCB group and 55.6% were in the ABC group. The rates of CD10, BCL-6, MUM-1 and BCL-2 positivity were 38.3%, 65.4%, 58% and 65.4%, respectively. Any significant difference was not found between the individual markers and subgroups in terms of OS and PFS in survival analysis.

Regarding the morphological features, DLBCLs are classified as GCB, ABC and unclassified groups by gene expression profiling. Masir et al [11] reported the GCB subtype in 37% and ABC subtype in 49% of patients. Wawire et al [12] reported the GCB cell type in 40.6% of 165

DLCBL patients in their study. We reported the GCB and ABC prevalence as 44.4% and 55.6%, respectively; and our data were compatible with the literature.

Prognostic factors in DLBCL are highly important in management and determining appropriate treatment modalities. For that reason, immunohistochemical markers have been studied before, but conflicting results are present in previous literature.

Table 2: Demographical and clinical features of patients regarding the immunohistochemical subgroups

Characteristics	ABC n (%)**	GCB n (%)**	Total (n/%)*	p <sup>a</sup>
Gender				
Male	21 (46.7)	18 (50)	39 (48.1)	0.765
Female	24 (53.3)	18 (50)	42 (51.9)	
Age( years)				
<60	28 (62.2)	17 (47.2)	45 (55.6)	0.177
≥60	17 (37.8)	19 (52.8)	36(44)	
Stage (Ann-Arbor)				
1	6 (13.3)	8 (22.2)	14 (17.3)	NA
2	24 (53.3)	12 (33.3)	36 (44.4)	
3	11(24.4)	8 (22.2)	19 (23.5)	
4	4 (8.9)	8 (22.2)	12 (14.8)	
B symptom				
Present	24 (53.3)	18 (50)	42 (51.9)	0.765
Absent	21 (46.7)	18 (50)	39 (48.1)	
Bulky mass				
Present	22 (48.9)	6 (16.7)	28 (34.6)	0.002
Absent	23 (51.1)	30 (83.3)	53 (65.4)	
LDH				
≤N	20 (44.4)	19 (52.8)	39 (48.1)	0.456
>N	25 (55.6)	17 (47.2)	42 (51.9)	
ECOG performance				
0	10 (22.2)	4 (11.1)	14 (17.3)	NA
1	21 (46.7)	21 (58.3)	42 (51.9)	
2	5 (11.1)	5 (13.9)	10 (12.3)	
3	7 (15.6)	5 (13.9)	12 (14.8)	
4	2 (4.4)	1 (2.8)	3 (3.7)	
EN involvement				
+	29 (64.4)	23 (63.9)	52 (64.2)	0.959
-	16 (35.6)	13 (36.1)	29 (35.8)	
IPI risk group				
0-1	25 (55.6)	21 (58.3)	46 (58.6)	NA
2	10 (22.2)	4 (11.1)	14 (17.3)	
3	7 (15.6)	6 (16.7)	13 (16)	
4-5	3 (6.7)	5 (13.9)	8 (9.9)	
IPI				
0	15 (33.3)	10 (27.8)	25 (30.9)	NA
1	10 (22.2)	11 (30.6)	21 (25.9)	
2	10 (22.2)	4 (11.1)	14(17.3)	
3	7 (15.6)	6 (16.7)	13 (16)	
4-5	3 (6.7)	5 (13.9)	8 (9.9)	
Treatment				
R-CHOP	25 (55.69)	25 (69.4)	50 (61.7)	0.201
CHOP	20 (44.4)	11 (30.6)	31 (38.3)	
Treatment Response				
Unevaluated	1 (2.2)	1 (2.8)	2 (2.5)	NA
CR	37 (82.2)	30 (83.3)	67 (82.7)	
PR	5 (11.1)	1 (2.8)	6 (7.4)	
SD	1 (2.2)	1 (2.8)	2 (2.5)	
PD	1 (2.2)	3 (8.3)	4 (4.9)	

p<sup>a</sup>: Pearson chi-square analysis \* : column % \*\* : row % NA: not applicable; IPI: International prognostic index; EN:Extranodal involvement; CR:Complete remission; PR: Parita remission; SD:Stabil disease; PD:Progressive disease

Table 3: The effects of subgroups and general patient characteristics on OS and PFS

		Number (%)	5 year OS (%)	p	3 year PFS (%)	pA
Age	<60	45(56)	91	6	85	4
	>60	36(44)	47		51	
Stage	1	14(17)	61	0.34	77	0.32
	2	36(44)	79		74	
	3	19(24)	53		50	
	4	12(15)	75		75	
IPI risk group	0-1	46(57)	84	5	83	6
	2	14(17)	45		55	
	3	13(16)	29		42	
	4-5	8(10)	62		62	
LDH	<N	39(48)	45	0.25	70	0.18
	>N	42(52)	68		69	
EN involvement	Yok	29(36)	69	0.45	63	0.30
	Var	52(64)	65		72	
KT group	RCHOP	50(62)	73	0.60	76	0.39
	CHOP	31(38)	67		64	
Response	D*	2(2)	0	<0.001	0	<0.001
	CR	67(84)	79		79	
	PR	6(7)	33		25	
	SD	2(2)	50		50	
	PD	4(5)	25		25	
CD10	(-)	50(62)	74	0.43	77	0.39
	(+)	31(38)	65		57	
BCL-6	(-)	28(35)	62	0.20	67	0.15
	(+)	53(65)	74		72	
MUM-1	(-)	34(42)	62	0.43	59	0.37
	(+)	47(58)	77		79	
BCL-2	(-)	28(35)	58	0.46	67	0.42
	(+)	53(65)	72		68	
GCB group		36(44)	63	0.55	60	0.51
ABC group		45(56)	77		78	

Table 4: 5-year OS rates in subgroups according to chemotherapy regimens

		5 year OS (%)	p
R-CHOP	GCB	78	0.64
	ABC	73	
CHOP	GCB	62	0.55
	ABC	75	

\* Pearson chi-square analysis

Akyurek et al [13] reported that BCL-6 rearrangement predicted significantly shorter overall survival while BCL-2 rearrangement had no prognostic impact on outcome in 239 patients with DLBCL. Bodoor et al [14] investigated the role of BCL-6, CD10, CD138 and MUM-1 expressions in the prognosis of patients with DLBCL and reported that BCL-6 expression was associated with better overall survival without any impacts of CD10 and MUM-1 on survival.

Culpin et al [15] assessed the prognostic validity of immunohistochemical markers and algorithms identified in the CHOP era in immunochemotherapy-treated DLBCL patients and reported that low CD10 (<10%), low LMO2 (<70%) or high BCL-2 ( $\geq$ 80%) predicted shorter OS and high BCL-2 ( $\geq$ 80%), low BCL-6 (<60%), low GCET1 (<20%) or low LMO2 (<70%) predicted shorter PFS. Hassan et al [16] reported that CD-10 expression in DLBCL was associated with

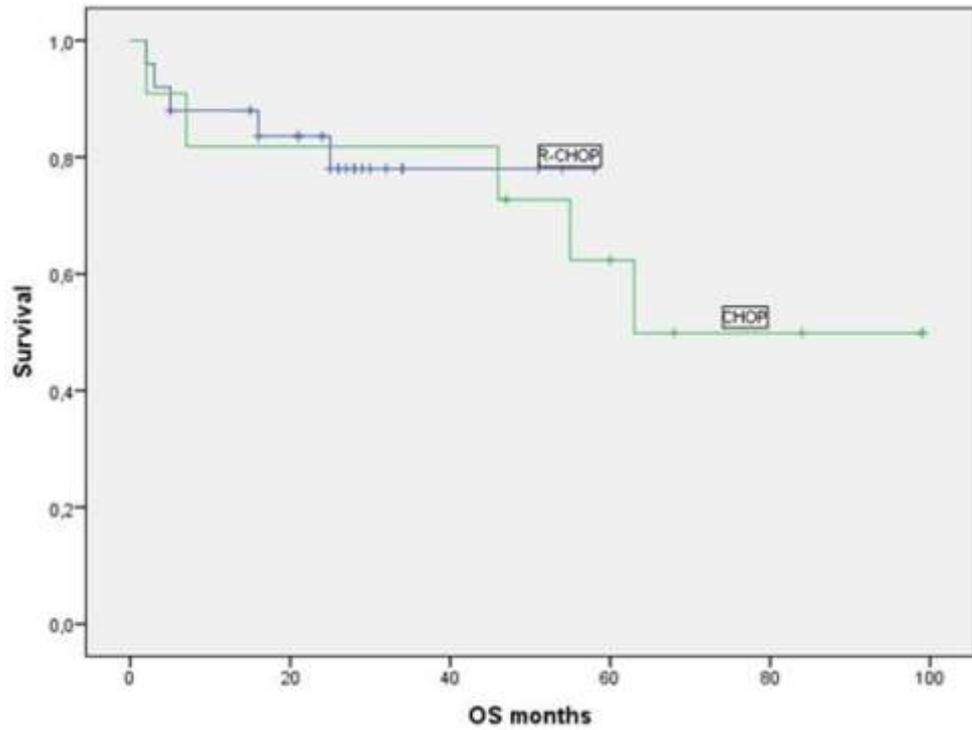


Figure 1a: Chemotherapy-related OS durations in the GCB subgroup (Kaplan-Meier curve)

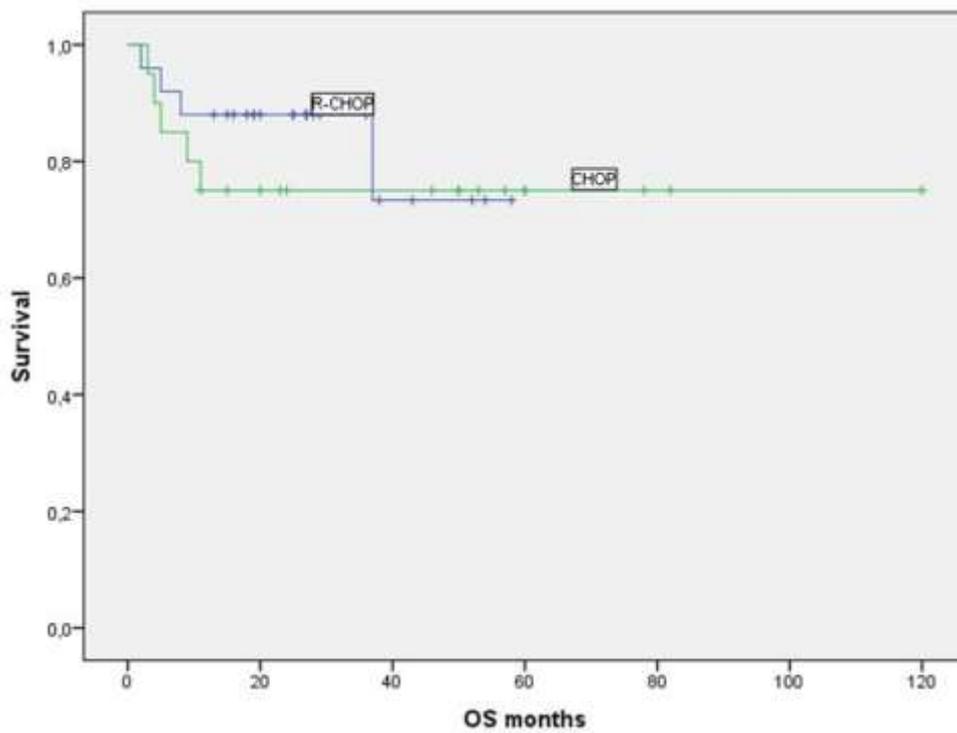


Figure 1b: Chemotherapy-related OS durations in the ABC subgroup (Kaplan-Meier curve)

better immediate clinical response whereas MUM-1 expression was associated with poor immediate clinical response. However, there was no statistically significant association of BCL-6 with an immediate clinical response that was noted after 6 cycles of chemotherapy. Jia et al [17] reported that, among Uygur patients diagnosed with DLBCL, expression of germinal center B (GCB) cell-expressed transcript-1, FOXP1, CD10, BCL-6, and MUM1 was associated with a significantly higher 3-year OS. Dwivedi et al [18] did not determine any statistically significant difference regarding the survival rates between GCB and non-GCB subtypes of DLBCL patients. Recently, in a detailed study of Lu et al [19], using immunohistochemistry and fluorescence in situ hybridization, the prognostic value of Hans algorithm was investigated in 306 cases who were treated with chemoimmunotherapy and GCB subtype was reported to have a better OS and PFS than non-GCB cases. On the other hand, it was also reported that double-positive (CD10(+), MUM1(+)) patients showed similar OS and PFS with the non-GCB group while the (triple-negative (CD10(-), BCL6(-), MUM(-) group) showed similar OS and PFS with the GCB group. Similarly, Chang et al [20] also did not determine any difference in survival of patients with GCB or non-GCB tumors. Although GCB tumors more frequently expressed CD10 and BCL-6 as well as the BCL2 and MYC rearrangements with less

frequent expression of MUM1. Abdulla et al reported that concurrent expression of MYC and BCL-2 proteins was strongly associated with inferior survival in DLBCL patients treated with R-CHOP [21].

The main limitations of this study are the retrospective design, the low number of patients and the low follow up time.

In the present study, the 5-year OS rate in patients receiving R-CHOP therapy was 78% in the GCB group, while it was 49% in the group receiving CHOP therapy. The 5-year OS difference in the GCB subgroup is remarkable at 29% among the treatment groups. A similar difference was not detected among the treatment groups in the ABC subgroup (73% in the R-CHOP group, 75% in the CHOP group). Despite this remarkable difference, the difference between the treatment groups in the GCB group was statistically nonsignificant and may be related to the limitations of the study, the low number of patients who reach the primary endpoint, and especially the number of patients.

In conclusion, we did not determine any significant effect of CD10, BCL-6, MUM-1, or BCL-2 positivity or GCB and non-GCB groups in the survival of patients with DLBCL. The addition of rituximab can contribute to treatment success, particularly in the GCB subgroup. Further, prospective, larger studies are warranted to determine the exact role of these markers in the prognosis of DLBCL.

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