Original Article

Shorter Overall Survival in Non-Germinal Center of Diffuse Large B-Cell Lymphoma Based on Hans' Criteria among Thai Patients

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Background: *Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma type in Thailand. Gene expression profiling (GEP) can classify DLBCL by the cell of origin in the germinal center B-cell (GCB) and non-GCB subtypes that differ in prognosis and may lead to different therapy. Nevertheless, GEP is not generally available. Hans et al. has developed a simple and inexpensive immunohistochemistry algorithm that can reliably categorize DLBCL. However, the validation data are still conflicting and no study has been conducted in Thailand.* ***Objective:*** *To compare the overall survival (OS) time of patients who were diagnosed as DLBCL of GCB vs. non-GCB subgroups as classified by immunohistochemistry.* ***Methods:*** *This study employed a retrospective cohort design. Patients with newly diagnosed cases of DLBCL, aged ≥ 18 years, who had adequate remaining tissue pathology for further study from January 2009 to September 2015, were enrolled. Patients with transformed lymphoma, HIV-associated lymphoma, primary mediastinal B-cell lymphoma, primary CNS lymphoma and primary effusion lymphoma were excluded. All were treated by doxorubicin-based chemotherapy. The paraffin-embedded tissue was immunohistochemically stained to determine the cell of origin using Hans' criteria. Demographic data were recorded. All patients were followed for clinical outcome. The Kaplan-Meier method was used to estimate overall and event-free survival distributions.* ***Result:*** *One hundred nineteen patients with DLBCL were investigated. The mean age was 56 ± 14 years with a median follow-up of 29 months. The median OS time for the non-GCB group was 37 months compared with unattained outcome for the GCB group [Hazard ratio (HR) 2.23, 95% confidence interval (CI): 1.10-4.52, p = 0.013]. The 3-year OS for the non-GCB was 49% compared with 74% for the GCB group (p = 0.02, log rank test). Using multivariate analysis, 4 factors were significantly associated with poor OS, i.e., high serum LDH (adjusted HR 2.40, 95%CI: 1.04-5.52, p = 0.04), stage 3-4 (adjusted HR 2.28, 95%CI: 1.06-4.88, p = 0.034), chemotherapy without rituximab (adjusted HR 3.21, 95%CI: 1.71-6.03, p < 0.001) and the non-GCB subtype (adjusted HR 2.08, 95%CI: 1.02-4.27, p = 0.045).* ***Conclusion:*** *The non-GCB subgroup determined using Hans' criteria was independently associated with shorter OS among Thai patients with DLBCL.*

Keywords : ● *Diffuse large B-cell lymphoma* ● *Prognosis* ● *Cell of origin*

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นิพนธ์ต้นฉบับ

ผู้ป่วยไทยที่เป็นมะเร็งต่อมน้ำเหลืองชนิดดิฟฟิวลาจบีเซลล์ ชนิดนอนเจอร์มินัลเซนเตอร์ตามเกณฑ์ของฮานส์ มีอัตราการรอดชีวิตที่สั้น

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ที่มา *Diffuse large B-cell lymphoma (DLBCL) เป็นมะเร็งต่อมน้ำเหลืองที่พบบ่อยที่สุดในประเทศไทย การใช้ gene expression profiling (GEP) สามารถแบ่ง DLBCL ตามจุดกำเนิดของเซลล์คือ germinal center B-cells (GCB) และ non-germinal center B-cells (non-GCB) ทั้งสองกลุ่มมีการพยากรณ์โรคที่แตกต่างกัน อาจนำไปสู่การรักษาที่ต่างกันด้วย แต่ GEP นั้นไม่สามารถทำได้ทั่วไป Hans และคณะ จึงนำ immunohistochemistry ซึ่งทำได้ง่าย ราคาไม่แพงและเชื่อถือได้มาใช้แทน อย่างไรก็ตามความเชื่อถือได้ของวิธีนี้ยังมีความขัดแย้งในบางการศึกษา ทั้งยังไม่เคยมีการศึกษาการพยากรณ์โรคด้วยวิธีนี้ในประเทศไทย* ***วัตถุประสงค์*** *เพื่อเปรียบเทียบอัตราการรอดชีวิต overall survival (OS) ของผู้ป่วย DLBCL ชนิด GCB และ non-GCB ด้วยการตรวจทาง immunohistochemistry ตาม Hans' criteria* ***วิธีการศึกษา*** *Retrospective cohort study ศึกษา DLBCL รายใหม่ ตาม WHO 2008 criteria อายุ ≥ 18 ปี ที่มีชิ้นเนื้อเพียงพอต่อการตรวจทางพยาธิวิทยา ช่วง มกราคม 2552 ถึง กันยายน 2558 เกณฑ์การคัดออกคือ indolent lymphoma ซึ่งมีการเปลี่ยนแปลงไปเป็น large-cell lymphoma, HIV associated lymphoma, primary mediastinal B-cell lymphoma, primary CNS lymphoma และ primary effusion lymphoma โดยผู้ป่วยทุกรายต้องได้รับการรักษาด้วยเคมีบำบัดที่มี Doxorubicin ชิ้นเนื้อได้รับการย้อมทาง immunohistochemistry เพื่อสามารถแยกจุดกำเนิดของเซลล์ ข้อมูลพื้นฐานของผู้ป่วยและผลของการรักษาได้รับการบันทึก Kaplan-Meier curve ถูกใช้เพื่อหาอัตราการรอดชีวิต* ***ผลการศึกษา*** *ผู้ป่วย 119 ราย อายุเฉลี่ย 56 ± 14 ปี มัธยฐานเวลาติดตาม 29 เดือน พบว่า non-GCB มี median OS 37 เดือน เทียบกับ GCB = not reach [Hazard ratio = 2.23 (95%CI: 1.10-4.52), p = 0.013] OS ที่ 3 ปีของ non-GCB เป็นร้อยละ 49 เทียบกับ GCB ร้อยละ 74 (p = 0.02, log rank test) จากการวิเคราะห์พหุตัวแปรมี 4 ปัจจัยที่สัมพันธ์กับการลดลงของอัตราการรอดชีวิตโดยรวม ได้แก่ serum LDH สูง (adjusted HR 2.40, 95%CI: 1.04-5.52, p = 0.04) โรคระยะที่ 3-4 (adjusted HR 2.28, 95%CI: 1.06-4.88, p = 0.034) การไม่ได้รับ rituximab (adjusted HR 3.21, 95%CI: 1.71-6.03, p < 0.001) และกลุ่ม non-GCB (adjusted HR 2.08, 95%CI: 1.02-4.27, p = 0.045)* ***สรุป*** *กลุ่ม non-GCB โดย Hans' criteria เป็นตัวแปรที่สัมพันธ์กับอัตราการรอดชีวิตที่สั้นลง ในผู้ป่วยไทยที่เป็น DLBCL*

คำสำคัญ : ● *Diffuse large B-cell lymphoma* ● *Prognosis* ● *Cell of origin*

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต 2560;27:411-21.

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma (NHL) in Thailand. It comprises approximately 64% of all Thai patients with NHL[1]. Worldwide, the proportion of DLBCL is approximately 30-40% of newly diagnosed NHL[2]. Clinical presentations of DLBCL include enlargement of lymph nodes or extranodal masses, fever, weight loss, night sweating and possible splenomegaly. The most often used staging system of the disease is Ann Arbor classification[3]. However, staging alone has some limitations in prognostication.[4]

Since 1993, the International Non-Hodgkin's Lymphoma Prognostic Factor Project[5] developed the International Prognostic Index (IPI) that is a clinical assessment scoring system for predicting outcome in patients with aggressive NHL before treatment. IPI is calculated by adding one point for each factor present; age greater than 60 years, Ann Arbor stage III or IV disease, elevated serum lactate dehydrogenase (LDH) level, performance status of 2-4, and more than one extranodal involvement.

In the past, investigative efforts were made to find other prognostic factors in addition to IPI. Rosenwald et al.[6] applied the molecular profiling technique with the use of cDNA microarray to classify type of DLBCL in germinal center B-cell-like (GCB), activated B-cell-like (ABC) or type 3 gene expression. This technique provided strong prognostic factors independent of IPI. Unfortunately, limitations for this method include the requirement of fresh frozen tissue (formalin-fixed tissue cannot be used), expensive technique and availability only in few institutes.

Hans et al.[7] developed the tissue microarray (TMA) technique to differentiate the type of DLBCL. This technique is inexpensive and more widely available. Their study compared TMA technique with cDNA microarray classification (gold standard). The sensitivity of TMA was 71% in the GCB and 88% in the non-GCB groups. The positive predictive value of TMA classification was 87% in the GCB and 73% in the non-GCB groups. Furthermore, the 5-year overall survival (OS) was 76% in the GCB and 34% in the non-GCB groups.

Nevertheless, the frequencies of GCB vs non-GCB subtypes, as well as the association with disease prognosis among patients with DLBCL, have not previously been reported in Thailand.

Materials and Methods

Patients

In this retrospective and prospective cohort study, patients with newly diagnosed cases of DLBCL using WHO 2008 criteria, aged ≥ 18 years, who had adequate remaining tissue pathology for further studies at King Chulalongkorn Memorial Hospital from January 2009 to December 2014, were enrolled. The patients with transformed lymphoma, HIV-associated lymphoma, primary mediastinal B-cell lymphoma, primary CNS lymphoma and primary effusion lymphoma were excluded. All patients were treated using doxorubicin-based chemotherapy. The paraffin-embedded tissue was immunohistochemically stained to determine the cell of origin by Hans' criteria.

Methods

Patients with a diagnosis of large cell lymphoma using code C83.3 of the ICD-10 (International classification of diseases, 10th revision) with histopathological confirmation were searched from the hospital medical database. The paraffin-embedded tissue was immunohistochemically stained (CD10, BCL6 and MUM1) to determine the cell of origin using Hans' criteria. All patients were followed for clinical outcomes.[8] Treatment outcome and survival time of the patients were recorded. The definition of OS was the time period from first diagnosis until death or lost follow-up. Progression free survival was defined as the time period from diagnosis to disease recurrence or death, which ever occurred first.

Outcomes

The median overall survival, three-year OS and three-year progression free survival (PFS) of germinal center B-cells (GCB) subtype and non-germinal center B-cells (non-GCB) subtype among patients with DLBCL classified using Hans' criteria were analyzed.

Statistical Analysis

Demographic data were analyzed for frequency or percentage distribution. Comparative data were calculated using Chi-Square or Fisher's exact test. Age of the patient and age comparison were calculated using mean SD and unpaired t-test. Survival Analysis was conducted using Kaplan-Meier methods to calculate OS and PFS. Log-rank testing univariate analysis was used for survival curve comparison. Log-rank test was analyzed for the relationship between variable and overall survival. Multivariate analysis was performed for the relative factors of OS and PFS using Cox regression analysis, enter method. The statistical significance was set at $p < 0.05$ (two-sided). All statistical analyses were performed using SPSS, version 17.0.

Results

Patient Characteristics

From January 2009 to December 2014, 119 patients with large cell lymphoma were investigated. They totaled 58 (48.7%) male patients with a mean age of 56 ± 14 years old (range, 18-81 years). Underlying hypertension and diabetes mellitus were found in 26.1% and 16%, respectively. Applying the International Prognostic Index (IPI/age-adjusted IPI), the frequency of low and low-intermediate risk patients was 46.2%. The high-intermediate risk and high risk group comprised 53.8%. Most patients were at stage 3-4 and had more than one extranodal site and elevated serum LDH level.

The patients were classified in two groups, depending on immunohistochemistry stain to determine the cell of origin. A total of 43 (36%) patients comprised the GCB group and 76 (64%) patients comprised the non-GCB group. The majority, 24 GCB cases (55.82%), had positive CD10 and BCL6. Six GCB cases (13.95%) had only positive CD10 while BCL6 positivity was found in 13 cases (30.23%).

According to patients' characteristics (Table 1, 2), no significant difference was observed between the GCB and non-GCB subtypes with respect to sex, age, underlying disease, serum LDH level, disease stage, extranodal site, performance status, IPI and chemotherapy regimen.

The OS of GCB and non-GCB groups

At the time of analysis, 44 patients (37%) had died from lymphoma, and 75 (63%) were still alive. The median follow-up time was 29 months. The median OS of the non-GCB group was lower than the GCB group (37 months vs unattained, HR = 2.23, 95%CI: 1.10-4.52, $p = 0.013$). The OS of the non-GCB group was significantly shorter than that of the GCB group (3-year OS, 49% vs 74%, $p = 0.02$, log rank test) as shown in Figure 1.

The result of Cox multivariate proportional hazards analysis revealed the independent prognostic factors for OS among patients with DLBCL comprised high serum LDH (adjusted HR 2.40, 95%CI: 1.04-5.52, $p = 0.040$), stage 3-4 (adjusted HR 2.28, 95%CI: 1.06-4.88, $p = 0.034$), chemotherapy without rituximab (adjusted HR 3.21, 95%CI: 1.71-6.03, $p < 0.001$) and non-GCB status (adjusted HR 2.08, 95%CI: 1.02-4.27, $p = 0.045$).

The PFS of GCB and non-GCB groups

At the median follow-up time of 29 months, the median PFS of the non-GCB group was shorter than that of the GCB group (37 months vs. unattained, HR = 1.99, 95%CI: 1.04-3.83, $p = 0.019$). The 3-year PFS of the non-GCB group was shorter than the GCB group (48% vs 72%, $p = 0.033$, log rank test) as shown in Figure 1.

Using Cox multivariate proportional hazards analysis, the independent prognostic factors for PFS among patients with DLBCL included high serum LDH (adjusted HR 2.21, 95%CI: 1.01-4.85, p = 0.047), stage 3-4 (adjusted HR 2.16, 95%CI: 1.05-4.47, p = 0.038), chemotherapy without rituximab (adjusted HR 3.20, 95%CI: 1.74-5.87, p < 0.001) and ECOG 2-4 (adjusted HR 1.90, 95%CI: 1.02-3.53, p = 0.043).

Table 1 Characteristics of diffuse large B-cell lymphoma patients in the study groups, classified by cell of origin

Category	N (%)		*p*-value
	GCB (n = 43)	Non-GCB (n = 76)	
Sex			0.246
Male	24 (55.8)	34 (44.7)	
Female	19 (44.2)	42 (55.3)	
Age (year); mean (SD)	53 (15)	58 (13)	0.068
Underlying disease			
Cardiovascular disease	0 (0.0)	5 (6.6)	0.158
Hypertension	11 (25.6)	20 (26.3)	0.930
Diabetes mellitus	6 (14.0)	13 (17.1)	0.652
Kidney disease	1 (2.3)	0 (0.0)	0.361
Serum LDH			0.353
Normal	16 (37.2)	22 (28.9)	
High	27 (62.8)	54 (71.1)	
Stage			0.076
1	1 (2.3)	11 (14.5)	
2	19 (44.2)	22 (28.9)	
3	12 (27.9)	17 (22.4)	
4	11 (25.6)	26 (34.2)	
Extranodal site			0.741
0	13 (30.2)	28 (36.8)	
≤ 1	23 (53.5)	38 (50.0)	
> 1	7 (16.3)	10 (13.2)	
ECOG			0.130
ECOG 0	22 (51.2)	25 (32.9)	
ECOG 1	8 (18.6)	22 (28.9)	
ECOG 2	9 (20.9)	13 (17.1)	
ECOG 3	4 (9.3)	11 (14.5)	
ECOG 4	0 (0.0)	5 (6.6)	
IPI / aaIPI			0.256
Low risk	9 (20.9)	14 (18.4)	
Low-intermediate risk	13 (30.2)	19 (25.0)	
High-intermediate risk	15 (34.9)	20 (26.3)	
High risk	6 (14.0)	23 (30.3)	
Chemotherapy regimen			0.658
Control group	18 (41.9)	35 (46.1)	
Rituximab group	25 (58.1)	41 (53.9)	

Table 2 Characteristics of diffuse large B-cell lymphoma patients in the study groups, classified by with or without rituximab treatment

Category	N (%)		*p*-value
	No Rituximab (n = 53)	Rituximab (n = 66)	
Sex			0.296
Male	23 (43.4)	35 (53.0)	
Female	30 (56.6)	31 (47.0)	
Age (year); mean (SD)	59 (14)	55 (14)	0.116
Underlying disease			
Cardiovascular disease	2 (3.8)	3 (4.5)	1.000
Hypertension	12 (22.6)	19 (28.8)	0.448
Diabetes mellitus	4 (7.5)	15 (22.7)	0.025
Kidney disease	0 (0.0)	1 (1.5)	0.368
Serum LDH			0.247
Normal	14 (26.4)	24 (36.4)	
High	39 (73.6)	42 (63.6)	
Stage			0.971
1	5 (9.4)	7 (10.6)	
2	19 (35.8)	22 (33.3)	
3	12 (22.6)	17 (25.8)	
4	17 (32.1)	20 (30.3)	
Extranodal site			0.484
0	21 (39.6)	20 (30.3)	
≤ 1	24 (45.3)	37 (56.1)	
> 1	8 (15.1)	9 (13.6)	
ECOG			0.055
ECOG 0	17 (32.1)	30 (45.5)	
ECOG 1	16 (30.2)	14 (21.2)	
ECOG 2	10 (18.9)	12 (18.2)	
ECOG 3	5 (9.4)	10 (15.2)	
ECOG 4	5 (9.4)	0 (0.0)	
IPI / aaIPI			0.490
Low risk	7 (13.2)	16 (24.2)	
Low-intermediate risk	16 (30.2)	16 (24.2)	
High-intermediate risk	16 (30.2)	19 (28.8)	
High risk	14 (26.4)	15 (22.7)	
Cell of origin			0.658
GCB	18 (34.0)	25 (37.9)	
Non-GCB	35 (66.0)	41 (62.1)	

Abbreviations: Non-GCB, non-germinal center B-cell-like; GCB, germinal center B-cell-like; LDH, lactate dehydrogenase; ECOG, Eastern Cooperative Oncology Group Performance Status; IPI, International Prognostic Index; aaIPI, age-adjusted International Prognostic Index

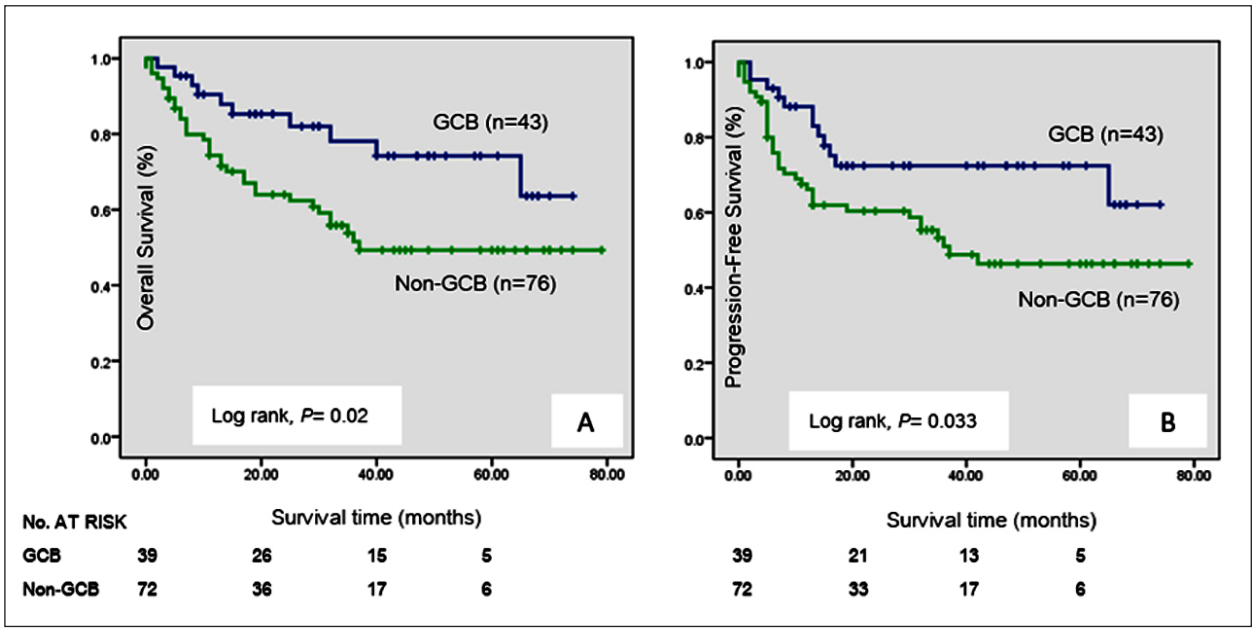

Figure1 Overall survival (**A**) and progression-free survival (**B**) of patients in the GCB and non-GCB groups

The OS and PFS between the rituximab and no rituximab groups

The comparison of survival analysis between patients treated with rituximab (rituximab group) and without rituximab (no rituximab group) showed that the group without rituximab had a shorter median OS than that of the rituximab group (36 months vs unattained, HR = 2.97, 95%CI: 1.59-5.53, $p < 0.001$). The three-year OS of the no rituximab group was shorter than that of the rituximab group (37% vs 72%, $p < 0.001$, log rank test). The median PFS of the no rituximab group was shorter than that of the rituximab group (32 months vs unattained, HR =1.99, 95%CI: 1.04-3.83, $p < 0.001$), The three-year PFS of the no rituximab group was shorter than that of the rituximab group (33% vs 74%, $p <0.001$, log rank test) as shown in Figure 2.

The combination of rituximab with standard chemotherapy improved the survival of patients in both the GCB and non-GCB groups

Patients in the GCB group, who received rituximab plus standard chemotherapy (rituximab group) had a significantly better survival than those treated with standard chemotherapy alone (3-yr OS, 85% vs 57%, $p = 0.032$; 3-year PFS, 87% vs 50%, $p = 0.003$, log rank test) as shown in Figure 3.

Using Cox multivariate proportional hazards analysis, the independent prognostic factors for OS among patients in the GCB group included no rituximab treatment (adjusted HR 4.97, 95%CI: 1.19-20.72, $p = 0.028$) and extranodal site > 1 site (adjusted HR 6.71, 95%CI: 1.71-26.33, $p = 0.006$). The independent prognostic factors for PFS among patients in the non-GCB group included the no rituximab treatment (adjusted HR 5.82, 95%CI: 1.53-22.10, $p = 0.010$) and extranodal site > 1 site (adjusted HR 3.63, 95%CI: 1.09-12.13, $p = 0.036$) as shown in Table 3.

Similarly, patients in the GCB group, who received rituximab plus standard chemotherapy (rituximab group) had a significantly better survival than that of those treated with standard chemotherapy alone (3-yr OS, 64% vs 28% hazards analysis). The independent prognostic factors for OS among patients in the non-GCB group included no rituximab treatment (adjusted HR 2.903, 95%CI: 1.434-5.874, $p = 0.003$), stage 3-4

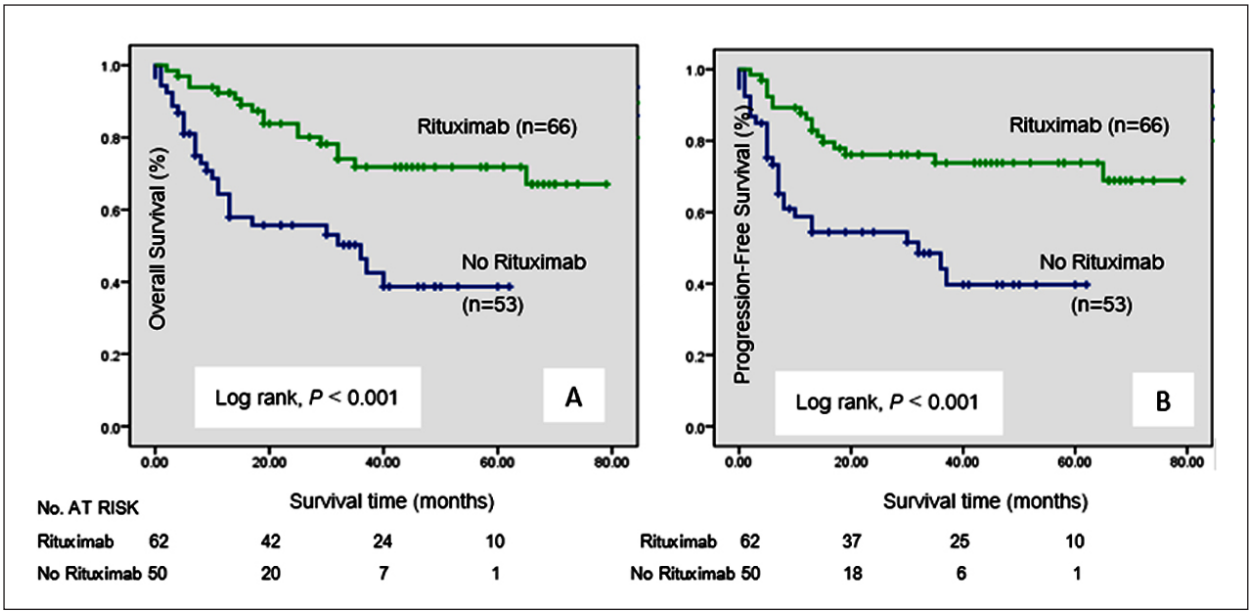

Figure2 Kaplan-Meier survival curves show overall survival (**A**) and progression free survival (**B**) among patients with DLBCL were compared between the rituximab and no rituximab groups

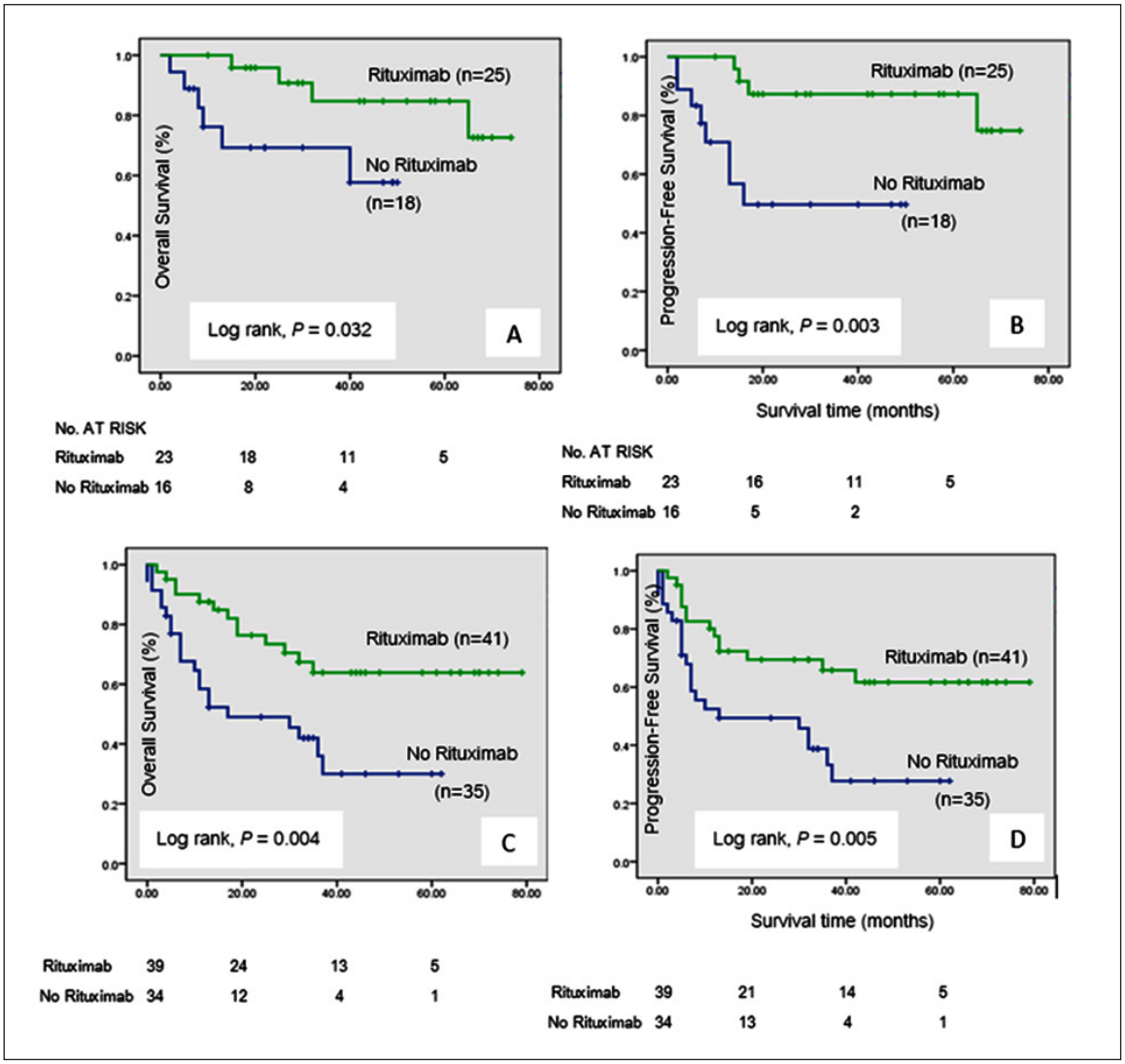

Figure 3 Kaplan-Meier survival curves show OS (**A**) and PFS (**B**) of the GCB group and OS (**C**) PFS (**D**) of the non-GCB group

Table 3 Multivariate hazard analysis in the GCB subgroup

Factor	Overall survival				Progression free survival			
	Univariable Model		Multivariable Model		Univariable Model		Multivariable Model	
	Unadjusted HR (95%CI)	p	Adjusted HR (95%CI)	p	Unadjusted HR (95%CI)	p	Adjusted HR (95%CI)	p
Age (> 60 vs ≤ 60)	0.67 (0.14, 3.18)	0.612	-	-	0.75 (0.20, 2.77)	0.661	-	-
LDH level (High vs Normal)	3.48 (0.73, 16.66)	0.119	-	-	3.81 (0.83, 17.53)	0.086	-	-
Stage (3-4 vs 1-2)	2.46 (0.63, 9.65)	0.197	-	-	1.80 (0.54, 5.98)	0.337	-	-
Extranodal site (> 1 vs ≤ 1)	5.34 (1.46, 19.56)	0.011	6.71 (1.71, 26.33)	0.006	3.63 (1.08, 12.18)	0.037	3.63 (1.09, 12.13)	0.036
ECOG (2-4 vs 0-1)	1.51 (0.43, 5.36)	0.524	-	-	1.77 (0.56, 5.58)	0.330	-	-
Chemotherapy (No Rituximab vs. Rituximab)	4.09 (1.02, 16.48)	0.047	4.97 (1.19, 20.72)	0.028	5.82 (1.53, 22.17)	0.010	5.82 (1.53, 22.10)	0.010

Abbreviations: Non-GCB, non-germinal center B-cell-like; GCB, germinal center B-cell-like; LDH, lactate dehydrogenase; ECOG, Eastern Cooperative Oncology Group Performance Status; OS, overall survival; PFS, progression-free survival; NS, nonsignificant

Table 4 Multivariate hazard analysis in the non-GCB subgroup

Factor	Overall survival				Progression free survival			
	Univariable Model		Multivariable Model		Univariable Model		Multivariable Model	
	Unadjusted HR (95%CI)	p	Adjusted HR (95% CI)	p	Unadjusted HR (95%CI)	p	Adjusted HR (95%CI)	p
Age (> 60 vs ≤ 60)	1.33 (0.68, 2.60)	0.408	-	-	1.32 (0.68, 2.53)	0.411	-	-
LDH level (High vs Normal)	3.17 (1.23, 8.20)	0.017	2.16 (0.81, 5.79)	0.124	2.76 (1.15, 6.63)	0.024	1.79 (0.71, 4.52)	0.222
Stage (3-4 vs 1-2)	3.76 (1.69, 8.33)	0.001	2.38 (1.01, 5.63)	0.047	4.029 (1.83, 8.88)	0.001	2.44 (1.01, 5.88)	0.047
Extranodal site (> 1 vs ≤ 1)	2.32 (0.95, 5.64)	0.063	-	-	2.414 (1.05, 5.55)	0.038	1.32 (0.55, 3.21)	0.537
ECOG (2-4 vs 0-1)	3.15 (1.59, 6.24)	0.001	2.13 (1.03, 4.38)	0.041	3.447 (1.76, 6.74)	< 0.001	2.12 (1.03, 4.35)	0.041
Chemotherapy (No Rituximab vs Rituximab)	2.64 (1.32, 5.29)	0.006	2.90 (1.43, 5.87)	0.003	2.53 (1.29, 4.97)	0.007	2.55 (1.28, 5.08)	0.008

Abbreviations: Non-GCB, non-germinal center B-cell-like; GCB, germinal center B-cell-like; LDH, lactate dehydrogenase; ECOG, Eastern Cooperative Oncology Group Performance Status; OS, overall survival; PFS, progression-free survival; NS, nonsignificant

(adjusted HR 2.38, 95%CI: 1.01-5.63, p = 0.047) and ECOG 2-4 (adjusted HR 2.13, 95%CI: 1.03-4.38, p = 0.041). The independent prognostic factors for PFS among patients in the non-GCB group included no rituximab treatment (adjusted HR 2.55, 95%CI: 1.28-5.08, p = 0.008), stage 3-4 (adjusted HR 2.44, 95%CI: 1.01-5.88, p = 0.047) and ECOG 2-4 (adjusted HR 2.12, 95%CI: 1.03-4.35, p = 0.041) as shown in Table 4.

The OS and PFS among patients with DLBCL who were treated with rituximab

The clinical features did not significantly differ between the GCB and non-GCB groups (Table 5). The survival analysis of the GCB and non-GCB groups did not significantly differ (3-yr OS, 85% vs 64%, p = 0.135; 3-yr PFS 87% vs 66%, p = 0.074, log rank test) as shown in Figure 4.

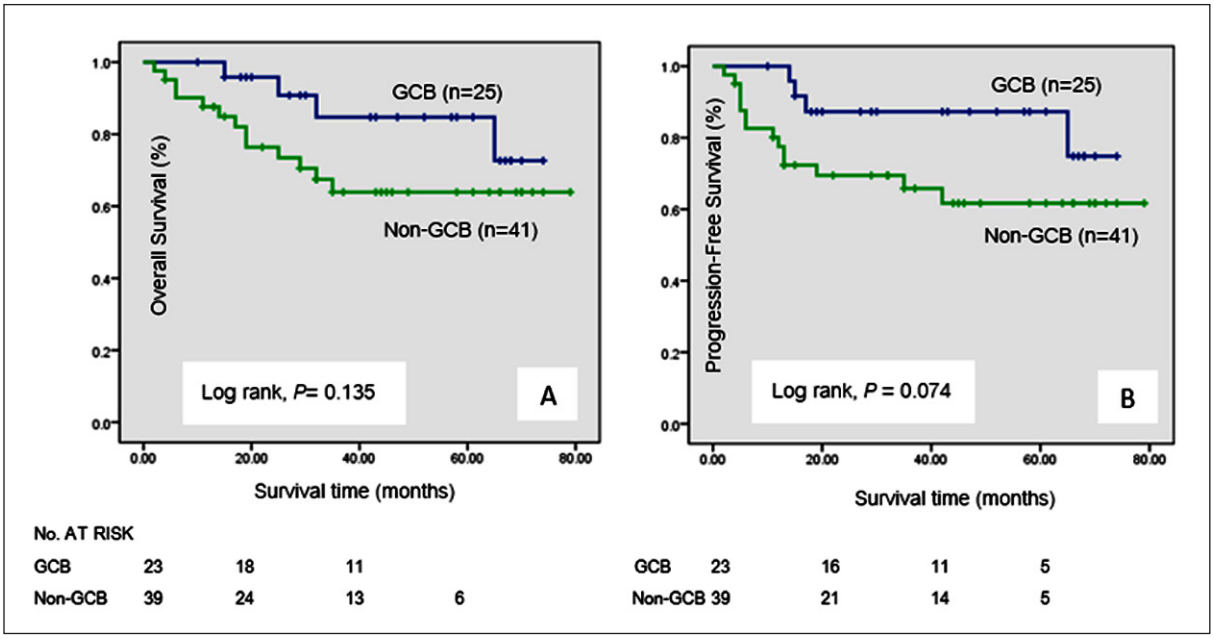

Figure 4 Kaplan-Meier survival curves show OS (A) and PFS (B) in rituximab group divided by cell of origin

Table 5 Clinical features of patients in the rituximab group

Feature	N (%)		p-value
	GCB (n = 25)	non-GCB (n = 41)	
Male	14 (56.0)	21 (51.2)	0.706
Age (years); Mean (SD)	50 (16)	57 (12)	0.050
High serum LDH	14 (56.0)	28 (68.3)	0.314
Stage			0.490
1 - 2	10 (40.0)	19 (46.3)	
3 - 4	15 (60.0)	22 (53.7)	
Extranodal site > 1	4 (16.0)	5 (12.2)	0.669
ECOG			0.620
ECOG0 - 1	18 (72.0)	26 (63.5)	
ECOG2 - 4	7 (28.0)	15 (36.6)	
IPI			0.228
Low risk	7 (28.0)	9 (22.0)	
Low-intermediate risk	5 (20.0)	11 (26.8)	
High-intermediate risk	10 (40.0)	9 (22.0)	
High risk	3 (12.0)	12 (29.3)	

Abbreviations: GCB, germinal center B-cell-like; Non-GCB, non-germinal center B-cell-like; LDH, lactate dehydrogenase; ECOG, Eastern Cooperative Oncology Group Performance Status; IPI, International Prognostic Index; aaIPI, age-adjusted International Prognostic Index

Discussion

DLBCL is a heterogeneous disease displaying different clinical presentation, prognosis and treatment response in each patient. Validated and reliable techniques to subclassify DLBCL subgroups can predict disease prognosis with more certainty and help treatment decision.

This study employed an analytic approach using a retrospective cohort and prospective design among newly diagnosed cases of patients with DLBCL, using Hans' criteria to classify cell of origin in GCB and non-GCB subgroups. The frequency of non-GCB was 64%, higher than that of western populations.

With a relatively long follow-up of 29 months, the non-GCB subgroup shows a shorter OS and PFS compared with the GCB subgroups. Multivariate analysis showed that the cell of origin was a prognostic factor independent of the other known factors, i.e., IPI and rituximab uses.

Compared with other studies, similar results were found for OS and PFS by Hans et al. However, Colomo et al.[9] showed no significant difference in term of response or OS according to immunophenotype.

The data analysis of treatment outcomes using rituximab revealed a significantly higher three-year OS and three-year PFS in the combined rituximab group compared with that of the no rituximab group, which was consistent with previous randomized trials. In 2002, Coiffier et al.[10] (GELA study) reported better treatment responses for both complete and unconfirmed complete response patients with combined rituximab-CHOP than that of CHOP alone (76% vs 63%, $p = 0.005$). Rituximab-CHOP also increased event-free survival and OS (two-year EFS: 57% vs 39%, $p < 0.001$; two-year OS: 70% vs 57%, $p = 0.007$). It has also been reported in a related study by Pfreundschuh et al.[11] (MInT study) in 2006 that patients receiving combined CHOP with rituximab treatment had higher EFS and OS than that of those receiving CHOP alone (three-year EFS: 79% vs 59%, $p < 0.0001$; three-year OS: 93% vs 84%, $p = 0.0001$). Our study was similar to these related international reports.[9,10]

In the present study, combined rituximab treatment in GCB and non-GCB groups could significantly increase OS and PFS in both groups (Table 6).

Regarding the prognosis of the patients classified by cell of origin, the non-GCB subgroup had poorer disease prognosis than that of the GCB group, similar to related reports by G. Lenz et al.[12] and Kai Fu et al.[13]

In the G. Lenz et al.[12] study, 414 patients with newly diagnosed cases of DLBCL underwent GEP test. A total of 233 received R-CHOP. G. Lenz et al. reported significantly higher OS and PFS in the GCB-DLBCL group than that found in the ABC-DLBCL group. In the Kai Fu et al.[13] study, 243 newly diagnosed DLBCL cases were classified in GCB and non-GCB subgroups by immunophenotype using Hans' criteria. From 243 patients, 131 received R-CHOP treatment and 112 received CHOP alone. When analyzing patients receiving R-CHOP treatment, the GCB group had higher three-year OS than that of the non-GCB group (85% vs 69%; $p = 0.032$). However, the present study revealed no significant differences in OS and PFS between GCB and non-GCB subgroups among patients receiving rituximab. This might be due to the relatively lower number of patients in this study.

Table 6 Comparison of OS and PFS between the rituximab and no rituximab groups in GCB and non-GCB patients

Cell of Origin	3-year overall survival			3-year progression free survival		
	Rituximab group (%)	No Rituximab group (%)	p Log rank test	Rituximab group (%)	No Rituximab group (%)	p Log rank test
GCB	85	57	0.032	87	50	0.003
Non-GCB	64	28	0.004	66	25	0.005

The limitations of our study were the retrospective population, comprising 96% of the subjects and the small sample size. Further prospective multicenter studies enrolling a larger number of patients is required.

Conclusion

The majority of patients with DLBCL in King Chulalongkorn Memorial Hospital was classified as non-GCB using Hans' criteria. This subtype was independently associated with shorter OS and PFS. More research is required to improve treatment outcomes in this subgroup of patients.

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