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Original Article

R-CHOP regimen can significantly decrease the risk of disease relapse and progression in patients with non-germinal center B-cell subtype diffuse large B-cell lymphoma

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Abstract

To further explore the role of rituximab when added to the CHOP-like regimen in the treatment of immunohistochemically defined non-germinal center B-cell subtype (non-GCB) diffuse large B-cell lymphoma (DLBCL), 159 newly diagnosed DLBCL patients were studied retrospectively based on the immunohistochemical evaluation of CD10, Bcl-6, MUM-1, and Bcl-2. Altogether, 110 patients underwent the CHOP-like regimen, and rituximab was added for the other 49 patients. Cox regression analysis showed that compared with the CHOP-like regimen, the rituximab-based regimen (R-CHOP regimen) significantly decreased the risk of disease relapse and progression in CD10-negative patients (P = 0.001), Bcl-6-negative patients (P = 0.01), and MUM-1-positive patients (P = 0.003). The risk of disease relapse in patients with non-GCB subtype (P = 0.002) also decreased. In contrast, patients with the opposite immunohistochemical marker expression profile and GCB subtype did not benefit from treatment with the R-CHOP regimen. In addition, non-GCB subtype patients had a significantly higher expression rate of Bcl-2 than GCB subtype patients (P = 0.042). Although univariate analysis found that both Bcl-2-positive and -negative patients had significantly higher event-free survival rates with the R-CHOP regimen, only Bcl-2 positivity (P = 0.004) maintained significance in the Cox regression analysis. We conclude that the addition of rituximab can significantly improve the prognosis of patients with non-GCB subtype DLBCL, which is closely related to the expression of CD10, Bcl-6, MUM-1, and Bcl-2.

Key words Diffuse large B-cell lymphoma, rituximab, chemotherapy, NF-κB, immunohistochemistry

Diffuse large B-cell lymphomas (DLBCLs), which represent approximately 40% of adult non-Hodgkin's lymphomas (NHLs), are a heterogeneous group of tumors that vary in immunophenotype, cytogenetics, and clinical features[1]. Gene expression profiling has revealed that DLBCL can be subdivided into three groups: germinal center B-cell (GCB)-like DLBCL, active B-cell (ABC)-like DLBCL, and type 3 DLBCL[2,3]. Because ABC-like DLBCL and type 3 DLBCL have similar significantly worse prognosis than GCB-like DLBCL, they are always grouped as non-GCB subtype. Hans *et al.*[4] found, using an algorithm based on CD10, Bcl-6, and MUM-1 expression, that immunostaining can be used to determine GCB and non-GCB subtypes of DLBCL and predict survival similar to gene expression profiling. Due to its convenience, this technique has been widely employed as a substitute for gene expression profiling for the study of DLBCL. Currently, no effective treatment strategies have been established for patients with non-GCB subtype DLBCL, although compared with patients with GCB subtype DLBCL, they do not have satisfactory long-term survival when treated with an

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anthracycline-based regimen[2-5]. However, some retrospective studies found that patients with non-GCB subtype DLBCL can have comparable long-term survival to those with GCB subtype DLBCL when treated with rituximab-based chemotherapy[6,7].

To further explore the role of rituximab in the treatment of non-GCB subtype DLBCL, clinical data from 159 DLBCL patients were analyzed retrospectively based on four popular markers: CD10, Bcl-6, MUM-1, and Bcl-2.

Materials and Methods

Patients and clinical data collection

Clinical data were collected from the database of the Department of Medical Oncology, Cancer Institute and Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College (CAMS & PUMC). Patients with primary DLBCL who were treated in our hospital and had immunostaining performed for at least one of the following proteins were eligible for the study: CD10, Bcl-6, MUM-1, and Bcl-2. The study protocol and sampling were approved by the Institutional Review Board of the Cancer Institute and Hospital of CAMS & PUMC. Informed consent was provided according to the Declaration of Helsinki. Clinical data were obtained at the time of diagnosis. Staging was performed according to the Ann Arbor staging system for Hodgkin's disease. At the time of diagnosis, each patient underwent a physical examination, a full blood count and biochemical profile analysis, serum lactate dehydrogenase (LDH) detection, chest X-ray, bone marrow aspiration, ultrasonography, and computed tomography or magnetic resonance imaging (MRI) scan. B symptoms were defined as fever of unknown reason (>38℃) for more than 3 days, more than 10% weight loss within 6 months, and night sweats. Bulky disease was defined as a tumor mass larger than 10 cm.

Treatment

At least six cycles of CHOP-like regimens, including the CHOP regimen (cyclophosphamide, vincristine, doxorubicin/epirubicin, and prednisone), etoposide added to the CHOP regimen, HyperCVAD regimen (fractionated cyclophosphamide, vincristine, epirubicin, and dexamethasone), BACOP regimen (bleomycin, doxorubicin/epirubicin, cyclophosphamide, vincristine, and prednisone), and CHEP regimen (cyclophosphamide, etoposide, epirubicin, and prednisone), were given to each patient. If rituximab (Roche, Basel, Swiss) was added to the regimen, it was administered the day before the start of chemotherapy at a dose of 375 mg/m^2 and repeated every 3 weeks. Locoregional radiotherapy was added for patients with stage I/II disease or bulky disease.

Patient following-up

Each patient was followed every 3 months for the first 2 years and every 6 months for the next 3 years till April 2011. Full blood count, biochemical profile analysis, ultrasonography, and computed tomography or MRI were conducted each time.

Immunohistochemical study

All tumor specimens were obtained by biopsy or surgical resection, fixed in 10% buffered formaldehyde, and embedded in paraffin. Immunohistochemical analysis with antibodies against CD20 (clone L26, Novocastra, Newcastle, UK), CD79a (clone HM57, Darko, Glostrup, Denmark), CD3 (clone SP7, Novocastra, Newcastle, UK), CD5 (clone 4C7, Zymed, San Diego, USA), and CD45RO (clone UCHL-1, Novocastra, Newcastle, UK) was conducted. All specimens were reviewed separately by two experienced pathologists, and a common consensus was reached in all cases. The WHO diagnostic criteria for NHL were adopted for diagnosis. Furthermore, CD10 (clone 56C6, Novocastra, Newcastle, UK), Bcl-6 (clone PG-B6p, Novocastra, Newcastle, UK), MUM-1 (clone MUM1p, Dako, Glostrup, Denmark), and Bcl-2 (clone 100-D5, Novocastra, Newcastle, UK) were tested upon clinical request. Cases were considered positive if 30% or more of tumor cells were stained with an antibody. Subtype determination was based on the algorithm developed by Hans *et al*[4].

Statistical analysis

SPSS 10.0 software was used for data analysis. Overall survival (OS) was estimated using the Kaplan-Meier method from the day of diagnosis to death or to the date of the last follow-up. Event-free survival (EFS) was estimated from the day of diagnosis to the time of disease progression or relapse or to the date of the last follow-up visit. The log-rank test was used to compare survival curves. For continuous variables, the *t* test or Mann-Whitney *U* test was employed to analyze the difference between two groups. χ^2 test was used to analyze categorical variables. All statistical analyses used 0.05 as the significance level (two-sided test).

Results

Patient characteristics

A total of 159 DLBCL patients, aged from 14 to 83

years (median, 50), were eligible for this retrospective analysis: 118 were treated with the CHOP regimen, 24 with etoposide added to the CHOP regimen, 8 with the BACOP regimen, 6 with the HyperCVAD regimen, and 3 with the CHEP regimen; rituximab was added to the CHOP regimen (R-CHOP regimen) in 49 cases. Patient characteristics are summarized in Table 1. The proportion of patients with a higher IPI score (high/high-intermediate risk) was significantly greater in patients treated with the R-CHOP regimen than in those treated with the CHOP-like regimen (26.5% vs. 14.5%, P = 0.037); no differences in other factors were observed between these two groups (Table 1).

Relationships between survival and clinical factors

The median follow-up time was 24 months (range, 1 to 105 months) for the whole group. The 2-year EFS and OS rates were 53.2% and 72.8% , respectively. In patients treated with the CHOP-like regimen, univariate analysis found that B symptoms, elevated β2-microglobulin levels, and a higher IPI score were associated with significantly lower 2-year EFS and 2-year OS rates; bulky disease was also associated with lower 2-year EFS rate (Table 2). Although more patients had a higher IPI score, the group treated with the R-CHOP regimen still showed significantly higher 2-year EFS rate (75.8% vs. 42.2%, P < 0.001) and OS rate (81.8% vs. 68.7%, P = 0.035) compared with the group treated with the CHOP-like regimen. Among patients treated with the CHOP-like regimen, no significant differences were observed betwan GCB and non-GCB subtypes (P > 0.05). Further analysis showed that almost all subgroup patients had significantly higher EFS rates when treated with the R-CHOP regimen, with the exception of patients with elevated β2-microglobulin levels and those who were older than 60 (Table 3). In terms of OS, only patients with a higher IPI score and without B symptoms benefited from treatment with the R-CHOP regimen. This result was most likely due to a relatively short follow-up period.

Immunohistochemistry

Immunohistochemistry of Bcl-2 was performed on 110 specimens, CD10 on 134 patients, Bcl-6 on 135 specimens, and MUM-1 on 137 specimens. The positive rate was 63.6% for Bcl-2, 20.1% for CD10, 34.8% for Bcl-6, and 80.3% for MUM-1. According to the algorithm

Table 1. Clinical characteristics of 159 patients with diffuse large B-cell lymphoma (DLBCL)

Variate	Total [cases (%)]	Treatment [cases (%)]		P	Subtype[a] [cases (%)]		P
		CHOP-like regimen	Rituximab-based regimen		GCB	Non-GCB	
Total	159(100)	110(69.2)	49(30.8)		33(25.6)	96(74.4)	
Age (years)				0.160			0.750
< 60	117(73.6)	84(76.4)	33(67.3)		24(72.7)	72(75.0)	
⩾ 60	42(26.4)	26(23.6)	16(32.7)		9(27.3)	24(25.0)	
Sex				0.320			0.021
Male	92(57.9)	66(60.0)	26(53.1)		22(66.7)	49(51.0)	
Female	67(42.1)	44(40.0)	23(46.9)		11(33.3)	47(49.0)	
B symptoms				0.140			0.025
Present	36(22.6)	22(20.0)	14(28.6)		5(15.2)	27(28.1)	
Absent	123(77.4)	88(80.0)	35(71.4)		28(84.8)	69(71.9)	
IPI				0.037			0.005
Low/low-intermediate	130(81.8)	94(85.5)	36(73.5)		31(93.9)	78(81.2)	
High/high-intermediate	29(18.2)	16(14.5)	13(26.5)		2(6.1)	18(18.8)	
Bulky disease				0.380			0.650
Present	18(11.3)	11(10.0)	7(14.3)		4(12.1)	10(10.4)	
Absent	141(88.7)	99(90.0)	42(85.7)		29(87.9)	86(89.6)	
β2-microglobulin elevation				1.000			0.770
Present	49(30.8)	34(30.9)	15(30.6)		11(33.3)	34(35.4)	
Absent	110(69.2)	76(69.1)	34(69.4)		22(66.7)	62(64.6)	

[a]In total, 129 patients have complete data for subtype classification. According to the algorithm developed by Hans *et al.*[4], 74.4% (96/129) of patients were classified with non-GCB subtype disease. IPI, international prognostic index; GCB, germinal center B-cell-like; non-GCB, non-germinal center B-cell-like.

Table 2. Univariate prognostic analysis on patients with DLBCL treated with the CHOP-like regimen

Variate	2-year EFS rate (%)	*P*	2-year OS rate (%)	*P*
Age (years)		0.083		0.810
< 60	36.9		69.1	
⩾ 60	58.7		67.7	
Sex		0.930		0.530
Male	40.0		69.9	
Female	44.8		66.7	
B symptoms		<0.001		0.002
Present	15.7		47.4	
Absent	49.4		75.7	
IPI		<0.001		<0.001
Low/low-intermediate	0		0	
High/high-intermediate	57.4		82.8	
Bulky disease		0.033		0.160
Present	10.9		51.1	
Absent	45.9		70.7	
β2-microglobulin elevation		<0.001		0.002
Present	30.0		55.0	
Absent	63.0		78.9	
Bcl-2 expression		0.005		0.090
Positive	32.8		63.9	
Negative	69.9		81.8	
CD10 expression		0.640		0.950
Positive	41.6		69.3	
Negative	41.2		67.6	
Bcl-6 expression		0.180		0.810
Positive	54.0		67.8	
Negative	36.1		68.3	
MUM-1 expression		0.018		0.130
Positive	38.4		71.5	
Negative	65.6		81.8	

EFS, event-free survival; OS, overall survival. Other abbreviations as in Table 1.

developed by Hans *et al.*[4], 74.4% (96/129) of patients were classified as non-GCB subtype.

In terms of the relationship between immunohistochemical markers, the positive rate of Bcl-2 was significantly higher in MUM-1-positive patients than in MUM-1-negative patients (73.6% vs. 35.7%, $P < 0.001$) and higher in patients with non-GCB subtype disease than in those with GCB subtype disease (68.4% vs. 53.8%, $P = 0.042$).

Survival analysis of patients treated with the CHOP-like regimen showed that the expression of Bcl-2 and MUM-1 indicated a poorer prognosis (Figures 1A and 1B). The 2-year EFS rate was significantly lower in patients with Bcl-2 (33.0% vs. 69.0%, $P = 0.005$) or MUM-1 expression (38.0% vs. 65.6%, $P = 0.018$) than in those without marker expression. CD10 and Bcl-6 did not show prognostic value in EFS (Figures 1C and 1D). There was no significant difference in either EFS ($P = 0.46$) or OS ($P = 0.79$) between GCB and non-GCB subtype patients.

As shown in Table 3, compared with the CHOP-like regimen, the R-CHOP regimen improved survival in selected populations. The R-CHOP regimen significantly improved 2-year EFS rate in CD10-negative, Bcl-6-negative, and MUM-1-positive patients (all $P < 0.01$). Accordingly, patients with the non-GCB subtype disease obtained more benefit from treatment with the R-CHOP regimen than GCB subtype patients ($P = 0.002$). For CD10-positive patients, Bcl-6-positive patients, MUM-1-negative patients, and patients with the GCB subtype disease, the R-CHOP regimen did not show a significant difference in EFS improvement compared with the CHOP-like regimen. In terms of Bcl-2 status, both Bcl-2-positive and -negative patients achieved significantly

Table 3. Survival comparison between patients treated with the CHOP-like regimen and rituximab-based regimen

Variate	2-year EFS rate (%)		*P*	2-year OS rate (%)		*P*
	CHOP	R-CHOP		CHOP	R-CHOP	
Age						
< 60	36.9	70.3	0.002	69.1	79.3	0.130
⩾ 60	58.7	87.5	0.150	67.7	87.1	0.150
Sex						
Male	40.0	73.8	0.009	69.9	82.6	0.150
Female	44.8	77.6	0.017	66.7	80.7	0.120
B symptoms						
Present	15.7	59.3	0.006	47.4	64.0	0.140
Absent	49.4	84.1	0.002	75.7	90.1	0.048
IPI						
Low/low-intermediate	47.7	80.8	0.002	78.4	84.3	0.230
High/high-intermediate	0	61.5	0.002	0	75.0	<0.001
Bulky disease						
Present	10.9	71.4	0.022	51.1	85.7	0.069
Absent	45.9	76.4	0.002	70.7	81.2	0.110
β2-microglobulin elevation						
Present	29.9	48.2	0.074	55.0	66.7	0.250
Absent	63.0	96.3	0.056	78.9	96.2	0.057
Bcl-2 expression						
Positive	32.8	74.9	0.001	63.9	84.7	0.053
Negative	69.9	100	0.029	81.8	100	0.098
CD10 expression						
Positive	41.6	80.0	0.230	69.2	100	0.130
Negative	41.2	78.0	<0.001	67.6	83.2	0.038
Bcl-6 expression						
Positive	54.0	83.0	0.052	67.8	88.5	0.260
Negative	36.1	71.1	0.006	68.3	88.1	0.025
MUM-1 expression						
Positive	38.4	77.9	<0.001	71.5	82.6	0.120
Negative	65.6	100	0.140	81.8	100	0.330
Subtype						
GCB	44.1	85.7	0.130	67.7	100	0.090
Non-GCB	42.0	74.3	0.002	69.6	79.9	0.170

Abbreviations as in Tables 1 and 2.

higher 2-year EFS from treatment with the R-CHOP regimen than from treatment with the CHOP-like regimen (both $P < 0.05$). In regard to OS, the difference was marginal in Bcl-2-positive patients ($P = 0.053$) and significant in CD10-negative ($P = 0.038$) and Bcl-6-negative patients ($P = 0.025$). However, the difference in MUM-1-positive patients was not significant.

Cox regression analysis further showed that the R-CHOP regimen significantly decreased the risk of disease relapse or progression in Bcl-2-positive patients ($P = 0.004$), CD10-negative patients ($P = 0.001$), Bcl-6-negative patients ($P = 0.01$), MUM-1-positive patients ($P = 0.003$), and patients with non-GCB subtype disease ($P = 0.002$) (Table 4). However, for patients with the opposite expression profile and patients with GCB subtype disease, the R-CHOP regimen showed no effect on the risk of disease relapse and progression (data not shown).

Discussion

DLBCL is the most common subtype of NHL and accounts for approximately 40% of NHL patients[1]. Gene expression profiling revealed that DLBCL can be subdivided into GCB and non-GCB subtypes[2,3]. Many retrospective studies have found that non-GCB subtype patients have significantly lower survival rates than GCB

Table 4. Multivariate Cox regression prognostic analysis on the EFS of selective populations

Variate	Bcl-2-positive			CD10-negative			Bcl-6-negative			MUM-1-positive			Non-GCB subtype		
	RR	95% CI	*P*	RR	95% CI	*P*	RR	95% CI	*P*	RR	95% CI	*P*	RR	95% CI	*P*
B symptoms															
Present vs. absent	4.0	1.4–11.7	0.011	2.6	1.1–6.3	0.038	1.9	0.84–4.2	0.120	2.6	1.1–6.1	0.026	2.6	1.2–5.9	0.022
IPI															
High/high-intermediate vs. low/low-intermediate	2.8	0.98–7.8	0.056	2.1	0.86–5.3	0.100	2.8	1.1–7.4	0.038	2.5	0.96–6.4	0.060	1.8	0.67–4.6	0.250
Bulky disease															
Present vs. absent	0.77	0.17–3.5	0.730	0.72	0.21–2.5	0.610	1.2	0.33–4.2	0.790	1.2	0.37–3.7	0.800	1.3	0.41–3.9	0.690
β2-microglobulin elevation															
Present vs. absent	2.1	0.85–4.9	0.110	2.4	1.1–4.9	0.021	2.1	0.89–4.9	0.088	3.9	1.6–9.2	0.002	3.2	1.5–6.9	0.003
Treatment															
CHOP vs. R-CHOP	4.9	1.7–14.6	0.004	4.6	1.9–10.9	0.001	4.1	1.4–11.8	0.010	4.8	1.7–13.3	0.003	4.2	1.7–10.5	0.002

RR, risk ratio; CI, confidence interval. Other abbreviations as in Tables 1 and 2.

subtype patients when treated with a CHOP-like regimen[2-5]. Therefore, a more effective regimen should be used to improve the survival of non-GCB subtype DLBCL patients. Nyman *et al.*[6] found that a R-CHOP regimen could eliminate the prognostic value of immunohistochemically defined GCB and non-GCB phenotypes in DLBCL. It was thus suggested that the R-CHOP regimen might significantly improve the prognosis of non-GCB subtype patients, making their survival comparable with GCB subtype patients.

To further explore this hypothesis, clinical data from 159 patients with newly diagnosed DLBCL were analyzed based on immunohistochemical examination of CD10, Bcl-6, MUM-1, and Bcl-2. Unfortunately, because the survival difference between non-GCB and GCB subtype patients did not reach a significant level in our study, the results of the Finland study[6] could not be reproduced. However, based on our survival analysis, we found that only non-GCB subtype patients benefited from treatment with the R-CHOP regimen, leading to significantly improved EFS. In GCB subtype patients, EFS was comparable between the R-CHOP regimen group and the CHOP-like regimen group. These results are similar to those of the Finland study, which indicated that the R-CHOP regimen is an effective way to improve the prognosis of non-GCB subtype patients. Furthermore, we also found that only CD10-negative patients, Bcl-6-negative patients, and MUM-1-positive patients benefited from treatment with the R-CHOP regimen, whereas patients with the opposite expression profile did not. These results were further validated by Cox regression analysis, which showed that the R-CHOP regimen only decreased the risk of disease relapse or progression in CD10-negative patients, Bcl-6-negative patients, MUM-1-positive patients, and patients with non-GCB subtype disease. In addition, Cox regression analysis proved that R-CHOP regimen decreased the risk of disease relapse or progression only in Bcl-2-positive patients, although both Bcl-2-positive and -negative patients were found to have a significantly higher EFS from treatment with the R-CHOP regimen in the univariate analysis. These interesting clinical findings indicate that rituximab has the ability to improve survival of non-GCB subtype patients, and this ability most likely correlates with the expression of the previously mentioned immunohistochemical markers.

CD10 is a cell surface marker that is expressed in many types of cells, including DLBCL[8-11]. Bcl-6 is a transcription suppressor that can inhibit lymphocyte activation and the differentiation of germinal center B cells to plasma cells[12-14]. Both CD10 and Bcl-6 have been found to be associated with increased apoptosis in B-cell lymphomas when overexpressed[15-17]. However, the definitive mechanism by which apoptosis is induced by CD10 and Bcl-6 is still unclear. Some studies have indicated that the Fas ligand signaling pathway may be involved because the expression of CD10 and Bcl-6 showed a significant positive correlation with the expression of Bid, Bak, and Bax and a negative correlation with the expression of Bcl-xL[18-20]. In addition, the expression of Bcl-6 was negatively correlated with Bcl-2 expression[16,21]. Therefore, it was suggested that the loss of CD10 and/or Bcl-6 expression might induce activation of the anti-apoptosis pathway concerning Bcl-2 family members. Because the overexpression of Bcl-2 family members can be suppressed by rituximab, it is reasonable that CD10-negative patients and Bcl-6-negative patients may benefit from treatment with a rituximab-based chemotherapy[22-24].

As shown in the present study, the EFS and OS

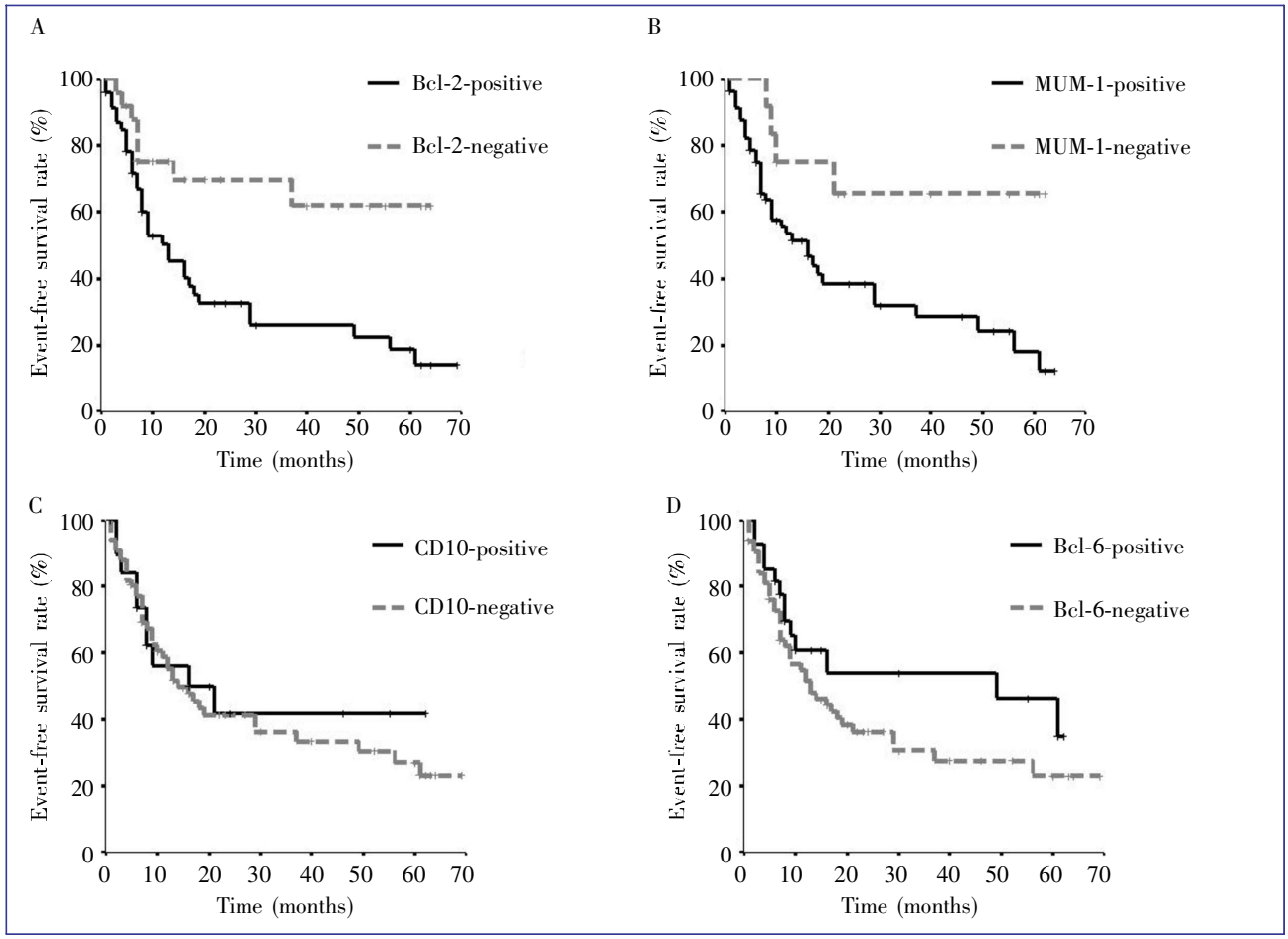

Figure 1. **Event-free survival (EFS) curves for patients with diffuse large B-cell lymphoma (DLBCL) treated with the CHOP-like regimen.** A, the EFS rate is lower in the patients with Bcl-6-positive tumors than in those with Bcl-6-negative tumors (P = 0.005). B, the EFS rate is lower in the patients with MUM-1-positive tumors than in those with MUM-1-negative tumors (P = 0.018). C, the EFS rate is similar between the patients with CD10-positive and -negative tumors (P = 0.64). D, the EFS rate is similar between the patients with Bcl-6-positive and -negative tumors (P = 0.18).

rates of the patients with CD10-negative or Bcl-6-negative tumors were significantly improved by the R-CHOP regimen. For CD10-positive and Bcl-6-positive patients, however, the R-CHOP regimen did not improve survival compared with the CHOP-like regimen alone. This outcome is most likely due to the tendency of self-apoptosis to occur in CD10- and Bcl-6-positive tumor cells because the CHOP-like regimen alone is effective enough to induce apoptosis and there is no need for the addition of rituximab.

MUM-1 is a transcription factor that is required for the proliferation of B and T lymphocytes and is induced by the binding of c-Rel to NF-κB motifs in its promoter[25]. It is also a potential mediator of NF-κB proliferative responses[26]. Given its biologic function, the expression of MUM-1 is a strong indicator of NF-κB activation. Furthermore, as shown in our study, the positive rate of Bcl-2 was significantly higher in MUM-1-positive patients than in MUM-1-negative patients. Because many studies have found that Bcl-2 and other Bcl-2 family members are target genes in the NF-κB pathway, the overexpression of Bcl-2 family members may provide other evidence of NF-κB pathway activation[27-31]. Some *in vitro* studies have found that rituximab has a dual suppression effect on both NF-κB activation and Bcl-2 overexpression[22-24], which would explain the results in our study demonstrating that the R-CHOP regimen can significantly improve the EFS of MUM-1-positive patients.

Bcl-2 is an important anti-apoptotic factor in normal B-cell development and differentiation. According to our analysis, it appears that Bcl-2 is closely associated with the expression of all three markers mentioned above. The overexpression of Bcl-2 is more likely to occur in CD10-negative, Bcl-6-negative, and MUM-1-positive patients, which is the typical expression pattern in non-GCB subtype patients. This result is in accordance with Bcl-2 expression being significantly higher in non-GCB subtype patients in our study. For ABC

subtype DLBCL patients, many studies have indicated that the constitutive activation of the NF-κB pathway appears to be responsible for Bcl-2 overexpression [32]. Moreover, 18q21 amplification was also found to be frequently detected in ABC subtype DLBCL patients [33]. For type 3 patients, the mechanism is unknown. Because gene expression profiling showed that these patients had lower expression levels of MUM-1, it has been suggested that some mechanisms other than NF-κB pathway activation might be involved in Bcl-2 overexpression.

Some *in vitro* studies have found that rituximab can sensitize lymphoma cells to chemotherapeutic drugs via down-regulating Bcl-2 family members [22,24]. In addition, clinical studies conducted by Mounier *et al.*[34,35] in elderly DLBCL patients proved that rituximab-based chemotherapy can significantly improve the survival of Bcl-2-positive patients. Although the same result was not reproduced in our elderly population due to the smaller number of cases (n = 21, P = 0.180), we demonstrated the efficacy of rituximab on Bcl-2-positive patients when the Cox regression analysis was conducted among the entire group of patients (P = 0.004) and among patients younger than 60 (P = 0.008). Therefore, Bcl-2 overexpression itself is the strongest indication for the addition of rituximab, no matter what type of mechanism is involved. Additionally, these results further validate the survival improvement ability of rituximab in non-GCB subtype patients, which may be exerted by either directly inhibiting the enhanced function of Bcl-2 or inhibiting another pathway that could induce the enhanced function of Bcl-2, such as NF-κB pathway activation.

In conclusion, the addition of rituximab to the CHOP-like regimen can significantly improve the prognosis of non-GCB subtype DLBCL patients. Suppressing the constitutive activation of the NF-κB pathway and/or perturbing Bcl-2-related anti-apoptotic proteins may be responsible. In addition, as shown in our study, not only non-GCB subtype DLBCL patients but also CD10-negative patients, Bcl-6-negative patients, MUM-1-positive patients, and Bcl-2-positive patients can benefit from treatment with rituximab. This result suggests that some GCB subtype patients, such as CD10-positive/Bcl-6-negative/MUM-1-negative patients and CD10-negative/Bcl-6-positive/MUM-1-negative patients, may be qualify for rituximab-based chemotherapy. This theory has been confirmed by preliminary results in the literature showing that not only non-GCB DLBCL patients but also GCB patients may benefit from rituximab-based chemotherapy[36,37]. However, the number of patients with those two expression modes was too small in our study, making further analysis impossible. Studies concerning this issue are needed, which could increase the target population of rituximab-based chemotherapy.

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