

Prognostic analysis of CD5 expression in double-hit diffuse large B-cell lymphoma and effectiveness comparison in patients treated with dose-adjusted EPOCH plus rituximab/R-CHOP regimens

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Objectives: To compare the efficacy of rituximab, dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide and doxorubicin (DA-EPOCH-R) with traditional rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP) regimens in CD5+ double-hit lymphoma (DHL) and to evaluate prognostic factors.

Methods: We retrospectively studied 139 patients with newly diagnosed DHL/THL diffuse large B-cell lymphoma (including 20 cases CD5+ and 119 cases CD5-), 87 cases were MYC/BCL2 DHL, 30 cases were MYC/BCL6 DHL, 22 cases were THL. MYC, BCL2 and BCL6 rearrangements were examined by fluorescence in-situ hybridization. CD5 is detected by immunohistochemistry (IHC).

Results: The objective response rate (ORR) difference between CD5+ and CD5- was significant (80.0% vs 63.8%, $P=0.003$). The median follow-up time was 18 months (range: 4–39 months). Progression-free survival (PFS) of CD5+ group was significantly worse than that of CD5- (28.1% vs 59.0%, $P=0.028$), while no significant difference was observed in overall survival (OS) (32.1% vs 59.9%, $P=0.057$). Compared with the two regimens, the 2-year survival rate of DA-EPOCH-R group was significantly superior than that of R-CHOP (63.6% vs 45.4%, $P=0.034$ for PFS; 67.4% vs 47.8%, $P=0.038$ for OS). Besides, CD5+ patients receiving DA-EPOCH-R had survival benefits compared with R-CHOP in PFS (85.7% vs 23.0%, $P=0.029$), but there was no statistical difference in OS (87.7% vs 34.4.0%, $P=0.064$). However, in DA-EPOCH-R protocol, there was no significant difference between CD5+ DHL (MYC/BCL2 and MYC/BCL6) and triple-hit lymphoma ($P=0.776$ for PFS; $P=0.728$ for OS). Multivariate analysis showed that CD5+ treatment regimen and disease stage were independent prognostic factors.

Conclusion: Our retrospective study shows that CD5+ has a poorer prognosis than CD5- patients. Based on its improved lifetime and good tolerance on CD5+ patients, which is expected to become the first-line treatment for high-risk DLBCL types based on more clinical research.

Keywords: CD5, DA-EPOCH-R, R-CHOP, diffuse large B-cell lymphoma, double-hit, treatment, prognosis

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Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma, which represents approximately ~40% of all cases.^{1,2} The category of DLBCL represents a heterogeneous group of neoplasms, different subsets of DLBCL

have a different underlying disease biology explaining differences in prognosis.^{3,4} In the rituximab time, R-CHOP regimen has greatly improved the survival rate of DLBCL patients, approximately 60% of patients with DLBCL are cured.⁵ However, there are still some high-risk DLBCL patients who show poor prognosis after receiving standard R-CHOP chemotherapy, with a less than 50% of 5-year survival rate.⁶ The 2016 WHO classification of the lymphoid hematopoietic system clearly defined the concurrent translocation of the MYC and BCL2/BCL6 genes as double-hit lymphoma (DHL).⁷ The result of DHL patients treated with R-CHOP regimen is poor, especially those with aggressive prognosis factors.⁸ Rituximab with DA-EPOCH (DA-EPOCH-R) has been shown to be effective in the treatment of DHL patients, and superior PFS has been reported in comparison with R-CHOP protocol.^{9–11}

CD5-positive (CD5+) DLBCL accounts for approximately 5–10% of all DLBCL,^{1,4,5} patients with CD5+ have distinctive clinic features including higher international prognostic index (IPI), higher frequency of extranodal sites involvement, easy central nervous system (CNS) involvement and relapse when compared with patients with CD5-negative DLBCL.^{12–16} Patients with CD5-positive DHL-DLBCL are rare and have poorer OS when treated with CHOP or R-CHOP regimens.^{17–20} R-EPOCH is a dose-adjusted infusional regimen that has shown improved outcome (versus R-CHOP) in untreated patients with aggressive and high-risk DLBCL.^{10,21,22} However, the effect in patients with CD5+ DHL-DLBCL is rarely reported.

In this study, we compared the survival outcome in CD5+ with CD5-negative DHL-DLBCL patients as well as the prognostic significance of CD5 expression in DHL-DLBCL patients treated with DA-EPOCH-R and R-CHOP, the purpose is to evaluate whether DA-EPOCH-R regimen is better than R-CHOP in CD5+ DHL-DLBCL patients.

Materials and methods

Patients selection

We collected 718 cases of newly diagnosed DLBCL patients who underwent fluorescence in-situ hybridization (FISH) detection from June 2015 to August 2018 in the database of First Affiliated Hospital of Zhengzhou University. One hundred and thirty-nine patients were conformed the DHL/THL diagnostic criteria (including 20 cases CD5+ and 119 cases CD5-); among them, 87

cases were MYC/BCL2 DHL, 30 cases were MYC/BCL6 DHL and 22 cases were MYC/BCL2/BCL6 THL. Patients were identified by at least three lymphoma pathologists in our hospital. Patients with primary mediastinal DLBCL, primary cutaneous DLBCL, lymphomatoid granulomatosis, T-cell/histiocyte-rich large B-cell lymphoma, plasmablastic lymphoma, small mature B-cell lymphoma and primary CNS lymphoma were excluded. The baseline clinical characteristics included age, gender, Ann Arbor stage, IPI score, serum lactate dehydrogenase (LDH) level, serum B2M level, extranodal sites involvement, COO subtype and CNS positive at involvement. The genetic abnormality of MYC and BCL2/BCL6 was detected by FISH. The Hans classification was used to analyze the cell of origin subtype, positive detection of CD antigen by immunohistochemistry.

Immunohistochemistry

Immunohistochemical studies were performed using formalin-fixed, paraffin-embedded (FFPE) tissue sections. The following panel of antibodies used was as follows: CD3, CD20, CD5, CD10, BCL-2, BCL-6, MUM1, FOXP-1, cyclin D1, SOX-11 and Ki-67. Cutoffs $\geq 50\%$ tumor cells with a strong expression for CD5 were considered positive. The cutoffs for positivity were $\geq 30\%$ for CD10, BCL-6 and MUM-1 to distinguish these tumors as germinal center or non-germinal center cell-like immunophenotype (Hans algorithm).²³ According to Visco-Young algorithm, the positive cutoff for FOXP1 was $\geq 60\%$.²⁴ The cutoffs for positive MYC or BCL-2 results were $\geq 40\%$ and $\geq 50\%$, respectively, as reported previously.

Fluorescence in situ hybridization

FISH was performed using LSI dual-color break-apart probes for MYC and BCL6, and dual-color, dual fusion probe for BCL2/IGH. For biopsy specimens, 4- μ m FFPE tissue sections were used. The signals from 200 nuclei were analyzed. All cutoffs were low ($\leq 5\%$) and all cases positive for rearrangements had a positive signal in $\geq 25\%$ of nuclei examined. Cases were designated as DHL if concurrent MYC and BCL2 or BCL6 were rearranged.

Treatment programs

All patients were treated with R-CHOP or DA-EPOCH-R regimen. The choice of therapy (DA-EPOCH-R versus R-CHOP) for these patients was up to the discretion of the oncology physician and patient's will. The DA-EPOCH-R

regimen was preferred for patients with high-risk clinical or pathologic features.

The R-CHOP regimen includes rituximab (375 mg/m^2) on day 1, followed by CHOP, which consisted of cyclophosphamide 750 mg/m^2 as an intravenous infusion on day 1, vincristine 1.4 mg/m^2 (the maximum dose 2 mg) as an intravenous infusion on day 1, doxorubicin 50 mg/m^2 as an intravenous infusion on day 1 and prednisone 100 mg/m^2 orally on days 1–5. The DA-EPOCH-R regimen comprised rituximab (375 mg/m^2) on day 1, followed by DA-EPOCH. Starting dose of DA-EPOCH composed of etoposide 50 mg/m^2 as a continuous intravenous infusion on days 1–4, vincristine 0.4 mg/m^2 as a continuous intravenous infusion on days 1–4, doxorubicin 10 mg/m^2 as a continuous intravenous infusion on days 1–4, cyclophosphamide 750 mg/m^2 intravenous on day 5 and prednisone 60 mg/m^2 orally on days 1–5. The meaning of the DA-EPOCH-R regimen is that when grade 4 myelosuppression was not reached at the end of the previous cycle of treatment, 20% of the original dose was increased in the subsequent cycles until patient got grade 4 myelosuppression. The cycle of both regimens was 21 days. All patients underwent blood tests before chemotherapy to rule out chemotherapy contraindications. Chemotherapy-related adverse reactions were graded according to the WHO criteria for cancer treatment results,²⁵ granulocyte colony-stimulating factor (G-CSF) was given when patients suffered from leukopenia or neutropenia and recombinant human thrombopoietin was applied for thrombocytopenia symptom. When necessary, the infusion of hemoglobin can be carried out. Symptomatic supportive care was given for non-hematological toxicities. Clinical information on patients meeting the inclusion criteria was obtained from the medical records from the First Affiliated Hospital of Zhengzhou University. Informed consent for the collection of medical information was obtained from all patients. This study was approved by the ethics committee of the First Affiliated Hospital of Zhengzhou University. This study was conducted in accordance with the Declaration of Helsinki.

Adverse reactions evaluation and follow-up

Efficacy was evaluated after completion of every two cycles of chemotherapy, and the final evaluation was conducted after completion of the last cycle chemotherapy 1 month later. 18F-FDG PET-CT scan was generally used to evaluate the standard uptake value of the lesion before treatment, and CT scan was used to evaluate the efficacy

of every 2 cycles. Evaluation of adverse reactions was implemented during each cycle. The responses were assessed in the light of modified Cheson criteria.^{26,27} Complete response (CR) was defined as the disappearance of all lesions for at least 4 weeks, including clinical indicators, laboratory and radiological findings. Partial response (PR) was an indicator of all clinical and imaging baseline abnormalities with a reduction of at least 50% and without new lesions. Stable disease (SD) means less than 50% of tumor shrinkage or less than 25% of tumor growth, at least 4 weeks. Progressive disease (PD) was defined as a greater than 25% increase in the tumour lesions or the appearance of new lesions. According to the WHO adverse reaction evaluation criteria,²⁵ toxicities were evaluated at each cycle from the first administration day to 1 month after completion of treatment. The principle of follow-up is visiting patients every 3 months within 1 year after the end of treatment, every semi-annual for the next 2 years and then annual follow-up until relapse.

Research end points and statistical analysis

The primary study end points were PFS and OS. OS was calculated from the date of diagnosis to the time of death or last follow-up. PFS was computed from the date of diagnosis to the time of last follow-up or a treatment failure, relapse, death. The second study end points were ORR. Statistical analyses were performed with SPSS version 22 and GraphPad Prism7. We used the chi-squared test and Fisher's exact test to compare the categorical variables. Two-year PFS and OS rates were compared between groups using the log-rank test, their 95% CIs were estimated by using the Kaplan–Meier method. Prognostic factors were analyzed using COX proportional hazards regression model. All *P*-values reported were bilateral, and $P < 0.05$ was considered to be statistically significant. Follow-up was completed in March 2019 for analysis.

Results

Patients clinical characteristics

One hundred and thirty-nine patients were diagnosed as DHL/THL-DLBCL by FISH test, 87 cases were MYC/BCL2 DHL, 30 cases were MYC/BCL6 DHL and 22 cases were MYC/BCL2/BCL6 THL. All patient's characteristics are listed in Table 1 and the composition of DHL and THL in two treatment programs is shown in Table 2. Among them, 20 (14.4%) were with CD5+ and 119

Table I Baseline clinical characteristics of patients

Patients' characteristics	Number of patients (%)						
	N=139	DA-EPOCH-R (n=63)	R-CHOP (n=76)	P-value	CD5 +(n=20)	CD5- (n=119)	P-value
Age (years)							
>60		33(52.4)	30 (39.5)	0.350	12(60.0)	51(42.9)	0.065
≤60		30(47.6)	46(60.5)		8(40.0)	68(57.1)	
Gender							
Male		37(58.7)	20(26.3)	0.289	15(75.0)	42(35.3)	0.286
Female		26(41.3)	56(73.7)		5(25.0)	77(64.7)	
Stage at diagnosis							
I-II		27(42.9)	22(28.9)	0.045	12(60.0)	37(31.1)	0.060
III-IV		36(57.1)	54(71.1)		8(40.0)	82(68.9)	
Serum B2M							
Elevated		39(61.9)	16(21.1)	0.052	14(70.0)	41(34.5)	0.182
Normal		24(38.1)	60(78.9)		6(30.0)	78(65.5)	
Serum LDH							
Three times higher than normal		38(60.3)	36(47.4)	0.061	6(30.0)	68(57.1)	0.013
Normal		25(39.7)	40(52.6)		14(70.0)	51(42.9)	
Extranodal disease sites							
0		26(41.3)	26(34.2)	0.347	11(55.0)	41(34.5)	0.327
≥2		37(58.7)	50(65.8)		9(45.0)	78(65.5)	
EBER							
Positive		13(20.6)	6(7.9)	0.690	2(10.0)	17(14.3)	0.482
Negative		50(79.4)	70(92.1)		18(90.0)	102(85.7)	
Bone marrow involvement							
Yes		20(31.7)	18(23.7)	0.093	7(35.0)	31(26.1)	0.051
No		43(68.3)	58(76.3)		13(65.0)	88(73.9)	
IPI							
0-2		21(33.3)	42(55.2)	0.140	10(50.0)	53(44.5)	0.992
3-5		42(66.7)	34(44.8)		10(50.0)	66(55.5)	
COO subtype							
GCB		48(76.2)	47(61.8)	0.073	14(70.0)	81(68.1)	0.078
Non-GCB		15(23.8)	29(38.2)		6(30.0)	38(31.9)	
CNS positive at relapse							
Yes		11(17.5)	15(19.7)	0.302	7(35.0)	19(16.0)	0.042
No		52(82.5)	61(80.3)		13(65.0)	100(84.0)	

Abbreviations: B2M, beta-2 microglobulin; CNS, central nervous system; COO, cell of origin; GCB, germinal centre B-cell; IPI, international prognostic index; LDH, lactate dehydrogenase; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; DA-EPOCH-R, rituximab, etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin.

(85.6%) were with CD5- lymphoma, and 63 (45.3%) were treated with the DA-EPOCH-R regimen and 76 (54.7%) with R-CHOP. The median age was 57 years (18–81 years), and 90 (64.7%) patients had a grade III/IV Ann Arbor stage. Seventy-six patients with 3–5 IPI score

accounted for 63.9%. Seventy-four (62.2%) patients had an elevated serum lactate dehydrogenase level. Eighty-seven (73.1%) patients had two or more extranodal sites involvement. Most clinical features at diagnosis were similar between each groups, including age, gender, serum

Table 2 The composition of DHL and THL in two regimens

	DA-EPOCH-R (n=63)	R-CHOP (n=76)	P-value
CD5+ DHL (MYC/BCL2)	2(3.1)	4(5.3)	0.790
CD5+ DHL (MYC/BCL6)	3(4.8)	3(3.9)	
CD5+ THL (MYC/BCL2/BCL6)	3(4.8)	5(6.6)	0.647

Abbreviations: DHL, double-hit lymphoma; THL, triple-hit lymphoma; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; DA-EPOCH-R, rituximab, etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin.

LDH, clinical stage, IPI score and frequency of involvement of bone marrow, CNS involvement and extranodal sites (Table 1, $P>0.05$). In our study, primary CNS lymphoma was excluded, because the treatment of primary central lymphoma and primary extranodal lymphoma invading the CNS is different. There were 26 patients CNS involved before treatment started, including 7 patients with CD5+, 19 patients with CD5-. CNS involvement was detected by assessing the patient's symptoms, and then we carried out MRI scans of the head and had CSF analysis pretreatment. Central invasion was confirmed based on cerebrospinal fluid flow and cytology. Patients with CNS involvement before treatment were treated with DA-R-EPOCH or R-CHOP combined with CNS intrathecal injection (methotrexate 12 mg, cytarabine 50 mg, dexamethasone 5 mg, 21 days per cycle). One clinical difference was that CD5+ DHL/THL-DLBCL patients showed relatively higher CNS involvement rate than CD5-DLBCL patients (35.0% versus 16.0%, $P=0.042$). Of the 139 patients, 11 patients (9.2%) received transplantation. Eight of 20 CD5+DLBCL patients received autologous stem cell transplant (SCT), five patients as consolidation at first CR and the other three patients as salvage treatment after 2 lines disease relapse. Of these eight patients, six relapsed after SCT and two patients died. Three of 119 CD5-DLBCL patients received allogeneic transplantation after 3 lines of therapy prior to SCT, two patients achieved PR prior to SCT, one had SD, those three patients relapsed after transplant and died of disease.

For the 11 transplanted patients, median time to SCT failure calculated from the time of the first SCT to relapse or death was 5.4 months (95% CI: 2.9–10.1).

Short-term efficacy

The median chemotherapy cycle was 6 (range: 4–8); all 139 patients can be evaluated, including 90 cases CR (64.7%), 13 cases PR (9.4%), 16 cases SD (11.5%) and 20 cases PD (14.4%). The ORR was 74.1%. The chi-squared test was used to compare ORR between two chemotherapy groups, and the results showed that the ORR in DA-EPOCH-R group was significantly higher than in R-CHOP group (84.1% vs 65.8%, $P<0.0001$); similarly, the difference in ORR between CD5+ and CD5- was also significant (80.0% vs 63.8%, $P=0.003$). The results are shown in Table 3.

Long-term efficacy

The median follow-up time was 18 months (range: 4–39 months). PFS of CD5+ group was significantly worse than that of CD5- (28.1% vs 59.0%, $P=0.028$), while no significant difference was observed in OS (32.1% vs 59.9%, $P=0.057$) (Figure 1). At the end of the last follow-up time, 12 (19.0%) patients treated with DA-EPOCH-R regimen and 22 (29.0%) cases treated with R-CHOP died. Compared with the two regimens, the 2-year survival rate of DA-EPOCH-R group was significantly greater than that of R-CHOP (63.6% vs 45.4%, $P=0.034$ for PFS; 67.4% vs 47.8%, $P=0.038$ for OS) (Figure 2). Besides, CD5+ patients receiving DA-EPOCH-R had

Table 3 Response rates of DA-EPOCH-R and R-CHOP regimen

Response	Number of patients (%)					
	DA-EPOCH-R (n=63)	R-CHOP (n=76)	P-value	CD5+(n=20)	CD5- (n=119)	P-value
CR	48(76.2)	42(55.3)	<0.0001	12(60.0)	64(53.7)	0.022
PR	5(7.9)	8(10.5)		4(20.0)	12(10.2)	
SD	4(6.4)	12(15.8)		2(10.0)	17(14.3)	
PD	6(9.5)	14(18.4)		2(10.0)	26(21.8)	
ORR	56(84.1)	50(65.8)	<0.0001	56(80.0)	76(63.8)	0.003

Abbreviations: R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; DA-EPOCH-R, rituximab, etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin; CR, complete response; PR, Partial Response; SD, Stable disease; PD, progressive disease; ORR, overall response rate.

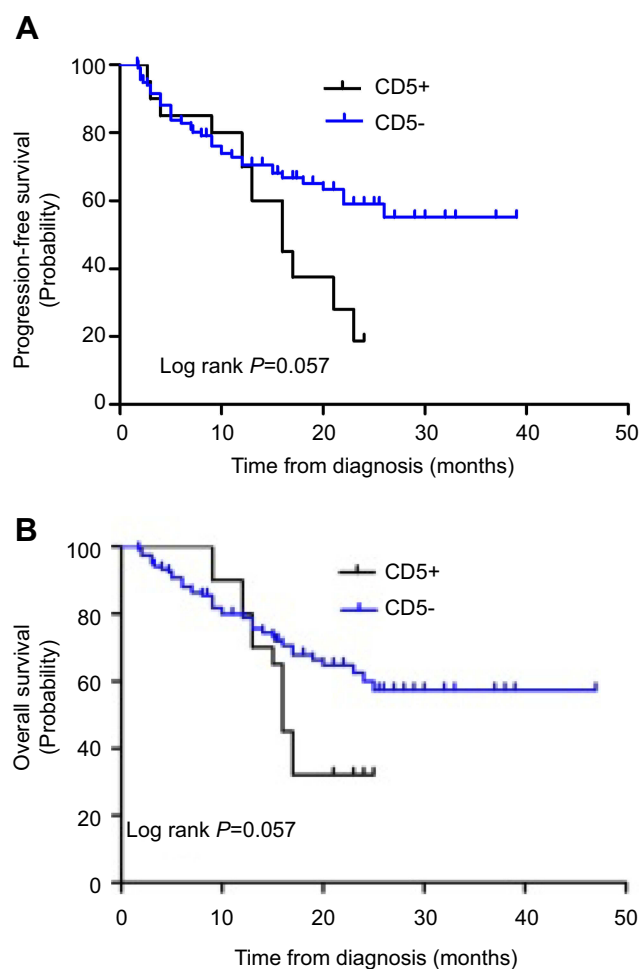


Figure 1 PFS (A) and OS (B) comparing in 20 cases CD5+ patients and 119 cases CD5- patients.

Abbreviations: OS, overall survival; PFS, progression-free survival; DA-EPOCH-R, rituximab, dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone.

survival benefits compared with R-CHOP in PFS (85.7% vs 23.0%, $P=0.029$), but there was no statistical difference in OS (87.7% vs 34.4.0%, $P=0.064$) (Figure 3). However, in DA-EPOCH-R protocol, there was no significant difference between CD5+ DHL (MYC/BCI2 and MYC/BCL6) and triple-hit lymphoma ($P=0.776$ for PFS; $P=0.728$ for OS). Multivariate analysis showed that CD5+ treatment regimen and disease stage were independent prognostic factors.

Univariate and multivariate analysis

Factors predictive of PFS and OS in univariate analysis were entered into multivariate analysis. Multivariate analysis showed that in DHL/THL-DLBCL patients with CD5+, the treatment regimen and disease stage were independent prognostic factors (Table 4). Patients treated with DA-EPOCH-R

regimen had lower risk of death than traditional R-CHOP (PFS:HR 0.09; 95% CI: 0.03–2.74; $P=0.017$; OS:HR 0.82; 95% CI: 0.56–5.26; $P=0.001$). Besides, patients with CD5+ had higher risk of death than patients with CD5- (PFS: HR 1.32; 95% CI:0.09–13.35; $P=0.011$; OS: HR 1.40; 95% CI:0.00–10.89; $P=0.009$).

Adverse effects

Adverse events (AEs) were evaluated in 139 patients, the most frequent AE was hematologic. The rate of grade 3/4 thrombocytopenia, anemia, leukopenia and neutropenia accounted for 36.7%, 38.1%, 25.8% and 28.1% of all patients, respectively. The non-hematological toxicity was mainly presented in 1/2 grade, including digestive tract toxicity, hepatic dysfunction, hypokalemia, cardiac toxicity, neurological toxicity and pulmonary infection. No

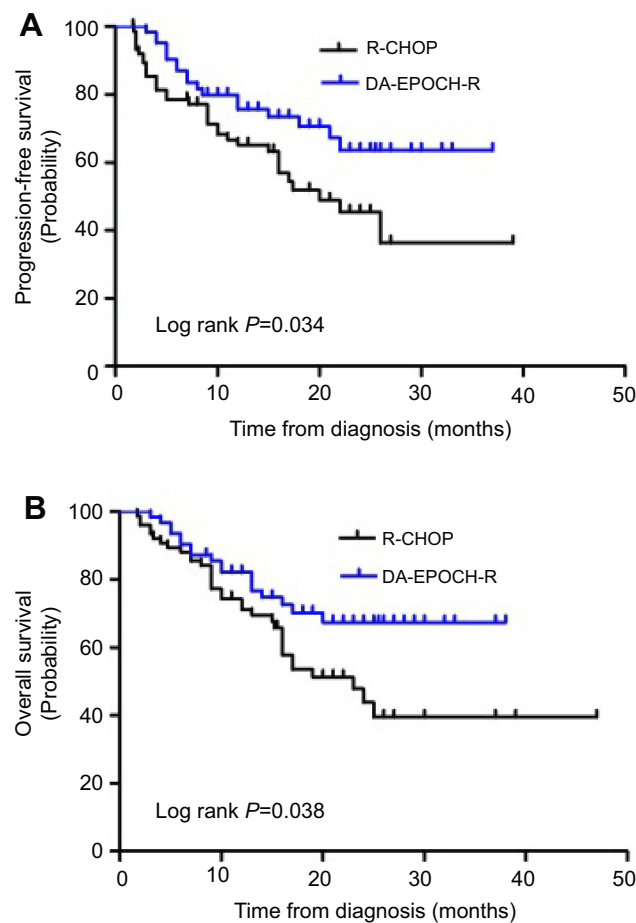


Figure 2 PFS (**A**) and OS (**B**) comparing DA-EPOCH-R and R-CHOP regimen in all patients.

Abbreviations: OS, overall survival; PFS, progression-free survival; DA-EPOCH-R, rituximab, dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone.

chemotherapy-related deaths happened, and the grade 3/4 myelosuppression in DA-EPOCH-R was more frequent than in R-CHOP, but there was no statistical difference ($P>0.05$) (Table 5).

Discussion

CD5-positive DHL-DLBCL is a rare and malignant lymphoma subtype. There is no standard treatment regimens. The innovation of our study is that we compared the therapeutic effects of DA-EPOCH-R and R-CHOP in CD5+ DHL lymphoma. Our study reveals that CD5+ is a poor prognosis factor in patients with DHL-DLBCL, and DA-EPOCH-R can significantly improve the survival time of CD5+ DHL-DLBCL patients compared to the traditional R-CHOP treatment regimen.

CD5+ DLBCL is a rare and aggressive type disease, which accounts for 5–10% of all cases of DLBCL.^{13,17,19} CD5+ patients have particular characters, which include older age,

elevated serum LDH level, advanced clinical stage, extranodal involvement and more frequent CNS relapse.^{28,29} Distinguishing CD5+ DLBCL from CD5-negative DLBCL is very important. At the molecular level, CD5+ DLBCL presents complex chromosomal aberrations and unmutated immunoglobulin heavy chain variable regions which may explain the aggressive course of CD5+ DLBCL.^{30,31} DHL is defined as both MYC and BCL2/BCL6 translocations or rearrangement. There are limited data about the frequency of MYC, BCL2 and BCL6 rearrangement or expression of MYC, BCL2 and BCL6 in CD5+ DLBCL cases, which are associated with poor prognosis in DLBCL patients.^{32,33} In our study, the ORR difference between CD5+ and CD5- was significant (80.0% vs 63.8%, $P=0.003$). PFS of CD5+ group was significantly worse than that of CD5- (28.1% vs 59.0%, $P=0.028$), while no significant difference was observed in OS (32.1% vs 59.9%, $P=0.059$). This may indicate that CD5-positive lymphoma has a poor prognosis.

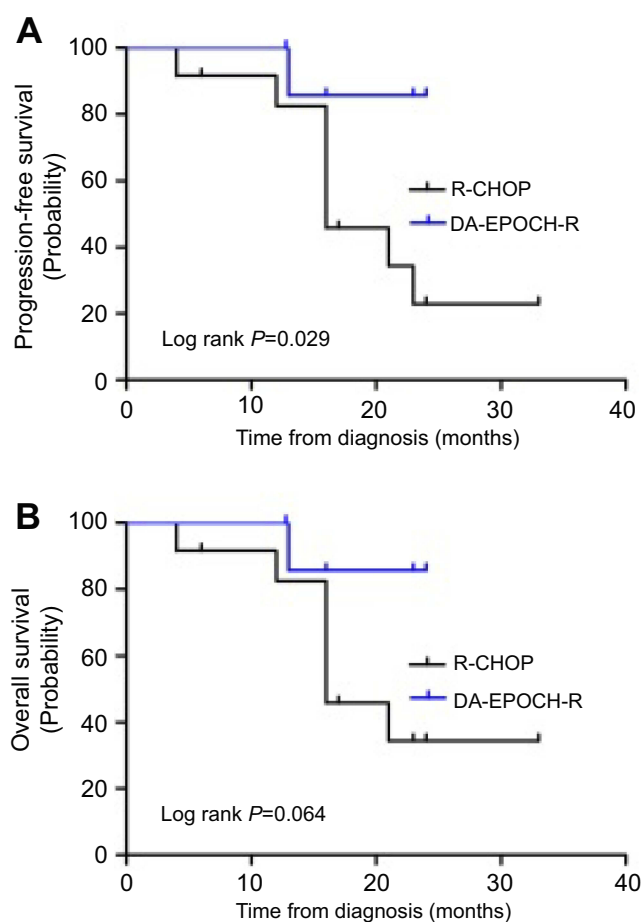


Figure 3 PFS (**A**) and OS (**B**) comparing in DA-EPOCH-R and R-CHOP regimen in 20 cases CD5+ patients.

Abbreviations: OS, overall survival; PFS, progression-free survival; DA-EPOCH-R, rituximab, dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone.

Because of the insufficient evidence for the optimal treatment in CD5+ DLBCL in the era of rituximab, physicians choose treatments based on DLBCL therapy strategy.^{22,24,34} R-EPOCH had shown improved outcome (versus R-CHOP) in untreated patients with aggressive DLBCL, and 5-year time to progression and OS were 81% and 88%, respectively.^{10,21} Recently, a retrospective clinical study obtained only 12.3% of 130 cases CD 5+ DLBCL, and after the front-line R-EPOCH therapy, the median OS was significantly worse in CD5+ than CD5-negative patients (28.13 months vs not reached, $pP=0.006$), and they also concluded that CD5 expression and DHL were independent prognosis factors for OS in multivariate analysis; however, there were only two CD5+ DHL-DLBCL patients.³⁵ Ennishi et al³⁴ showed that CD5 expression was associated with poorer 2-year event-free survival (18% vs 73%) and OS (45% vs 91%, $pP=0.01$) when receiving rituximab plus CHOP therapy. Nitsu et al¹³

reported similar results, and the CR rate and PFS were lower in patients with CD5+ DLBCL than in CD5- DLBCL with the addition of rituximab chemotherapy. Taken together, no study focuses on the prognostic significance of CD5 expression in patients with DHL-DLBCL who received DA-EPOCH-R as front-line therapy. Our study analyzed the effects of R-CHOP and DA-EPOCH-R in the treatment of patients with DHL, and the 2-year survival rate of DA-EPOCH-R group was significantly greater than R-CHOP (63.6% vs 45.4%, $P=0.034$ for PFS; 67.4% vs 47.8%, $P=0.038$ for OS). PFS of CD5+ group was significantly worse than that of CD5- (28.1% vs 59.0%, $P=0.028$), while no significant difference was observed in OS (32.1% vs 59.9%, $P=0.057$). CD5+ patients receiving DA-EPOCH-R had survival benefits compared with R-CHOP in PFS (85.7% vs 23.0%, $P=0.029$), but there was no statistical difference in OS (87.7% vs 34.4.0%, $P=0.064$). To further explore the

Table 4 Univariate and multivariate analysis for prognosis

Parameter	HR	PFS	P-Value	HR	OS	P-Value
		95% CI			95% CI	
Univariable analyses						
Age >60 years	1.87	0.48–5.56	0.633	1.36	0.19–7.65	0.071
Stage (III/IV vs. I/II)	3.61	0.36–10.02	2.041	2.86	0.50–12.45	0.013
ECOG PS (≥2)	0.56	0.34–0.94	0.059	0.46	0.27–0.76	0.053
Elevated LDH	1.27	0.77–2.11	0.348	1.88	0.53–4.46	0.618
Elevated B2M	2.60	0.33 –3.09	0.097	1.43	0.24–5.78	0.066
Extranodal involvement (≥2 sites)	1.45	0.87 –2.42	0.154	0.92	0.55–1.54	0.748
IPI (0–2 vs.3–5)	0.90	0.31–1.01	0.054	0.44	0.24–0.80	0.107
Bone marrow involvement	2.06	0.64 –6.67	0.228	2.26	0.07–6.89	0.333
Non-GCB (versus GCB)	1.08	0.62–1.85	0.793	1.33	0.77–2.32	0.304
Ki67>75% (vs ≤75%)	0.97	0.58–1.61	0.919	1.24	0.74–2.06	0.411
FISH (Translocation)						
Both MYC and BCL2 vs. both MYC and BCL6	0.94	0.49–1.79	0.845	1.55	0.80–3.01	0.194
Treatment						
DA-EPOCH-R versus R-CHOP	0.39	0.23–0.65	0.004	0.49	0.29–0.84	0.008
CD5(positive vs negative)	2.40	0.00–13.31	0.020	1.10	0.08–7.72	0.039
Multivariable analyses						
Stage (III/IV vs. I/II)	1.61	0.36–19.92	0.005	1.86	0.50–31.45	0.002
CD 5(positive vs negative)	1.32	0.09–13.35	0.011	1.40	0.00–10.89	0.009
DA-EPOCH-R versus R-CHOP	0.09	0.03–2.74	0.017	0.82	0.56–5.26	0.001

Abbreviations: B2M, beta-2 microglobulin; GCB, germinal centre B-cell; FISH, fluorescence in-situ hybridization; IPI, international prognostic index; LDH, lactate dehydrogenase; ECOG PS, Eastern Cooperative Oncology Group Performance Score; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; DA-EPOCH-R, rituximab, etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin.

Table 5 Treatment-related adverse reaction between R-CHOP and DA-EPOCH-R regimen

Event	Grade I/II	R-CHOP	Grade III/IV	R-CHOP	P-Value
	DA-EPOCH-R		DA-EPOCH-R		
Hematologic					
Anemia	19(30.2)	35(46.1)	25(39.7)	28(36.8)	0.193
Leukopenia	12(19.5)	22(29.0)	19(30.2)	17(22.4)	0.391
Neutropenia	18(28.6)	37(48.7)	13(20.6)	26(34.2)	0.230
Thrombocytopenia	20(31.7)	19(25.0)	31(49.2)	20(26.3)	0.189
Non-hematologic					
Digestive tract toxicity	22(34.9)	16(21.2)	11(17.5)	4(5.3)	0.420
Hepatic dysfunction	29(46.0)	21(27.6)	16(25.4)	6(7.9)	0.319
Hypokalemia	12(19.0)	6(7.9)	4(6.3)	8(10.5)	0.923
Cardiac toxicity	11(17.5)	5(6.6)	3(4.8)	6(7.9)	
Neurological toxicity	13(20.6)	9(11.8)	6(9.5)	6(7.9)	
Pulmonary infection	12(19.0)	4(5.3)	4(6.3)	0(0)	
Mucosal damage	2(3.2)	0(0)	0(0)	0(0)	

Abbreviations: R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; DA-EPOCH-R, rituximab, etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin.

therapeutic effects of DA-EPOCH-R on CD5+ DHL and CD5+ THL, we separately analyzed the survival of patients with DHL and THL; however, there was no significant difference between CD5+ DHL (MYC/BCL2 and

MYC/BCL6) and triple-hit lymphoma ($P=0.776$ for PFS; $P=0.728$ for OS). Considering the short observation period time and the lesser number of CD5+ DHL-DLCL patients, some bias may exist in analysis, and further

clinical studies are still needed to confirm these findings in the future.

In recent years, SCT has been shown to be an effective therapy compared with salvage chemotherapy for those chemo-sensitive DLBCL patients in the first relapse.^{36,37} CD5+ DLBCLs are a distinct subgroup of DLBCL with poor prognosis. However, there are few reports on the efficacy of salvage or consolidation transplantation in CD5-positive DLBCL patients. A recent retrospective study suggested that stem cell transplantation fails to salvage the majority of CD5-positive patients who received initial rituximab-containing chemotherapy.³⁸ In our research, 11 patients underwent SCT, including eight autologous SCT in CD5+DLBCL patients and three allogeneic transplantations in CD5-DLBCL patients. Of these patients, six relapsed after SCT, two patients died and three patients died after transplant recurrence. For the 11 transplanted patients, median time to SCT failure calculated from time of the first SCT to relapse or death was 5.4 months. Our study suggests that hematopoietic stem cell transplantation has a limited therapeutic effect in patients with CD5+ or CD5- DLBCL, which is similar to other studies. However, due to the lesser number of patients with transplants in this study, we did not specifically compare the value of hematopoietic stem cell transplantation in CD5+ and CD5-DLBCL. It is necessary to further increase the sample size and conduct prospective clinical studies.

The administration of the DA-EPOCH-R regimen is a continuous intravenous infusion, and the adverse reactions are obvious. Our results showed that the grade 3/4 myelosuppression in DA-EPOCH-R was higher than in R-CHOP, but there was no statistical difference ($P>0.05$). These AEs can be dealt with well and returned to normal quickly after given symptomatic treatment, and there were no chemotherapy-related deaths.

Conclusion

Our retrospective research indicates that CD5 expression in DHL-DLBCL is associated with poorer prognosis. Compared with traditional R-CHOP chemotherapy, DA-EPOCH-R is an effective and well-tolerated regimen for CD5+DHL-DLBCL, and it is expected to be the first-line treatment for CD5+ DHL-DLBCL. Besides, evaluation of CD5 expression in DLBCL at the beginning of diagnosis contributes to make stratification. However, due to the potential limitations of single

center, small sample, retrospective analysis, it still needs further prospective, multicenter and large-scale studies to conform those results.

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Disclosure

The authors report no conflicts of interest in this work.

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