Original Article

Prognostic Significance of MYC, BCL2, and BCL6 Rearrangements in Patients With Diffuse Large B-Cell Lymphoma Treated With Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone Plus Rituximab

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BACKGROUND: Diffuse large B-cell lymphomas (DLBCLs) are a biologically heterogeneous group in which various gene alterations have been reported. The aim of this study was to investigate the frequency and prognostic impact of *BCL2*, *BCL6*, and *MYC* rearrangements in cyclophosphamide, doxorubicin, vincristine, and prednisone plus rituximab (R-CHOP)-treated DLBCL cases. **METHODS:** Tissue microarrays were constructed from 239 cases of DLBCL, and the expressions of *CD10*, *BCL6*, *MUM1/IRF4*, and *BCL2* were evaluated by immunohistochemistry. *MYC*, *BCL2*, and *BCL6* rearrangements were investigated by interphase fluorescence in situ hybridization on tissue microarrays. Survival analysis was constructed from 145 R-CHOP–treated patients. **RESULTS:** *MYC*, *BCL2*, and *BCL6* rearrangements were detected in 14 (6%), 36 (15%), and 69 (29%) of 239 DLBCL patients. Double or triple rearrangements were detected in 7 (3%) of 239 DLBCL cases. Of these, 4 had *BCL2* and *MYC*, 2 had *BCL6* and *MYC*, and 1 had *BCL2*, *BCL6*, and *MYC* rearrangements. The prognosis of these cases was extremely poor, with a median survival of 9 months. *MYC* rearrangement was associated with significantly worse overall survival ($P = .01$), especially for the cases with GC phenotype ($P = .009$). *BCL6* rearrangement also predicted significantly shorter overall survival ($P = .04$), especially for the non-GC phenotype ($P = .03$). *BCL2* rearrangement had no prognostic impact on outcome. International Prognostic Index ($P = .004$) and *MYC* rearrangement ($P = .009$) were independent poor prognostic factors. **CONCLUSIONS:** Analysis of *MYC* gene rearrangement along with *BCL2* and *BCL6* is critical in identifying high-risk patients with poor prognosis. ***Cancer*** **2012;118:4173-83.** © *2011 American Cancer Society.*

KEYWORDS: diffuse large B-cell lymphoma, *MYC*, *BCL2*, *BCL6*, rearrangement, R-CHOP, prognosis.

INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most common histologic subtype of non-Hodgkin lymphoma, and comprises a heterogeneous group of diseases with varied morphologic, immunophenotypic, and molecular features.[1] Although more than half of the patients are cured with a combination of rituximab with anthracycline-based chemotherapy, such as the R-CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone plus rituximab; immunochemotherapy) regimen, responses are heterogeneous, and many patients die of their disease.[2,3]

The International Prognostic Index (IPI) is considered the most important prognostic factor for survival and the best method to identify high-risk patients who are unlikely to achieve a long-term remission with standard chemotherapy.[3,4] However, even within identical IPI risk groups, a substantial variability in outcome has been observed.[3] In addition, gene expression profiling has identified DLBCL subtypes based on cell of origin, including germinal center (GC), activated B-cell subtype, and unclassified DLBCL.[5,6] Hans and colleagues proposed an algorithm to discriminate GC from non-GC phenotypic DLBCL, based on *CD10*, *BCL6*, and *MUM1/IRF4* expression, determined by immunohistochemistry.[7] Patients with GC phenotype had better survival than those with non-GC phenotype.[7,8] The prognostic value of this algorithm has been confirmed among patients treated with anthracycline-based chemotherapy by some studies but not others.[9-13] In addition, even for patients receiving immunochemotherapy, the predictive value of the immunohistochemically defined GC and non-GC phenotypes remains controversial.[14,15]

Because the patients with DLBCL have widely various clinical outcomes, the discovery of prognostic markers that accurately predict outcome is an important area of investigation. Recurrent chromosomal translocations of *MYC*, *BCL2*, and *BCL6* have been reported in DLBCLs.[16-20] The *MYC* translocation is a characteristic cytogenetic event for Burkitt

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lymphoma (BL), but has also been reported to occur in DLBCLs with a frequency of 3% to 16%.[13,18,21-27] Such cases have an adverse prognosis with chemoresistance and shortened survival, but little is known about the relative significance of *MYC* in the context of other established prognostic features.[21-26] Moreover, it is unclear whether there are identifiable clinicopathologic presentations that predict the likelihood of *MYC* aberrancy.

In contrast to BL, *MYC* aberrations in DLBCL are usually associated with multiple cytogenetic abnormalities and other genetic lesions, such as concurrent rearrangements of *BCL2* and/or *BCL6*. Patients with so-called double-hit or triple-hit lymphomas have an extremely poor prognosis, with few survivors beyond 6 months.[28-32] In the updated 2008 World Health Organization (WHO) classification, double-hit and triple-hit lymphoma are classified as B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL.[33]

BCL6 is the most frequent cytogenetic abnormality in DLBCL, occurring in up to 30% to 35% of cases. *BCL2* gene rearrangement also occurs in 20% to 30% of cases of DLBCL.[11,13,16-20,27] Clinical studies investigating the prognostic impact of *BCL2* and *BCL6* gene rearrangements on patients of DLBCL have yielded conflicting results.[11,13,16-20,25,27,34,35] Conversely, there are very few data on the prognostic impact of chromosomal translocations involving *MYC*, *BCL2*, and *BCL6* genes in patients with DLBCL treated with R-CHOP.[13,21,24,35]

In this study, the frequency of *MYC*, *BCL2*, and *BCL6* rearrangements was investigated in DLBCLs using interphase fluorescence in situ hybridization (FISH) analysis. In addition, the clinicopathological features and prognostic implications of these gene aberrations in DLBCLs treated with R-CHOP chemotherapy were examined.

MATERIALS AND METHODS

Patients

Biopsies from 250 DLBCL patients diagnosed from January 2001 to June 2009 in the Pathology Departments of Gazi and Hacettepe Universities were included in this study. The diagnosis of DLBCL was based on the 2008 WHO classification criteria, and BL cases were excluded. Of the 250 patients, 239 cases in which FISH was successfully performed were analyzed in this study. These cases included 117 males and 122 females, with a median age of 57 years (range, 16-95). Follow-up data were available for 178 of these patients. Clinical data were obtained from the referring oncologists and medical records. One hundred forty-five patients received R-CHOP therapy, whereas 29 patients were treated with CHOP or CHOP-like chemotherapy regimens, and 4 patients received palliative therapy only. The characteristics of the patients who received R-CHOP therapy were similar to the remaining cases. For the purpose of achieving homogeneity of the patient population, the survival analysis was constructed from 145 patients who had been uniformly treated with R-CHOP therapy. This group consisted of 69 males and 76 females, with a median age of 56 years (range, 17-95). The median follow-up for this group was 26 months (range, 2-96). At the time of analysis, 43 (30%) of 145 patients had died, and 102 (70%) were reported to be alive.

This retrospective study was approved by the Gazi University Medical School Research Ethics Board and is in accordance with the Declaration of Helsinki.

Immunohistochemistry

The hematoxylin and eosin-stained slides from each tumor block were reviewed, and representative areas with the highest percentage of tumor cells were selected for tissue microarray (TMA) construction. TMA blocks were constructed with a tissue microarrayer. Immunohistochemistry was performed on 4-μm TMA sections using *CD10*, *BCL6*, *MUM1*/*IRF4*, *BCL2*, and Ki-67. The sections were stained using automated immunohistochemistry on the Ventana Benchmark XT using standard protocols. Immunoreactivity was determined without any knowledge of survival or other clinical data. The cases were then subclassified as GC and non-GC phenotype using the algorithm described by Hans and colleagues.[7] For evaluation of *CD10*, *BCL2*, *BCL6*, and *MUM1*/*IRF4*, the staining was interpreted as positive if 30% or more of the tumor cells showed expression.[7] The Ki-67 antibody was used to determine the proliferation rate, and Ki-67 $\geq$80% was defined as "high proliferation" as previously described.[36]

FISH Analysis

Interphase FISH analysis was performed on 3-μm TMA tissue sections using split signal FISH DNA probes for *MYC*/8q24, *BCL2*/18q21, and *BCL6*/3q27 (Dako, Glostrup, Denmark) according to the manufacturer's instructions. The slides were analyzed with an Olympus BX-51 fluorescence microscope (Olympus, Tokyo, Japan). At least 100 intact, nonoverlapping nuclei were assessed by 2 pathologists, and the percentages of positive nuclei were averaged. A sample was considered positive for the rearrangement if >10% of nuclei exhibited breaking apart.

Table 1. Clinicopathological Characteristics of Patients with *MYC*, *BCL2*, and *BCL6* Rearranged in DLBCL (N=239)

Characteristic	All DLBCL	*MYC* Rearranged	*P*	*BCL2* Rearranged	*P*	*BCL6* Rearranged	*P*
Patients, No. (%)	239	14 (6)		36 (15)		69 (29)	
Age, y							
Median [range]	57 [16-95]	49 [17-74]	.11	59 [18-77]	.77	59 [17-95]	.14
<60, No. (%)	141 (59)	11 (79)	.16	19 (53)	.46	36 (52)	.19
≥60, No. (%)	98 (41)	3 (21)		17 (47)		33 (48)	
Sex, No. (%)							
Male	117 (49)	8 (57)	.59	14 (39)	.20	34 (49)	1
Female	122 (51)	6 (43)		22 (61)		35 (51)	
Presentation, No. (%)							
Nodal	148 (62)	9 (64)	1	20 (56)	.02	39 (56)	.30
Extranodal	91 (38)	5 (36)		16 (44)		30 (44)	
Stage, No. (%)							
I-II	65 (27)	4 (31)	.56	8 (30)	.20	22 (45)	.60
III-IV	92 (39)	9 (69)		19 (70)		27 (55)	
Unknown	82 (34)	1		10		20	
IPI score, No. (%)							
Low, low-intermediate risk (0-2)	80 (34)	4 (33)	.04	11 (48)	.15	24 (60)	.69
High-intermediate, high risk (3-5)	48 (20)	8 (67)		12 (52)		16 (40)	
Unknown	111 (46)	0		13		29	
Phenotype, No. (%)							
GC	117 (49)	8 (57)	.59	20 (56)	.47	34 (49)	1
Non-GC	122 (51)	6 (43)		16 (44)		35 (51)	
BCL2 protein expression, No. (%)	123 (55)	9 (64)	.58	23 (70)	.08	35 (53)	.76
BCL6 protein expression, No. (%)	118 (51)	9 (64)	.40	18 (53)	.85	37 (54)	.56
CD10 expression, No. (%)	77 (33)	5 (36)	1	15 (43)	.24	23 (34)	1
Ki-67 ≥ 80%, No. (%)	83 (35)	10 (77)	.005	15 (44)	.34	28 (42)	.29

Abbreviations: DLBCL, diffuse large B-cell lymphoma; GC, germinal center phenotype; IPI, International Prognostic Index.

Statistical Analysis

Pearson chi-square test or Fisher exact test was used to test for differences in the frequencies between groups. Continuous variables were compared using the Mann-Whitney *U* test. Spearman test was used to analyze correlations between different variables.

Progression-free survival (PFS) was defined as the time interval from initial diagnosis to progressive disease under therapy, failure to achieve complete remission, additional therapy, relapse, or death from any cause. Overall survival (OS) was defined as the time from initial diagnosis to death or the last contact. Patients were censored for OS at the time of the last contact if currently alive and for PFS if no relapse or disease progression had occurred at the time of their last visit. PFS and OS were estimated according to Kaplan-Meier and compared by log-rank test. Multivariate analyses were performed with the use of a Cox regression model to estimate hazard ratios for an evolving event. All *P* values are based on 2-tailed statistical analysis, considering *P* values $<.05$ as significant. Statistical analyses were performed using SPSS (version 15.0; SPSS Inc, Chicago, Ill) statistical software.

RESULTS

FISH was successfully performed in 239 cases, which were analyzed in this study (Table 1). A GC phenotype was found in 49% and a non-GC phenotype in 51% of the tumors, as defined by the Hans' algorithm. No significant differences were observed with regard to age (<60 vs ≥60 years), sex, primary site of presentation (nodal vs extranodal), stage (I-II vs III-IV), IPI (low- vs high-risk), or high Ki-67 proliferation index among GC and non-GC subgroups. However, BCL2 protein was expressed in a higher proportion of non-GC than GC DLBCLs ($P = .03$).

MYC, BCL2, *and* BCL6 *Rearrangements*

MYC, *BCL2*, and *BCL6* gene rearrangements were observed in 6%, 15%, and 29% of the cases, respectively (Table 1). There was no significant difference between GC and non-GC phenotype cases with regard to *MYC*, *BCL2*, and *BCL6* rearrangements.

MYC *Rearrangements*

MYC rearrangements were observed in 14 (6%) of 239 DLBCL patients (Fig. 1A). *MYC* rearrangement was more frequent in the high-risk IPI group ($P = .04$). Cases

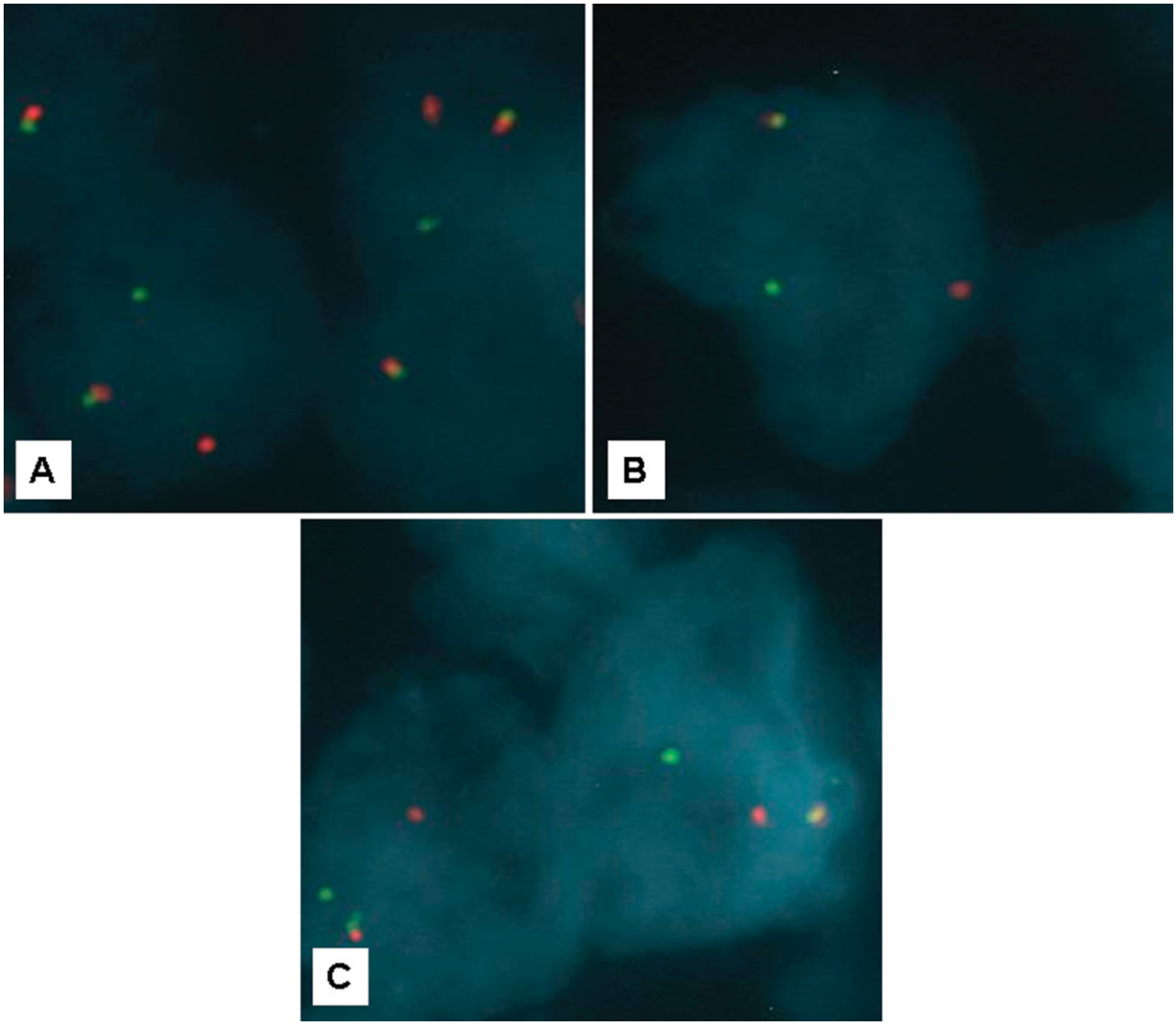


Figure 1. Fluorescence in situ hybridization (FISH) analysis using split signal FISH DNA probes shows (A) *MYC* rearrangement, (B) *BCL2* rearrangement, and (C) *BCL6* rearrangement in diffuse large B-cell lymphoma cases (original magnification, ×1000).

with *MYC* rearrangement were more likely to have a high proliferation rate (Ki-67 $\geq$ 80%, $P = .005$). However, there was no association with other clinical features, including age, sex, nodal/extranodal disease, or stage (Table 1).

BCL2 *Rearrangements*

BCL2 rearrangements were observed in 36 (15%) of 239 DLBCL patients (Fig. 1B). *BCL2* rearrangement correlated with overexpression of the BCL2 protein ($P = .08$); 70% of cases with *BCL2* rearrangement had BCL2 protein expression.

BCL2 rearrangements were more common in nodal than in extranodal presentations ($P = .02$). There was no association with other clinical features, including age, sex, stage, or IPI score (Table 1).

BCL6 *Rearrangements*

BCL6 rearrangements were detected in 69 (29%) of 239 DLBCL patients (Fig. 1C). *BCL6* rearrangement showed no significant association with clinical features, including age, sex, nodal/extranodal primary site of presentation, stage, or IPI score. No association was found between *BCL6* rearrangement and either BCL6 or BCL2 protein expression. No relationship between *BCL6* rearrangement and a high Ki-67 proliferation index was observed.

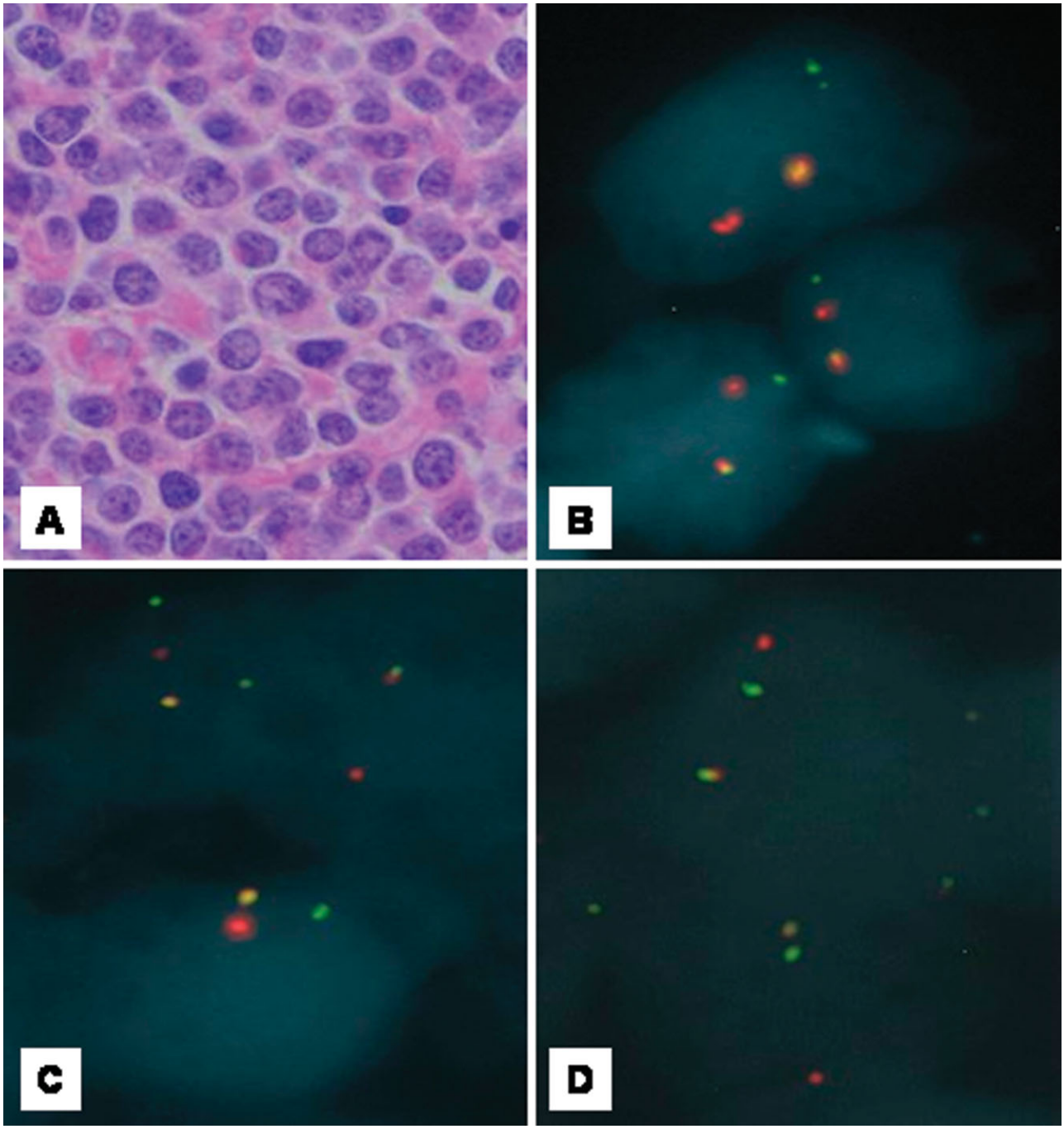


Figure 2. Triple-hit lymphoma case is shown: (A) diffuse large B-cell lymphoma not otherwise specified (H & E, original magnification, ×400), (B) split signals demonstrating presence of *MYC* rearrangement, (C) *BCL2* rearrangement, and (D) *BCL6* rearrangement (B-D original magnification, ×1000).

Double or Triple Rearrangements

Double or triple rearrangements were detected in 7 (3%) of 239 DLBCL cases: concurrent *BCL2*/*MYC* rearrangement in 4 cases and *BCL6*/*MYC* in 2 cases. An additional case had rearrangements involving all 3 genes (*BCL2*/*BCL6*/*MYC*).

Double-hit or triple-hit lymphoma patients included 4 females and 3 males with a median age of 49 years (range, 17-68). No patient had a prior diagnosis of lymphoma at the time of clinical presentation. Four cases had primary extranodal and 3 primary nodal presentations. The majority of patients had stage III or IV disease

Table 2. Univariate Analysis of the Clinicopathological Characteristics in 145 DLBCL Patients Treated With Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone Plus Rituximab

Characteristic	DLBCL, No. (%)	PFS *P*	OS *P*
Patients	145		
Age			
<60 years	87 (60)	.20	.50
≥60 years	58 (40)		
Sex			
Male	69 (48)	.50	.60
Female	76 (52)		
Presentation			
Nodal	88 (61)	.10	.20
Extranodal	57 (39)		
Stage			
I-II	53 (37)	.01	.03
III-IV	67 (46)		
Unknown	20 (17)		
IPI score			
Low, low-intermediate risk (0-2)	64 (44)	<.001	<.001
High-intermediate, high risk (3-5)	35 (24)		
Unknown	46 (32)		
Phenotype			
GC	68 (47)	.10	.30
Non-GC	77 (53)		
BCL2 protein expression	75 (52)	.40	.20
BCL6 protein expression	77 (53)	.20	.34
CD10 expression	44 (30)	.20	.08
Ki-67 ≥ 80%	54 (37)	.90	.90
MYC rearranged	11 (8)	.10	.01
BCL2 rearranged	24 (17)	.40	.20
BCL6 rearranged	42 (29)	.10	.04

Abbreviations: DLBCL, diffuse large B-cell lymphoma; GC, germinal center phenotype; IPI, International Prognostic Index; OS, overall survival; PFS, progression-free survival.

(6 of 7, 86%) and high-risk IPI scores (6 of 7, 86%). Bone marrow involvement was present in 1 patient (1 of 7, 14%) and central nervous system involvement in 1 patient (1 of 7, 14%).

The morphology and immunophenotype of the tumor cells was consistent with DLBCL, not otherwise specified (NOS) in 6 cases and B-cell lymphoma, unclassifiable in 1 case, according to the 2008 WHO classification. One case had coexisting follicular lymphoma grade 3B and DLBCL at diagnosis. BCL2 protein expression was found in 6 (86%) of 7 cases. The majority of cases (5 of 7, 71%) had a GC phenotype, whereas 2 cases had a non-GC phenotype. The majority of these cases (6 of 7, 86%) had a high proliferation rate (Ki-67 ≥80%). Morphological features consistent with DLBCL-NOS and rearrangements of *MYC*, *BCL2*, and *BCL6* are shown in the case of triple-hit lymphoma in Figure 2.

Survival Analysis

We studied clinicopathological characteristics and their association with PFS and OS by univariate analysis for 145 R-CHOP–treated patients (Table 2). Among clinical parameters, an IPI score >2 (high-risk IPI) was associated with worse OS ($P < .001$), as was advanced stage (stage III or IV, $P = .03$). By univariate analysis, *CD10*, *BCL6*, *MUM1/IRF4*, and *BCL2* expressions and high proliferation rate were not prognostic for PFS or OS. Similarly, no significant difference in PFS ($P = .1$) or OS ($P = .3$) was observed between the GC and non-GC phenotypes.

Considering FISH analysis, patients with *MYC* gene rearrangements had significantly worse OS ($P = .01$; Fig. 3A) than those without *MYC* rearrangement, but not PFS ($P = .1$). In particular, when analyzed according to GC and non-GC phenotypes, the prognostic implication of *MYC* rearrangement was statistically significant only for the GC phenotype, in which patients with *MYC* rearrangement had significantly shorter OS ($P = .009$) and tended to have worse PFS ($P = .09$). However, no significant prognostic impact of *MYC* rearrangement on PFS ($P = .6$) or OS ($P = .1$) was observed for the patients with non-GC phenotype.

BCL6 rearrangement had a significantly unfavorable impact on OS ($P = .04$; Fig. 3B) but not on PFS ($P = .1$). When analyzed according to GC and non-GC phenotypes, cases with *BCL6* rearrangement had significantly inferior OS ($P = .03$) if they had non-GC phenotype, but not if they had GC phenotype ($P = .3$). *BCL2* rearrangement was not significantly associated with PFS ($P = .4$) or OS ($P = .2$; Fig. 3C) in either the GC or the non-GC subgroup.

All 6 patients with double-hit lymphoma (*BCL2/MYC* or *BCL6/MYC* rearrangements) were treated with R-CHOP, and 1 patient with triple-hit lymphoma (*BCL2/BCL6/MYC* rearrangements) received only CHOP chemotherapy. The prognosis of the cases with double-hit lymphoma and triple-hit lymphoma was extremely poor, with a median survival of 9 months and a 2-year OS rate of 14% (PFS, $P = .003$ and OS, $P < .001$; Fig. 4). Only 1 *BCL2/MYC* double-hit lymphoma case was currently in complete remission at 30 months after the diagnosis.

In a multivariate analysis incorporating IPI, stage, and *BCL6* and *MYC* rearrangements, only IPI ($P = .01$) and *MYC* rearrangement ($P = .02$) remained as independent prognostic factors for OS, whereas the prognostic significance of *BCL6* rearrangement disappeared. With respect to PFS, only IPI ($P = .001$) was independently associated with PFS.

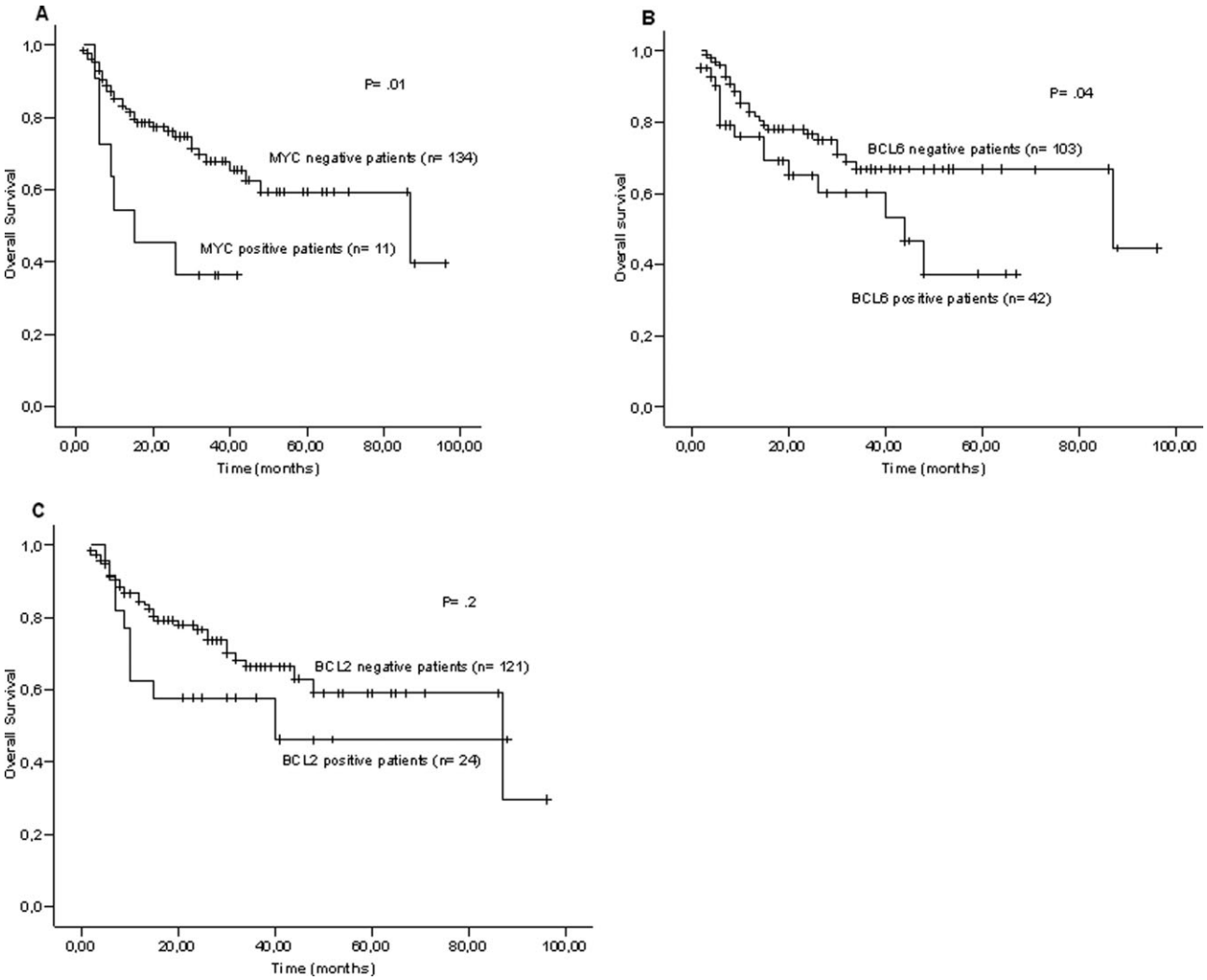


Figure 3. Overall survival curves are shown according to (A) *MYC* rearrangement status, (B) *BCL6* rearrangement status, and (C) *BCL2* rearrangement status. Patients with *MYC* and *BCL6* rearrangements showed inferior overall survival to those without *MYC* and *BCL6* rearrangement.

DISCUSSION

Several studies have examined the prognostic factors for predicting survival and for determining optimal therapeutic strategies in DLBCL. In the present study, GC/non-GC phenotype according to Hans' algorithm had no prognostic impact, in agreement with the majority of studies among R-CHOP–treated patients.[13-15,37] This might be explained by the addition of rituximab to chemotherapy, as it may modify the prognostic impact of BCL6 or BCL2 protein expressions.[13,34,35]

Recently, the prognostic impact of chromosomal translocations involving *MYC*, *BCL2*, and *BCL6* genes have been widely investigated in DLBCLs, but only a small number of studies have been conducted in the rituximab era (Table 3).[13,16,19-25,35,38] In this study, the frequency of *MYC* rearrangement was 6%, which is comparable to the previous studies, which have reported in 3% to 16% of DLBCL.[13,18,21-27] Several studies have analyzed the clinicopathologic presentations predicting the likelihood of *MYC* rearrangement. MYC rearrangement was significantly associated with a high proliferation rate, whereas the other studies did not show any correlation between a high proliferation rate and the presence of *MYC* rearrangement.[21-24]

In this study, *MYC*-rearranged DLBCLs were more frequently found in the high-risk IPI group. Barrans and colleagues found patients with *MYC*-rearranged DLBCL were significantly associated with an advanced stage and a higher IPI score.[21] Niitsu and colleagues showed that patients with *MYC*-rearranged DLBCL significantly

correlated with poor performance status, bone marrow involvement, high IPI score, and elevated lactate dehydrogenase levels.[23] On the basis of these findings, one can recommend the routine screening for *MYC* rearrangements in DLBCL cases, with high-risk features and/or a high proliferation rate.

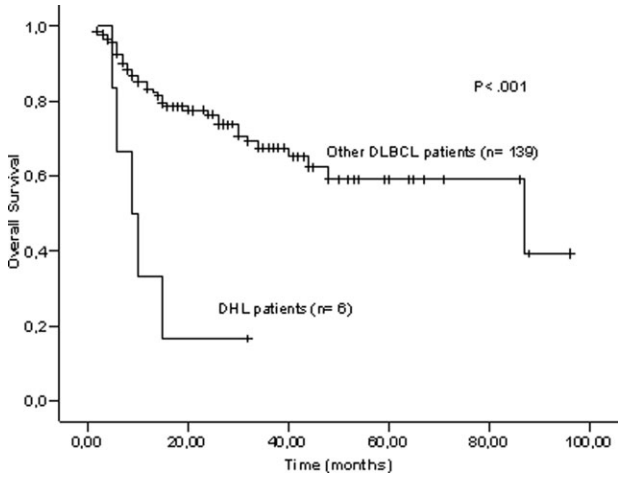


Figure 4. Overall survival curve of patients with double-hit lymphoma (DHL) shows inferior survival to that of the other diffuse large B-cell lymphoma (DLBCL) cases.

DLBCL with *MYC* rearrangements has a poor prognosis, with outcomes that are inferior to both BL and to other types of DLBCL. When treated with CHOP-like or augmented CHOP-like regimens, the 5-year survival is only 44% compared with 67% for DLBCL without a *MYC* aberration.[23] Furthermore, the negative impact of *MYC* expression supersedes the favorable prognosis of GC phenotype of DLBCL, with significantly inferior event-free survival and OS compared with either non-GC phenotype or non–*MYC*-associated DLBCL treated with CHOP.[22]

The presence of *MYC* rearrangement retains its negative prognostic significance even in patients treated with rituximab and anthracycline-based immunochemotherapy, and is an independent predictor of outcome in DLBCL.[21,24] In our study, patients with *MYC* gene rearrangement showed significantly worse OS than nonrearranged patients, especially in GC phenotype DLBCL. The *MYC* rearrangement and IPI score remained as independent prognostic indicators by multivariate analysis in this study. Savage and colleagues found that for DLBCL,

Table 3. Summary of Recent Studies with *MYC*, *BCL2*, and *BCL6* Rearrangements in Diffuse Large B-Cell Lymphoma Patients

Gene	Study	No. of Patients	Frequency of the Abnormality, %	Clinicopathologic Parameters	Treatment Regimen	Survival
MYC	Our study	239	6	High-risk IPI group and high proliferation rate	R-CHOP (145 cases)	Worse OS
	Savage 2009[24]	135	9	High risk of central nervous system relapse and high proliferation rate	R-CHOP	Worse OS and PFS
	Barrans 2010[21]	245	14	High IPI score and high stage	R-CHOP	Worse OS
	Klapper 2008[22]	177	8	Low-risk IPI group	CHOP or CHOEP	Worse OS
	Niitsu 2009[23]	252	11	High IPI score, poor performance status, high lactate dehydrogenase level and bone marrow involvement	CHOP or CyclOBEAP	Worse OS and PFS
	Copie-Bergman 2009[13]	68	3	NA	R-CHOP	NS
	Yoon 2008[25]	156	9	High IPI score	CHOP or CHOP-like	Worse OS
BCL2	Our study	239	15	Nodal presentation	R-CHOP (145 cases)	NS
	Copie-Bergman 2009[13]	71	20	NA	R-CHOP	NS
	Barrans 2003[20]	137	13	NS	CHOP	Worse OS for GC type
BCL6	Our study	239	29	NS	R-CHOP (145 cases)	Worse OS
	Shustik 2010[35]	164	20	High IPI score	CHOP or R-CHOP	Worse OS for R-CHOP
	Copie-Bergman 2009[13]	69	30	NA	R-CHOP	Worse OS
	Iqbal 2007[38]	133	19	NA	CHOP	NS
	Barrans 2002[19]	111	25	NS	NA	Worse OS
	Offit 1994[16]	102	22	Extranodal presentation	CHOP	Favorable OS

Abbreviations: CHOEP, CHOP plus etoposide; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; CyclOBEAP, cyclophosphamide, vincristine, bleomycin, etoposide, doxorubicin, prednisone; GC, germinal center phenotype; IPI, International Prognostic Index; NA, not available; NS, not significant; OS, overall survival; PFS, progression-free survival; R-CHOP, CHOP plus rituximab.

MYC rearrangements had a negative prognostic impact by multivariate analysis for both PFS and OS, as well as a higher risk of central nervous system relapse even when treated with R-CHOP.[24]

Even in pediatric patients with DLBCL, the presence of 8q24 rearrangements independently reduces event-free survival by 6-fold.[39] By contrast, Copie-Bergman and colleagues found that presence of *MYC* rearrangement did not predict shortened OS in DLBCLs treated with R-CHOP.[13]

Half of our cases with *MYC* rearrangement also had *BCL2* and/or *BCL6* rearrangements. The poor clinical outcome of the group of tumors with *MYC* rearrangement is likely to be a consequence of the synergistic interaction between multiple dysregulated genes.

Because of the very poor outcome and resistance to lower-dose therapies, an intensive chemotherapy regimen used for BL (CODOX-M/IVAC) has been examined in patients with aggressive B-cell lymphomas characterized by complex patterns of cytogenetic abnormalities, but this therapy did not improve survival in this group of patients in 1 study.[40]

BCL6 gene rearrangement is the most common chromosomal translocation identified in DLBCL, occurring in up to 30% to 35% of cases.[13,16,18,19,27,38] The frequency of *BCL6* rearrangement was 29% in our study. It has been reported that *BCL6* rearrangement occurs more frequently in patients with extranodal disease and, interestingly, has an association with non-GC phenotype.[13,16,18,19,38] However, in our study, no significant correlation was found between *BCL6* rearrangement and the site of presentation. We also did not find a correlation between *BCL6* rearrangement and BCL6 protein expression, consistent with recent observations.[19,35] It has been suggested that *BCL6* rearrangements does not directly lead to *BCL6* expression. However, *BCL6* mutations and a number of alternative translocation partners may be related to deregulation of *BCL6* expression.[38] Ohno and colleagues found that nonimmunoglobulin/*BCL6* translocation correlated with low-level BCL6 protein expression, and was associated with a poor prognosis.[41]

Different studies investigating the prognostic significance of the *BCL6* rearrangement in DLBCL have yielded conflicting results, which probably reflects the heterogeneity of the disease and various molecular techniques used. For example, *BCL6* rearrangement has been associated with a favorable prognosis in DLBCL patients treated with CHOP-like chemotherapy in some studies, but not in others.[11,16,18,19,38]

In the present study, *BCL6* rearrangement was also associated with an inferior outcome in patients treated with R-CHOP on univariate analysis, but was not an independent prognostic factor. Otherwise, *BCL6* rearrangement did not correlate with the presence of high-risk clinical features. In agreement with our results, investigators have reported results of 2 other large series and have also demonstrated that *BCL6* rearrangement is associated with significantly worse OS in patients treated with R-CHOP.[13,35] Shustik and colleagues noted that *BCL6* rearrangement correlated with the presence of high-risk clinical features, but was not a prognostic factor independent of the IPI score.[35] Although we did not find any correlation among *BCL6* rearrangement and GC/non-GC phenotype, the poor survival effect of *BCL6* rearrangement was particularly evident in non-GC phenotype DLBCL in our study. Thus, *BCL6* rearrangement could be a biomarker of more aggressive disease in the non-GC subgroup.

In the present study, the frequency of *BCL2* rearrangement was 15% in DLBCL patients. Moreover, no significant difference in the frequency of *BCL2* rearrangement was observed between GC and non-GC phenotype cases. However, we observed that *BCL2* rearrangement correlated with BCL2 protein expression, which was more frequently observed in non-GC phenotype cases. It has been suggested that alternative mechanisms other than (14;18)(q32;q21), such as 18q21 amplification or activation of nuclear factor-κB pathway, may be primarily responsible for the up-regulation of *BCL2* expression in the non-GC subgroup.[42] In our cohort, 24% of DLBCL cases showed amplification at *BCL2* locus (data not shown). Of these cases, roughly 2/3 had non-GC phenotype, and 1/3 had GC phenotype. This observation lends further evidence to the presence of alternative mechanisms for *BCL2* overexpression in non-GC DLBCL. The prognostic role of *BCL2* rearrangement is still controversial. In the present study, *BCL2* rearrangement was not predictive of OS in patients treated with R-CHOP, in accordance with most reports.[11,13,18,43] Barrans and colleagues demonstrated that GC type DLBCL patients with t(14;18) had significantly worse survival.[20]

Double-hit and triple-hit lymphomas are rare neoplasms, with estimated frequencies ranging from 1% to 12% of DLBCLs. *BCL2*/*MYC* double-hit lymphomas (62%) formed the great majority of double-hit lymphomas. *BCL6*/*MYC* double-hit lymphomas were relatively rare (8% of all cases), and triple-hit lymphomas that involved *BCL2*/*BCL6*/*MYC* (16%) were more frequent than *BCL6*/*MYC* double-hit lymphoma cases in these studies.[11,21,24-27] In our study, in accordance with the reported cases in the literature, most patients presented as

de novo DLBCL.[29-32] However, in a recent study on 20 patients with *BCL2*/*MYC* double-hit lymphoma, low-grade follicular lymphoma history was reported in 6 (30%) of the cases.[44]

In conclusion, our findings and other studies confirm that double-hit and triple-hit lymphomas are characterized by aggressive clinical behaviors. Patients usually present with poor prognostic parameters, including advanced stage, B symptoms, elevated lactate dehydrogenase, poor performance status, bone marrow and central nervous system involvement, and a high IPI score. Patients with these lymphomas have poor prognosis and resistance to multiagent chemotherapy, even if treated with R-CHOP or high-intensity treatment regimens. This poor outcome is likely related to synergistic action of *MYC*-induced growth promotion and the antiapoptotic effect provided by *BCL2*. The presence of *MYC* rearrangement in DLBCL was associated with a poor prognosis, and is an independent predictor of shortened OS in the R-CHOP treatment era. Because there are no clinical, morphological, or immunophenotypic features to identify such cases, all DLBCL cases should be analyzed for *MYC* abnormality using FISH, especially if the proliferation index is >80%. In addition, determination of *BCL2* and *BCL6* rearrangements is also imperative to identify double-hit and triple-hit lymphoma, which have clinically more aggressive behavior and extremely poor prognosis. *BCL6* rearrangement was also associated with worse prognosis in DLBCL, particularly in the non-GC subgroup. Patients with these abnormalities have a poor response to R-CHOP, and novel therapeutic approaches should be developed for these cases.

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