Table 1. Characteristics of patients ($n = 32$) with relapsed or refractory B-cell non-Hodgkin's lymphoma who participated in this study

Characteristic	No.	%
Total	32	100
Median age in years (range)	54 (28–67)	
Male/female	17/15	53/47
Histology		
Indolent	17	53
Follicular grade 1/2	17	53
Aggressive	15	47
DLBCL	11	34
MCL	2	6
Large cell transformation of indolent lymphoma	2	6
ECOG performance status at entry		
0/1	30	94
2	2	6
Stage at entry		
1	5	16
2	5	16
3	5	16
4	17	53
LDH at entry		
Normal	18	56
High	14	44
No. of sites of extranodal involvement		
0	13	41
1	14	44
2 or more	5	16
IPI score at study entry		
1	12	38
2	10	31
3	10	31
No. of prior treatment regimens		
1	22	69
2	4	16
3	3	6
4 or more	3	9
Prior platinum-containing therapy	1	3
Prior rituximab-containing therapy	20	63
Prior radiation therapy	4	13
Prior radioimmunotherapy (^{90}Yttrium-ibritumomab)	1	3
Prior autologous stem cell transplant	2	6
Refractory to last chemotherapy	10	31
Relapsed disease		
Previous remission duration ≤1 year	8	25
Previous remission duration >1 year	14	44

DLBCL, diffuse large B-cell lymphoma; ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index; LDH, lactate dehydrogenase; MCL, mantle cell lymphoma.

25%. One patient who experienced hyperglycemia, which was difficult to control with insulin after the third cycle, received only 12 mg of dexamethasone for the fourth cycle at the discretion of the responsible physician.

Response. The objective response of all 32 evaluable patients is summarized in Table 3. ORR was 84% (95% CI [67–95%]), including CR or CRu in 24 patients (75% [57–89%]), and partial response in three patients (9%). The ORR and CR rates in indolent lymphoma were 100% (84–100%; 17 of 17) and 94% (71–99%; 16 of 17), respectively, and those in aggressive lymphoma were 67% (38–88%; 10 of 15) and 53% (27–79%; 8 of 15), respectively. Response was observed both in patients who previously received rituximab (17 of 20, 85%) and those who did not (10 of 12, 83%) ($P = 0.37$). In patients with aggressive B-NHL with ($n = 8$) or without ($n = 7$) prior rituximab exposure, ORR was 63% and 71%, respectively ($P = 0.39$), and CR rate was 57% and 50%, respectively ($P = 0.38$). The CR rate was higher in patients with longer than 1 year of response duration after last treatment (93%, 13 of 14) than in patients with 1 year or shorter of response duration or refractory disease after last treatment (61%, 11 of 18) ($P = 0.047$). Other factors such as International Prognostic Index (IPI) score at study entry, response to the first treatment were not significantly associated with overall or complete response to CHASER (data not shown). Two patients who had previously undergone autologous SCT also experienced responses (CR and partial response, respectively). Three patients achieved only stable disease and proceeded to different salvage regimens after two, two and four cycles, respectively. Two patients had rapidly progressive disease after one and two cycles, respectively, and eventually received different salvage regimens.

Stem cell collection and SCT. Although not required to enter the study, all patients aged 65 years ($n = 30$) were offered at study entry an option of peripheral blood stem cell harvesting, to be carried out after the second (and third, if necessary) course of CHASER for future SCT. Out of 30 patients, stem cell collection was not attempted in eight patients: three patients with follicular

Table 2. Toxicity observed in patients with relapsed or refractory B-cell non-Hodgkin's lymphoma during salvage chemoimmunotherapy incorporating cyclophosphamide, cytarabine, etoposide, dexamethasone, and rituximab (n = 32)

	Grade 1	Grade 2	Grade 3	Grade 4
Hematologic				
Neutropenia (%)	0 (0)	0 (0)	0 (0)	32 (100)
Thrombocytopenia (%)	0 (0)	0 (0)	4 (13)	28 (88)
Febrile neutropenia (%)	0 (0)	0 (0)	25 (78)	0 (0)
Gastrointestinal				
Nausea/vomiting (%)	9 (28)	4 (13)	0 (0)	0 (0)
Diarrhea (%)	6 (19)	1 (3)	0 (0)	0 (0)
Elevated liver enzymes (%)	14 (44)	4 (13)	2 (6)	0 (0)
Neurological				
Peripheral neuropathy (%)	2 (6)	0 (0)	0 (0)	0 (0)
Syncope (%)	0 (0)	0 (0)	1 (3)	0 (0)
Pain (%)	1 (3)	4 (13)	0 (0)	0 (0)
Edema (%)	4 (13)	0 (0)	0 (0)	0 (0)

Table 3. Responses observed in patients with relapsed or refractory B-cell non-Hodgkin's lymphoma (B-NHL) after treatment with salvage chemoimmunotherapy incorporating cyclophosphamide, cytarabine, etoposide, dexamethasone, and rituximab

Type of B-NHL	Prior treatment	Total no.	CR or Cru	Overall response
Indolent B-NHL	All	n = 17	n = 16 94% (71–99%)	n = 17 100% (84–100%)
	Previous rituximab	n = 12	n = 11 92% (62–99%)	n = 12 100% (78–100%)
	Rituximab-naive	n = 5	n = 5 100% (55–100%)	n = 5 100% (55–100%)
Aggressive B-NHL	All	n = 15	n = 8 53% (27–79%)	n = 10 67% (38–88%)
	Previous rituximab	n = 8	n = 4 50% (16–84%)	n = 5 63% (24–91%)
	Rituximab-naive	n = 7	n = 4 57% (18–90%)	n = 5 71% (29–96%)
Total	All	n = 32	n = 24 75% (57–89%)	n = 27 84% (67–95%)
	Previous rituximab	n = 20	n = 15 75% (51–91%)	n = 17 85% (62–97%)
	Rituximab-naive	n = 12	n = 9 75% (43–95%)	n = 10 83% (52–98%)

Ranges in parentheses indicate 95% confidence interval. CR, complete response; CRu, complete response unconfirmed.

lymphoma declined this option; two patients had undergone autologous SCT prior to CHASER; and three patients had poor control of disease during CHASER (two progressive disease and one stable disease). As a result, stem cell collection was attempted in 22 patients. Three had insufficient mobilization of CD34 positive cells in peripheral blood; one of these patients had had three prior regimens including one cladribine-containing regimen. The remaining 19 patients successfully completed stem cell collection, with a median CD34 count of 4.0×10^6/kg body weight (range 1.9–23.4×10^6) by a median of two rounds of apheresis (range 1–3 rounds). All collected stem cell sources were free of malignant B cells, determined by flow cytometric analyses. In six patients with follicular lymphoma with MBR/JH rearrangement detected by seminested polymerase chain reaction (using primer sets LJH-P, TGAGGAGACGGTGACC and MBR-P, CCAAGTCATGTGCATTTCCACGTC for the first step, and VLJH-P, GTGACCAGGGTNCCTTGGCCCCAG and MBR-P for the second step). Negativity of tumor cell contamination in the stem cell sources was confirmed by the same method (data not shown). Two of 19 patients with aggressive NHL had suboptimal response (stable disease) on imaging studies after CHASER, thus proceeded to other salvage regimens. One patient who had adequate stem cell collection refused to undergo SCT. As a result, a total of 16 patients (50%) underwent autologous SCT as an immediate next treatment after CHASER treatment. One patient who had undergone autologous SCT prior to CHASER underwent allogeneic SCT as an immediate next treatment after CHASER.

TTF and OS. The Kaplan–Meier estimates of TTF and OS are shown in Fig. 1. The median TTF and OS durations for the entire group were 24.5 months and not reached, respectively. The median TTF in patients with indolent and aggressive lymphoma was 24.5 months and not reached, respectively. The median OS duration in patients with indolent and aggressive lymphoma was not reached and 39.3 months, respectively. Neither TTF nor OS duration was significantly different by IPI score at study entry, response duration after last chemotherapy (refractory or ≤1 year *vs* >1 year), previous rituximab exposure, or response to the first treatment (log–rank test, data not shown).

Discussion

Patients with relapsed or refractory NHL have limited options and poor prognosis. Even in patients who might be candidates for autologous SCT, it is critical to reduce the tumor size with

doi: 10.1111/j.1349-7006.2007.00662.x

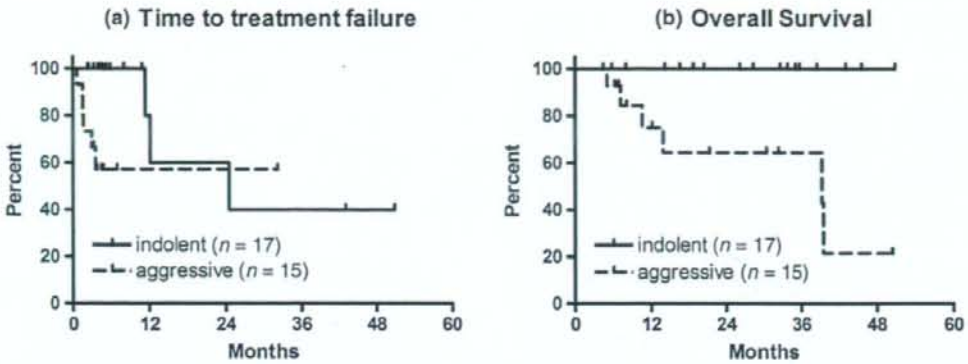

Fig. 1. Overall survival (OS) and time to treatment failure (TTF) in patients with relapsed or refractory B-cell non-Hodgkin's lymphoma undergoing salvage chemoimmunotherapy incorporating cyclophosphamide, cytarabine, etoposide, dexamethasone, and rituximab. Solid lines indicate survival curves of patients with indolent lymphoma ($n = 17$). Dashed lines indicate those of patients with aggressive lymphoma ($n = 15$). (a) The median TTF in patients with indolent and aggressive lymphoma was 24.5 months and not reached, respectively. Those who had stem cell transplant were censored for TTF at the initiation of conditioning regimen. (b) The median OS duration in patients with indolent and aggressive lymphoma was not reached and 39.3 months, respectively.

Table 4. Comparison of CHASER, R-DHAP, R-ESHAP and R-ICE in relapsed or refractory aggressive B-cell non-Hodgkin's lymphoma (doses are per course)

	CHASER	R-DHAP[9]	R-ESHAP[7]	R-ICE[6]
Rituximab	375 mg/m² × 1	375 mg/m² × 1	375 mg/m² weekly × 8	375 mg/m² × 1
Cytarabine	2 g/m² × 2	2 g/m² × 2	2 g/m² × 1	–
Etoposide	100 mg/m² × 3	–	40 mg/m² × 4	100 mg/m² × 3
Steroid	Dexamethasone 40 mg × 3	Dexamethasone 40 mg × 4	Methylprednisolone 500 mg × 5	–
Platinum agent	–	Cisplatin 25 mg/m² × 4	Cisplatin 25 mg/m² × 4	Carboplatin AUC 5 × 1
Non-platinum alkylator	Cyclophosphamide 1200 mg/m² × 1	–	–	Ifosfamide 5 g/m² × 1
No. of patients	15	53	26	36
Prior rituximab exposure (%)	53	4	19†	0
CR rate % (95% CI)	53 (27–79)	32 (20–46)	46 (27–65)	53 (36–69)
OR rate % (95% CI)	67 (38–88)	62 (48–75)	92 (82–100)	78 (61–90)

†L. Hicks *et al.*, 2007, personal communication; –, not included in treatment; AUC, area under the curve; CI, confidence interval; CR, complete response; OR, overall survival.

an effective salvage regimen prior to SCT. For those who are not candidates for transplant, a treatment regimen to induce a durable response is the sole key for long-term survival. The present study showed the significant activity of the new combination salvage regimen CHASER in patients with relapsed or refractory B-NHL who may or may not have undergone prior rituximab-containing treatment such as R-CHOP.

Although rituximab has been studied in salvage settings as an additional drug to commonly used combination chemotherapy, such as ESHAP[7] DHAP[9,10], and ICE (ifosfamide, carboplatin and etoposide)[6,10], currently available data are from studies recruiting mostly rituximab-naive patients (Table 4). Therefore, it remains to be shown whether R-ICE (rituximab with ICE) or R-DHAP is still as effective in patients who were previously treated with a rituximab-containing regimen.[10] It is noteworthy in our study that CHASER produced high CR rates in relapsed or refractory B-NHL after rituximab-containing chemotherapy, and that the activity seems comparable to those of other platinum-containing regimens in patients with aggressive B-NHL (Table 4). Randomized trials would be needed to further compare the efficacy of CHASER with other regimens. Also, careful long-term follow-up is needed to assess the potential late effect of rituximab, such as delayed neutropenia as has recently been recognized.[11–14]

Both CHASER and R-ESHAP contain high-dose cytarabine, etoposide, steroid, and rituximab in common. In the original study of ESHAP, Velasquez *et al.* initially compared ESHA with ESHAP[3], revealing that the addition of cisplatin significantly improved the response rate (33% *vs* 75% at initial phase of the study, but the response rate of ESHAP at the end of the study was 64%), despite only moderate activity of single agent cisplatin against NHL (response rate 26%[15]). Further addition of rituximab to ESHAP seems even more active, and in a phase II study of R-ESHAP in patients with aggressive B-NHL ($n = 26$, 21 were rituximab-naive), a response rate of 92% (95% [CI 84–100%]) including a CR rate of 46% (95% [27–65%]) was observed. CHASER contains 1200 mg/m² of cyclophosphamide instead of cisplatin, producing comparable response rates to R-ESHAP. Virtually all patients with relapsed or refractory B-NHL were exposed to cyclophosphamide at 750 mg/m² as a part of CHOP therapy, however, a higher dose of cyclophosphamide seems to play a significant role in overcoming resistance in this setting. Furthermore, one major benefit of using cyclophosphamide instead of cisplatin is absence of renal toxicity.

One important aspect of salvage regimens for relapsed or refractory NHL is their stem cell mobilizing effect. In our study, 19 of 22 attempts at stem cell collection were successful, but it should be noted that one of three who experienced poor stem cell mobilization had been heavily pretreated. Furthermore, addition of rituximab to the CHASE regimen might add an *in vivo* purging effect and allow tumor-free stem cell collection. Further studies are necessary to determine whether *in vivo* purged autologous SCT will improve outcomes compared to non-purged SCT.

In conclusion, CHASER showed favorable tolerability, significant antitumor activity, and stem cell mobilizing effects in patients with relapsed or refractory B-NHL with or without prior rituximab-containing treatment such as R-CHOP. This promising result warrants the further investigation of CHASER in large-scale multicenter trials and comparison to other salvage regimens.

Acknowledgments

This study was supported in part by Health and Labour Science Grants for Clinical Cancer Research from the Ministry of Health, Labour and Welfare, Japan. We thank Dr Eisei Kondo for his laboratory work.

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doi: 10.1111/j.1349-7006.2007.00662.x

European Journal of Haematology ISSN 0902-4441

ORIGINAL ARTICLE

Low absolute lymphocyte count is a poor prognostic marker in patients with diffuse large B-cell lymphoma and suggests patients' survival benefit from rituximab

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Abstract

Objectives: To evaluate the prognostic value of absolute lymphocyte count (ALC) at diagnosis in patients with diffuse large B-cell lymphoma (DLBCL). *Methods:* In a large cohort of patients with DLBCL treated with CHOP (n = 119) or RCHOP (n = 102) in our institution, we evaluated the prognostic value of ALC at diagnosis with regards to treatment response, overall (OS) and progression-free survival (PFS). Use of rituximab, all International Prognostic Index (IPI) determinants, β2microglobulin level, presence of B symptoms or bulky disease, and ALC were evaluated. *Results:* Low ALC ($<1.0 \times 10^9$/L) was associated with advanced stage, performance status ≥2, elevated lactate dehydrogenase, number of extranodal involvement ≥2, B symptoms, elevated β2microglobulin and higher IPI risk group. Low ALC was associated with lower CR rate by univariate analysis (odds ratio = 3.29, P = 0.024) but not by multivariate analysis. By univariate analysis using Cox proportional hazard model, low ALC was associated with shorter OS [hazard ratio (HR) = 2.89, $P < 0.001$] and PFS (HR = 2.91, $P < 0.001$). Multivariate analysis revealed that low ALC was associated with shorter OS (HR = 2.51, P = 0.003) and PFS (HR = 2.72, $P < 0.001$), independent of above-mentioned parameters. Subclass analyses revealed that the use of rituximab improves OS in patients with low ALC (HR = 0.42, P = 0.05) but not in those with high ALC (HR = 0.83, P = 0.71). This observation was most obvious in patients with higher IPI score. *Conclusion:* Low ALC is a poor prognostic marker in patients with DLBCL and suggests patients' survival benefit from rituximab.

Key words absolute lymphocyte count; diffuse large B-cell lymphoma; prognostic factor; rituximab

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Accepted for publication 14 July 2008

doi:10.1111/j.1600-0609.2008.01129.x

Prognostication of patients with diffuse large B-cell lymphoma (DLBCL) is important in determining optimal treatment approaches. Numbers of prognostic factors have been studied, but some require expensive molecular testing and thus not clinically applicable. Inexpensive and readily available prognostic factors are practical and helpful.

Low absolute lymphocyte count (ALC) at diagnosis is associated with poor prognosis in patients with advanced Hodgkin lymphoma (1) as well as follicular lymphoma (2). A recent preliminary study with short follow-up duration also suggested a potential prognostic value of ALC in DLBCL (3). While International Prognostic Index (IPI) is currently the most valuable prognostic indicator in patients with aggressive lymphoma, ALC was not included in the parameters analyzed (4). We performed a retrospective study evaluating the prognostic value of low ALC using our large cohort of patients with DLBCL, about half treated with CHOP and the rest with RCHOP.

Patients and methods

This retrospective study was approved by the institutional review board. We reviewed 221 consecutive newly

diagnosed patients with non-HIV-associated DLBCL who were treated with CHOP (n = 119; before approval of rituximab) or RCHOP (n = 102; after approval) based therapy at Aichi Cancer Center Hospital between January 1999 and January 2007. Age (≤60 or >60), performance status (PS, ≤1 or ≥2), B symptoms (present or absent), stage (≤2 or ≥3), number of extranodal involvement (≤1 or ≥2), bulky disease (largest diameter of the disease ≥10 cm, present or absent) serum lactate dehydrogenase (LDH) levels (normal or elevated), ALC at diagnosis, IPI group (scored from 0 to 5 by age >60, stage ≥3, PS ≥2, LDH higher than upper limit of normal range and number of extranodal involvement ≥2, and risk groups were classified as low by score 0/1, low-intermediate by score 2, high-intermediate by score 3 and high by score 4/5), initial treatment (CHOP or RCHOP) were collected and incorporated as potential prognostic factors in various analyses. Serum β2microglobulin level was collected if available but excluded from the survival analyses because of many missing data.

The Fisher exact tests were used for the descriptive statistical analyses on categorical data. Overall survival (OS) and progression free survival (PFS, time from diagnosis to disease progression, relapse or death of any cause) were calculated using Kaplan–Meier method (5) and was compared between two groups by log-rank test. Logistic regression models were used to evaluate the associations between multiple characteristics and complete response (CR). Patient characteristics were also analyzed for their association with PFS and OS using Cox proportional hazard models. In this model, characteristics with P-values <0.10 in the univariate analyses were included in the multivariate analyses, and a backward elimination with a P-cutoff of 0.05 was used. All computations were performed in STATA version 9.0 (StataCorp, College Station, TX, USA).

Results

Patient characteristics

Patient characteristics are summarized in Table 1. There was no significant difference in baseline characteristics between CHOP and RCHOP group. In patients with early-stage non-bulky disease, involved field radiation therapy was performed following three courses of CHOP (n = 37) or RCHOP (n = 38) therapy. Patients younger than 65 with age-adjusted IPI score of 2 or 3 were generally offered an option of upfront autologous stem cell transplantation after induction therapy, and 20 such patients (11 after CHOP and nine after RCHOP) underwent this treatment.

The median value of ALC of entire population was 1.20×10^9/L (range 0.10–4.64 $\times 10^9$/L). ALC was significantly higher in IPI low risk (median ALC 1.49×10^9/L), and the values were not significantly different among low-intermediate (median 0.97×10^9/L), high-intermediate (median 0.93×10^9/L) and high-risk (median 0.83×10^9/L) groups (Fig. 1). Low ALC [$< 1.2 \times 10^9$/L (median value)] was associated with advanced stage, PS ≥2, elevated LDH, number of extranodal involvement ≥2, B symptoms, elevated β2microglobulin and higher IPI risk group. Using different cutoff value of ALC (0.8, 1.0 and 1.4×10^9/L) revealed essentially the same result (data using the cutoff value of 1.0×10^9/L are shown in Table 1).

Treatment response

Response to initial treatment was evaluable in 210 patients, among whom CR rate was 91.9%. CR rates in patients with low and high ALC after CHOP were 85.0% (34/40) and 97.3% (72/74), respectively (P = 0.021). Those after RCHOP were 87.5% (35/40) and 92.9% (52/56), respectively (P = 0.483). Univariate analysis using logistic regression model for the chance of achieving CR revealed that elevated LDH, PS ≥2, number of extranodal involvement ≥2 and presence of B symptoms were significantly associated with lower chance of achieving CR. Low ALC [$< 1.2 \times 10^9$/L (median value)] was not significantly associated with low CR rate {odds ratio of low ALC ($< 1.2 \times 10^9$/L) = 2.63 [95% confidence interval (CI) 0.894–7.77], P = 0.079}. Other cutoff values (0.8, 1.0 and 1.4×10^9/L) were also tested in association with CR rate, and the association was significant when cutoff value of 1.0×10^9/L was used [odds ratio of low ALC ($< 1.0 \times 10^9$/L) for low CR rate = 3.29 (95% CI 1.17–9.30), P = 0.024]. The cutoff value of 1.0×10^9/L was also found to be optimal in the survival analyses as shown later. Higher IPI risk group was also associated with lower CR rate [RR = 1.68 (1.11–2.55), P = 0.014]. Multivariate analysis revealed that only PS ≥2 [RR = 5.47 (1.87–16.0), P = 0.002] and elevated LDH [RR = 4.66 (1.25–17.3), P = 0.022] were independently associated with lower CR rate.

Overall survival

The median follow-up duration in the entire population, CHOP and RCHOP groups were 47, 67 and 29 months, respectively. Two-year OS rates in CHOP and RCHOP groups were 82.1 ± 3.6% and 87.0 ± 3.7%, respectively. The Kaplan–Meier OS estimate curves were first plotted according to ALC groups (<0.61, 0.61–0.80, 0.81–1.00, 1.01–1.20, 1.21–1.40, 1.41–1.60 and $> 1.60 \times 10^9$/L) to find the optimal cutoff value to define low and high ALC groups. This revealed that OS was generally longer in patients with higher ALC and curves

Table 1 Patient characteristics

Parameters	*n* (total 221)	ALC < 1.0×10^9/L	*P*-value	Rituximab	*P*-value
All	221	86		102	
Age (yr)					
≤60	106	37	0.270	47	0.686
>60	115	49		55	
Stage					
1/2	136	35	<0.001	62	0.890
3/4	85	51		40	
PS					
0/1	184	62	0.001	85	1.000
≥2	37	24		17	
LDH					
Normal	119	29	<0.001	51	0.344
High	102	57		51	
Number of extranodal involvement					
0/1	177	58	<0.001	83	0.736
≥2	44	28		19	
B symptoms					
Absent	188	65	0.003	84	0.346
Present	33	21		18	
IPI					
Low	117	26	<0.001	50	0.508
Low-intermediate	37	19		20	
High-intermediate	36	21		19	
High	31	20		13	
Bulky disease (≥10 cm)					
No	202	79	1.000	90	0.150
Yes	19	7		12	
Serum β2microglobulin					
<3.0 mg/dL	98	33	0.036	43	0.683
≥3.0 mg/dL	32	18		16	
NA	91	35		43	
Treatment					
CHOP	119	42	0.269	0	–
RCHOP	102	44		102	
ALC					
<1.0×10^9/L	86	86	–	44	0.269
≥1.0×10^9/L	135	0		58	

PS, Eastern Cooperative Oncology Group Performance Status; LDH, serum lactate dehydrogenase level; B symptoms, presence of at least one of the followings – night sweat, weight loss >10% over 6 months and recurrent fever >38.3°C; IPI, International Prognostic Index; ALC, absolute lymphocyte count; NA, not available.
P-values were calculated by Fisher exact test.

were grossly separated at a cutoff value of 1.0×10^9/L (data not shown). To confirm the optimal cutoff values for determining 'low ALC', we next performed sensitivity analysis, where among candidate cutoff values of 0.8, 0.9, 1.0, 1.1, 1.2, 1.3 and 1.4×10^9/L, the maximal hazard ratio (HR) was produced with the cutoff value of 1.0×10^9/L [HR = 2.89 (95% CI 1.61–5.17)]. Low ALC was thus defined to be $< 1.0 \times 10^9$/L for further survival analyses. The Kaplan–Meier OS estimate curves, calculated according to treatment (CHOP and RCHOP) and ALC (high and low) are shown in Fig. 2A. In CHOP group, 2-yr OS rates in patients with high and low ALC were 90.7 ± 3.6% and 66.5 ± 7.3%, respectively. Those in RCHOP group were 92.1 ± 3.8% and 79.8 ± 7.0%, respectively. By univariate analysis using Cox proportional hazard model, low ALC was associated with shorter OS duration in the entire population [HR = 2.89 (1.61–5.17), $P < 0.001$] or in CHOP group [HR = 3.61 (1.81–7.20), $P < 0.001$] but the difference was not significant in RCHOP group [HR = 1.78 (0.599–5.32), $P = 0.298$].

Multivariate analysis for OS incorporating all the characteristics except IPI risk group revealed that PS ≥2 [HR = 3.34 (1.82–6.15), $P < 0.001$], low ALC [HR = 2.51 (1.38–4.58), $P = 0.003$] were independently associated with shorter OS. In this model, rituximab was forced in the analysis [HR = 0.530 (0.276–1.02), $P = 0.057$]. Furthermore, when IPI risk group (analyzed

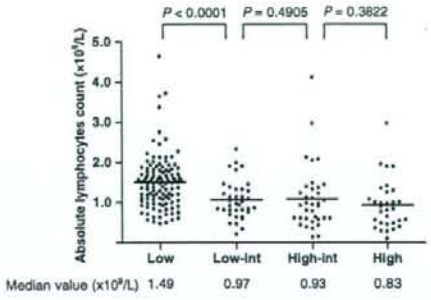

Figure 1 Absolute lymphocyte count according to IPI risk group.) *P*-values were calculated by non-parametric non-paired *t*-test (Man-Whitney test).

as a linear parameter) was incorporated instead of five IPI factors (i.e. age, PS, LDH, stage and number of extranodal involvement were omitted), low ALC was associated with shorter OS [HR = 2.11 (1.12–3.95), $P = 0.019$], along with IPI group [HR 1.50 (1.16–1.92), $P = 0.002$], where rituximab was again forced in the model [HR = 0.531 (0.278–1.02), $P = 0.056$, Table 2]. Removing rituximab from the final model showed the similar result for both analyses. Analyzing IPI risk group as a categorical parameter also showed essentially the same result [HR of low ALC = 2.05 (1.09–3.86), $P = 0.026$, Table 2].

Given that the baseline patient characteristics were similar in CHOP and RCHOP group (Table 1), OS was next compared between CHOP and RCHOP groups, according to ALC group. Use of rituximab was associated with longer OS in low ALC group [HR = 0.42 (0.18–1.00), $P = 0.05$] but not in high ALC group [HR = 0.83 (0.31–2.21), $P = 0.71$]. This suggests that the prognostic significance of ALC became smaller in the era of rituximab, as shown earlier, because the absolute survival benefit from rituximab is larger in low ALC group than in high ALC group (Fig. 2A). To further evaluate the significance of ALC and rituximab use, we next performed subgroup analyses of OS based on IPI risk group (Fig. 2B,C). In this analyses, we defined two IPI risk group [score '0–1' ($n = 117$) and '2–5' ($n = 104$)] because of significantly higher ALC distribution only in '0–1' group (Fig. 1), and limited number of patients in each low-intermediate, high-intermediate and high-risk group. The use of rituximab in patients with IPI '2–5' group with low ALC was associated with longer OS [HR = 0.35 (0.12–0.98), $P = 0.045$], but not in IPI '2–5' with high ALC [HR = 1.02 (0.33–3.13), $P = 0.978$], or in IPI '0–1' with low ALC [HR = 0.83 (0.15–4.44), $P = 0.824$] or in IPI '0–1' with high ALC [HR = 0.42 (0.05–3.67), $P = 0.432$].

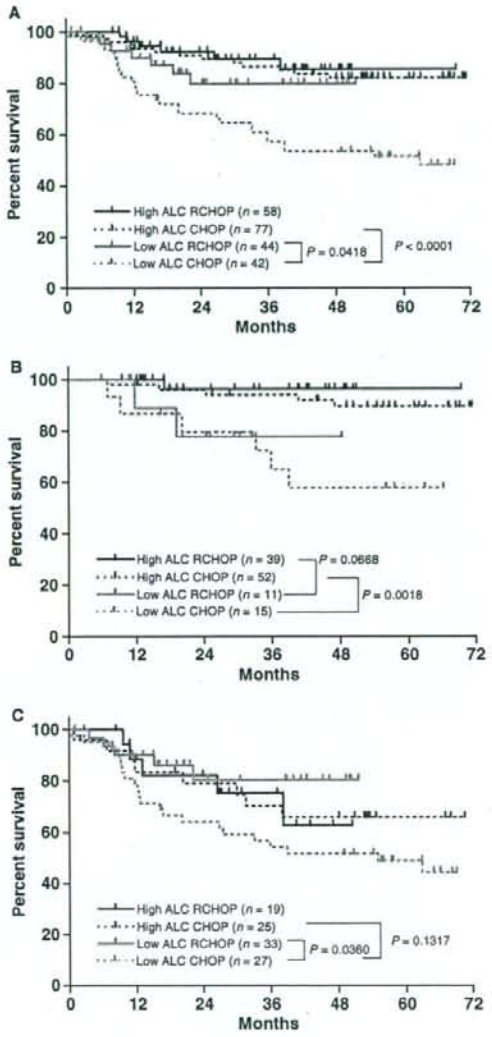

Figure 2 Overall survival according to absolute lymphocyte count and use of rituximab.) (A) All patients; (B) IPI score 0–1; (C) IPI score 2-5. *P*-values were calculated by Log-rank test. *P*-value for any survival comparison was >0.1 if not shown.

Progression free survival

We also performed analyses for PFS. Two-year PFS rates in CHOP group and RCHOP group were 72.8 ± 4.1% and 81.2 ± 4.2%, respectively. In CHOP group, 2-yr PFS rates in high and low ALC groups were 82.8 ± 4.3% and 54.6 ± 7.7%, respectively. In RCHOP group, those were

Table 2 The result of multivariate analyses for OS and PFS when IPI group was analyzed either as a linear parameter or a categorical parameter.

	Hazard ratio	95% CI	*P*-value
For OS			
Low ALC (<1.0 × 10^9/L)	2.11	1.12–3.95	0.019
IPI as a linear parameter	1.50	1.16–1.92	0.002
Rituximab (forced in the model)	0.531	0.278–1.02	0.056
For OS			
Low ALC (<1.0 × 10^9/L)	2.05	1.09–3.86	0.026
IPI			
Low-intermediate vs. low	1.93	0.831–4.49	0.126
High-intermediate vs. low	1.29	0.511–3.27	0.588
High vs. low	3.26	1.19–7.11	0.003
B symptoms	2.37	1.16–4.83	0.018
Rituximab	0.471	0.243–0.915	0.026
For PFS			
Low ALC (<1.0 × 10^9/L)	2.17	1.25–3.76	0.006
IPI as a linear parameter	1.53	1.22–1.91	<0.001
Rituximab	0.452	0.255–0.801	0.007
For PFS			
Low ALC (<1.0 × 10^9/L)	2.22	1.28–3.87	0.005
IPI			
Low-intermediate vs. low	1.52	0.703–3.28	0.287
High-intermediate vs. low	1.72	0.813–3.62	0.156
High vs. low	3.80	1.95–7.44	<0.001
Rituximab	0.457	0.258–0.810	0.007

84.0 ± 5.3% and 77.8 ± 6.6%, respectively. By univariate analysis using Cox proportional hazard model, low ALC was associated with shorter EFS duration in the entire population [HR = 2.91 (1.75–4.86), $P < 0.001$] or in CHOP group [HR = 3.90 (2.12–7.16), $P < 0.001$] but the difference was not significant in RCHOP group [HR = 1.68 (0.647–4.35), $P = 0.287$]. Multivariate analysis for PFS incorporating all characteristics except IPI group revealed that PS ≥2 [HR = 3.40 (1.98–5.83), $P < 0.001$], low ALC [HR = 2.72 (1.61–4.60), $P < 0.001$] and rituximab [HR = 0.433 (0.242–0.772), $P = 0.005$] were independently associated with shorter PFS. When IPI group as a linear parameter was incorporated instead of five IPI factors, low ALC was again associated with shorter PFS [HR = 2.17 (1.25–3.76), $P = 0.006$], along with IPI group [HR 1.53 (1.22–1.91), $P < 0.001$] and rituximab [HR = 0.452 (0.255–0.801), $P = 0.007$, Table 2]. Analyzing IPI risk group as a categorical parameter also showed similar result [HR of low ALC = 2.22 (1.28–3.87), $P = 0.005$, Table 2].

Discussion

ALC is an objective and reproducible test result, which can be obtained by basic laboratory equipment. Our study demonstrated that low ALC is a poor prognostic factor with regards to OS and PFS. Such prognostic value of ALC is in agree with other recently published studies (3, 6, 7). Although the actual mechanisms of this association between low ALC and poor prognosis is unclear, possibilities include: (i) low ALC may be associated with already immunosuppressed condition, suggesting that the host tends to have an inadequate immunological reaction; (ii) low ALC may be a consequence of lympholytic cytokines produced by lymphoma cells, and such lymphoma may already have a resistant character by itself; or (iii) the combination of these two or other.

The prognostic value of ALC was most remarkable in patients treated with CHOP without rituximab. The difference of prognostic impact between CHOP and RCHOP groups is largely because of the improvement of survival by rituximab in patients with low ALC. Particularly in the group of IPI score 2–5, treatment with rituximab significantly improved survival of patients with low ALC, but not significantly that of patients with high ALC. Analogy of this prognostic value of low ALC is that of expression of BCL2, which was a significant poor prognostic indicator before the emergence of rituximab but not in the era of rituximab (8).

Obvious limitation of this comparison is that salvage regimens might or might not have contained rituximab in relapsed patients in CHOP group (although this would not affect OS), and that the patients were not randomized (although characteristics shown in Table 1 were similar in the two groups). Although not using rituximab in addition to CHOP in any patients with DLBCL may not be justifiable given the little toxicity and significant potential benefit (9, 10), it should be noted that the absolute survival benefit is likely larger in patients with low ALC than in those with high ALC, in the era of multiple target therapy agents (currently approved or not) which may lead to expanding costs with significant impact on the economy.

Acknowledgements

We thank Ryoko Yamauchi and Aki Kobayashi for their excellent secretarial support.

Conflict of interest

None.

Authors' contributions

All authors contributed to the patient care and data collection. Y. O. designed the study, analyzed data and wrote the paper. K. Y. analyzed the data and edited the paper. H. K. and Y. Kuwatsuka edited the paper. H. T. and Y. Kagami reviewed the paper. Y. M. supervised the patient care and edited the paper.

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De novo CD5+ diffuse large B-cell lymphoma: results of a detailed clinicopathological review in 120 patients

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Acknowledgments: we thank the collaborators from the institutions for providing patients' data and specimens. A list of participating institutes is given in the Appendix. This paper was presented in part at the 49th Annual Meeting of the American Society of Hematology, Atlanta, December 2007.

Funding: this work was supported in part by Grants-in-Aid for Cancer Research (15-11, 19-8) from the Ministry of Health, Labour and Welfare, Japan.

Manuscript received January 24, 2008. Revised version arrived on March 26, 2008. Manuscript accepted April 15, 2008.

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ABSTRACT

Background
De novo CD5-positive diffuse large B-cell lymphoma (CD5+ DLBCL) is clinicopathologically and genetically distinct from CD5-negative (CD5-) DLBCL and mantle cell lymphoma. The aim of this retrospective study was to clarify the histopathological spectrum and obtain new information on the therapeutic implications of CD5+ DLBCL.

Design and Methods
From 1984 to 2002, 120 patients with CD5+ DLBCL were selected from 13 collaborating institutes. We analyzed the relationship between their morphological features and long-term survival. The current series includes 101 patients described in our previous study.

Results
Four morphological variants were identified: common (monomorphic) (n=91), giant cell-rich (n=13), polymorphic (n=14), and immunoblastic (n=2). Intravascular or sinusoidal infiltration was seen in 38% of the cases. BCL2 protein expression in CD5+ DLBCL was more frequent than in CD5- DLBCL (p=0.0003). Immunohistochemical analysis in 44 consecutive cases of CD5+ DLBCL revealed that 82% of these cases (36/44) were non-germinal center B-cell type DLBCL. The 5-year overall survival rate of the patients with CD5+ DLBCL was 38% after a median observation time of 81 months. Patients with the common variant showed a better prognosis than those with the other three variants (p=0.011), and this was confirmed on multivariate analysis. Overall, 16 patients (13%) developed central nervous system recurrence.

Conclusions
Our study revealed the morphological spectrum of CD5+ DLBCL, found that the incidence of central nervous system recurrence in this form of lymphoma in high, confirmed that CD5+ DLBCL frequently expresses BCL2 protein and showed that it is mainly included in the non-germinal center B-cell type of DLBCL.

Key words: diffuse large B-cell lymphoma, CD5, histopathology, BCL2, central nervous system.

Citation: Yamaguchi M, Nakamura N, Suzuki R, Kagami Y, Okamoto M, Ichinohasama R, Yoshino T, Suzumiya J, Murase T, Miura I, Ohshima K, Nishikori M, Tamaru J, Taniwaki M, Hirano M, Morishima Y, Ueda R, Shiku H and Nakamura S. De novo CD5+ diffuse large B-cell lymphoma: results of a detailed clinicopathological review in 120 patients. Haematologica 2008; 93:1195-1202. doi: 10.3324/haematol.12810

Introduction

Diffuse large B-cell lymphoma (DLBCL) constitutes the largest category of aggressive lymphomas, and is considered to have heterogeneous biological properties.[1,2] The phenomenon of CD5 expression in DLBCL evolving *de novo*, and not as a result of the transformation of chronic lymphocytic leukemia and mantle cell lymphoma, was first described by Matolcsy *et al.* in 1995.[3] Since then, accumulating clinicopathological evidence has gradually clarified that *de novo* CD5-positive (CD5$^+$) DLBCL constitutes a unique subgroup of DLBCL.[4-13] *De novo* CD5$^+$ DLBCL is associated with onset in old age, female predominance, advanced stage at diagnosis, the presence of B symptoms, high levels of lactate dehydrogenase, and the frequent involvement of extranodal sites. The genetic analysis of this lymphoma has suggested that it may originate from somatically mutated CD5$^+$ progenitor B cells.[5,6,8,13] Moreover, an analysis using cDNA microarray and comparative genomic hybridization technology demonstrated that *de novo* CD5$^+$ DLBCL is distinct from CD5$^-$ DLBCL and mantle cell lymphoma.[12,14-17] Cytogenetic analysis identified a subgroup of patients with *de novo* CD5$^+$ DLBCL with chromosomal abnormalities in 8p21 or 11q13 who have a poor prognosis.[18]

We reported that *de novo* CD5$^+$ DLBCL tumors usually show a centroblastic morphology, and 19% show an intravascular or sinusoidal growth pattern.[11] However, CD5 is expressed in some cases of intravascular large B-cell lymphoma[19-22] and T-cell-rich B-cell lymphoma,[23] and cases of CD5$^+$ follicular lymphoma[24,25] and CD5$^+$ Burkitt's lymphoma[26] have been reported. The relationship between these tumors and *de novo* CD5$^+$ DLBCL remains to be clarified. We reported that *de novo* CD5$^+$ DLBCL shows an aggressive clinical course, with a 5-year overall survival rate of 34%.[11] However, the median observation period in our previous study was 33 months; the results should, therefore, be confirmed by long-term survival analysis.

To clarify the histopathological spectrum of CD5$^+$ DLBCL and obtain new information on the therapeutic implications, we performed a detailed clinicopathological review and long-term follow-up analysis in a larger number of patients with *de novo* CD5$^+$ DLBCL.

Design and Methods

Patients

We selected 120 patients with *de novo* CD5$^+$ DLBCL from 13 collaborating institutes. All patients were diagnosed between 1984 and 2002 as having DLBCL according to the WHO classification,[2] and they had no past history of any other lymphoproliferative disorders. All specimens for histological and immunophenotypic studies were obtained at the initial presentation of the patients, and were examined for CD5 antigen expression by means of flow cytometry and/or immunohistochemistry. All patients were immunohistochemically confirmed to be cyclin D1-negative. The current series includes 101 of 109 *de novo* CD5$^+$ DLBCL cases described in our previous study.[11] Seven patients who fulfilled the diagnostic criteria for intravascular large B-cell lymphoma[2] and one patient with follicular colonization were excluded. The study was approved by the Ethics Committee of Mie University Graduate School of Medicine, and complied with the Helsinki Declaration.

Clinical information was obtained from the hospital records or supplied by the physicians at the collaborating centers.

Morphological evaluation

Tissue was fixed in 10% formalin and embedded in paraffin. Sections (5 μm thick) were stained with hematoxylin and eosin. We examined all the 120 initial diagnostic specimens of the *de novo* CD5$^+$ DLBCL cases, consisting of 85 lymphatic tissues such as lymph node, Waldeyer's ring, and spleen and 35 extranodal tissues with lymphomatous involvement. All cases were blindly reviewed twice by three of the authors (*MY, NN, and SN*). If discrepancies occurred, we discussed the cases while using a multiheaded microscope to reach a consensus.

Immunophenotypic study

Immunohistochemical and flow-cytometric analyses were performed as described previously.[27,28] The monoclonal antibodies used were Leu4 (CD3), Leu1 (CD5), and CALLA (CD10) (Becton Dickinson, Mountain View, CA, USA); J5 (CD10) and B1 (CD20) (Coulter, Hialeah, FL, USA); H107 (CD23) (Nichirei, Tokyo, Japan); MHM6 (CD23), BerH2 (CD30), UCHL1 (CD45RO), HM57 (CD79a), anti-immunoglobulin (Ig)G, anti-IgA, anti-IgM, anti-IgD, anti-kappa, and anti-lambda (DAKO, Carpentaria, CA, USA); 4C7 (CD5) and NCL-CD10 (CD10) (Novocastra, Newcastle, UK), and cyclin D1 (IBL, Gunma, Japan). More than 20% positivity of the tumor cells was considered to indicate positivity for the purposes of this study. Based on preliminary data that the incidence of CD5 positivity in DLBCL examined with paraffin material is approximately half of that examined using frozen sections, and that it can be increased using more sensitive immunohistochemical methods (*Yamaguchi M et al., presented at the Annual Meeting of the Japanese Society of Lymphoreticular Tissue Research, 2000*), CD5 expression was examined primarily by flow cytometry and/or immunohistochemistry in the frozen sections from 104 cases of *de novo* CD5$^+$ DLBCL. In the remaining 16 cases, CD5 expression was examined immunohistochemically using paraffin-embedded sections. In fact, 75% or more of the neoplastic cells were confirmed to be positive for CD5 in the cases examined using paraffin-embedded material alone.

BCL2 protein expression was examined by means of immunohistochemistry using paraffin sections and a monoclonal antibody (BCL2, DAKO). Paraffin-embedded material for this study was available in 96 out of 120 cases. Staining for BCL2 was performed at the Aichi Cancer Center, and the data were compared with those for 150 cases of CD5$^-$ DLBCL, which were sequentially diagnosed at the Aichi Cancer Center during the same period as the *de novo* CD5$^+$ DLBCL cases. The reaction

for BCL2 protein was classified as positive if more than 50% of lymphoma cells were stained.[29]

We also classified *de novo* CD5+ DLBCL into two subgroups, i.e., germinal center B-cell and non-germinal center B-cell types.[30] From the file of histological consultation for diagnosis at the Aichi Cancer Center in the period from 2000 to 2004, 44 cases of *de novo* CD5+ DLBCL were selected for this analysis. Staining for CD10, BCL6 (NCL-BCL6, Novocastra), and MUM1 (MUM1p, DAKO) was performed on paraffin sections.[30] Cases were considered positive if 30% or more of the neoplastic cells were stained with an antibody. Subsequently, each case was classified into germinal center or non-germinal center B-cell types according to the criteria of Hans *et al.*[30]

Statistical analysis

Correlations between the two groups were examined with the χ^2 test and Fisher's exact test. Patients' survival data were analyzed with the Kaplan-Meier method and were compared by means of the log-rank test. Univariate and multivariate analyses were performed with the Cox proportional hazard regression model, and data were analyzed with STATA software (version 9.0, STATA Corp., College Station, TX, USA).

Results

Histopathological review and characterization of morphological variants

At a low magnification, total or partial effacement of the nodal architecture with a diffuse (118 patients, 98%) or vaguely nodular pattern (2 patients, 2%) of tumor cell proliferation was observed. In ten patients (8%), these tumor cells were distributed throughout the interfollicular area, while the follicles which had retained their mantle cuffs were spared.

In the current study, particular attention was paid to the presence or absence of intravascular and/or sinusoidal patterns. Although the extent of such patterns varied in each case, they were seen in 45 cases examined (38%). In the specimens of lymph node obtained from 31 patients, tumor cells infiltrated diffusely and focal intrasinusoidal infiltration was observed simultaneously. In the specimens of bone marrow from seven patients, spleen from two patients, and Waldeyer's ring from one patient, lymphoma cells were observed mainly in the sinusoids. In the other patients, a specimen was taken from the tumor in the nasal cavity, stomach, breast, and testis. In those specimens, lymphoma cells infiltrated diffusely, and focal intravascular infiltration was also observed. There was no significant difference in the incidence of intravascular and/or sinusoidal patterns between lymphatic (34/85, 40%) and extranodal (11/35, 31%) specimens.

The size of tumor cells was medium-to-large in 19 cases, mixed medium and large in 14 cases, and large in 87 cases. The tumor cells generally showed a scant or moderate rim of pale baso- or amphophilic cytoplasm. Of note, bi-nucleated tumor cells with a *snowman-like* morphology were frequently observed in our series (101 out of 120 cases, 85%) (Figures 1A and 2B). Apoptotic cells were observed in 21% of the cases.

We classified *de novo* CD5+ DLBCL according to cytomorphological features (Figure 1). In 91 (76%) of 120 patients, monomorphic proliferation of typical centroblasts was observed, although a few scattered giant cells were seen in nine patients. We regarded these features as the prototype of *de novo* CD5+ DLBCL and referred to it as the common variant. In 13 (11%) out of the remaining patients, there was an increase in very large cells with giant or multiple nuclei, varying from 10 to 30% in area and intermixed with centroblasts and immunoblasts. We referred to this as the giant cell-rich variant. This could correspond to the anaplastic variant of DLBCL according to the WHO classification.[2] While the giant cell-rich variant was thus shown to have a polymorphous composition, monomorphous areas with relatively small cells were also usually identified, suggesting that there is a histological continuum between the common and giant cell-rich variants. CD30 was positive in 23% of the cases (3/13). In 14 patients (12%), tumor cells showed irregularly shaped nuclei,

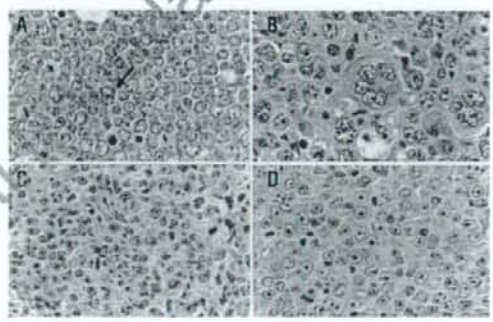

Figure 1. Cytomorphologic features of four variants of *de novo* CD5+ DLBCL. The cells, varying from medium to large in size, are uniform, with a pale basophilic or amphophilic cytoplasm. (A) Common variant, which can be described as the monomorphic or centroblastic variant. *Snowman-like*, bi-nucleated cells were seen (arrow). (B) Giant cell-rich variant. (C) Polymorphic variant, characterized by polymorphous proliferation with medium and large-sized cells. The immunoblastic variant (D) was rare in our case series.

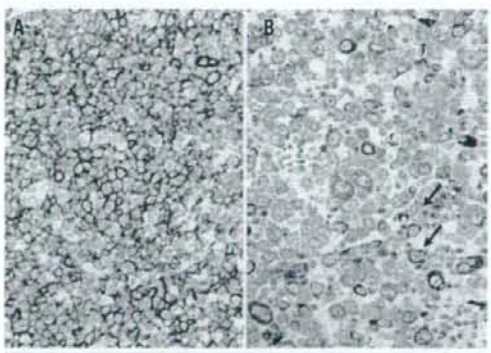

Figure 2. Immunohistochemical features of *de novo* CD5+ DLBCL. Lymphoma cells are positive for CD5 (A) and BCL2 (B). *Snowman-like*, bi-nucleated cells can be seen (arrow).

i.e., indented or multilobated, and were usually characterized by a mixed morphology, which was referred to as the polymorphic variant. Pure proliferation of immunoblasts was seen in only two patients (1%), and was termed the immunoblastic variant. Intravascular/sinusoidal infiltration was observed in 26% of the common variants, 62% of the giant cell-rich variants, 14% of the polymorphic variants, and 0% of the immunoblastic variants. The giant cell-rich variant was associated with intravascular/sinusoidal infiltration more frequently than the common variant (p=0.01).

Clinical features according to morphological variants

The patients' main characteristics and therapeutic results according to morphological categorization are summarized in Table 1. We compared the clinical characteristics between the current group of 120 patients with *de novo* CD5$^+$ DLBCL and 384 patients with CD5$^-$ DLBCL in our previous study.[11] Our previous findings on the clinical features of *de novo* CD5$^+$ DLBCL such as an older age, at onset, female predominance, frequent extranodal involvement, and higher International Prognostic Index (IPI)[31] score were confirmed in the current group of 120 patients (*data not shown*).

Table 1. Clinical features of the patients with *de novo* CD5$^+$ diffuse large B-cell lymphoma.

	Total (n=120) (%)	*Common (n=91) (%)*	*Giant cell-rich (n=13) (%)*	*Polymorphic (n=14) (%)*	*Immunoblastic (n=2) (%)*
Age at diagnosis, years.					
Median	66	66	63	67/71	62/69
Range	22-91	22-91	36-81	52-89	62,69
Over 60 years old	84 (70)	64 (70)	9 (69)	9 (64)	2 (100)
Sex (male:female)	58:62	40:51	9:4	8:6	1:1
Performance status >1	39 (33)	27 (30)	4 (31)	6 (43)	2 (100)
Serum LDH level >normal	85 (71)	61 (67)	11 (85)	11 (79)	2 (100)
Stage III/IV	73 (61)	54 (59)	9 (69)	8 (57)	2 (100)
Extranodal involvement	75 (63)	55 (60)	8 (62)	11 (79)	1 (50)
More than one site	29 (24)	20 (22)	4 (31)	5 (36)	0 (0)
International Prognostic Index					
Low	30 (25)	25 (27)	1 (8)	4 (29)	0 (0)
Low-intermediate	30 (25)	26 (29)	4 (31)	0 (0)	0 (0)
High-intermediate	19 (16)	11 (12)	4 (31)	4 (29)	0 (0)
High	41 (34)	29 (32)	4 (31)	6 (43)	2 (100)
B-symptoms present	49/117 (44)	35/88 (40)	5 (38)	7 (50)	2 (100)
Complete response rate	77/114 (68)	64/86 (74)	5/12 (42)	7/14 (50)	1/2 (50)
5-year OS rate	(38)	(44)	(15)	(21)	(0)

LDH: lactate dehydrogenase; OS: overall survival.

The clinical features, including the five factors of the IPI,[31] were not significantly different among the four morphological variants of *de novo* CD5$^+$ DLBCL. The bone marrow, liver, and spleen were the most frequently involved anatomical sites irrespective of the morphological variant (*data not shown*).

Atypical lymphocyte concentrations (range, 11 to 78%) were noted at presentation in the peripheral blood smear of four cases, whose white blood cell counts ranged from 6,000 to 41,000/mm^3. None of these patients showed marked splenomegaly and the morphology of leukemic cells differed from that of B-cell prolymphocytic leukemia cells.

Immunophenotypic features

BCL2 protein was expressed in 86 out of 96 tumors, and observed in more than 70% of the tumor cells in almost all positive cases (Figure 2B). This incidence was significantly higher than that in the CD5$^-$ DLBCL cases (105/150, 70%; p=0.0003).

As for the molecular classification system established by Hans *et al.*,[30] 36 of 44 cases (82%) of *de novo* CD5$^+$ DLBCL were classified as the non-germinal center B-cell type. Thirty patients (68%) showed the CD10$^-$BCL6$^-$MUM1$^+$ immunophenotype. CD10 was positive in seven patients (16%), BCL6 was negative in 79% of the cases examined (33/42), and MUM1 was positive in 95% of the cases (42/44). Only one patient showed the CD10$^+$BCL6$^+$MUM1$^-$ immunophenotype.

Among the four morphological variants, the common variant was positive for Ig-κ more frequently than either the giant cell-rich (p=0.05) or polymorphic (p=0.03) variant. As for other expression of other antigens there were no significant differences among the morphological variants of *de novo* CD5$^+$ DLBCL (*data not shown*).

Therapeutic outcome and long-term survival according to histopathological variants

Clinical follow-up data and information about the first-line therapy were available for all patients. The treatment consisted of chemotherapeutic regimens including anthracycline for 104 patients and without anthracycline for three. No patient was treated with rituximab in the first-line therapy. Seven patients with localized disease were treated with radiotherapy or surgical resection alone as first-line therapy. Six patients who did not receive any therapy because of their poor performance status all died of their disease. A complete response was achieved on first-line therapy in 77 (68%) out of the 114 patients who received treatment. Seven patients were lost to follow-up within 5 years after the diagnosis. The median observation time of surviving patients was 81 months. The 2-year overall survival rate of all 120 patients, estimated by the Kaplan-Meier method, was 52%, and the 5-year overall survival rate was 38% (Figure 3A).

We collected data on sites of involvement at relapse/progression. Among all 120 patients with *de novo* CD5$^+$ DLBCL, 16 patients (13%) developed central nervous system (CNS) recurrence (Table 2). All these patients were treated with anthracycline-containing chemotherapy as a front-line treatment. One patient had brain

involvement at diagnosis. She achieved a complete response following front-line therapy, but develop recurrence in the thoracic spinal cord. The other patients did not show any CNS involvement at diagnosis. Twelve patients experienced CNS relapse after achieving a complete response. Of these, eight experienced isolated CNS relapse while the CNS relapse was associated with a systemic relapse in the others. Four patients experienced CNS disease progression during the first-line treatment. The median age of all 16 patients with CNS relapse was 64 years (range, 28 to 85). Of note, all but three patients were over 60 years old. Seven were male and nine were female. The serum lactate dehydrogenase level was elevated in 13 of these patients and performance status was higher than one in seven patients. Five patients showed more than one extranodal site of involvement. Nine patients were categorized as having a high-intermediate or high risk, according to the IPI. The median time from diagnosis to CNS recurrence was 16 months. We compared therapeutic outcome and survival data in the 120 paients with *de novo* CD5+ DLBCL according to the morphological variants. The complete response rate was lowest (42%) in patients with the giant cell-rich variant of *de novo* CD5+ DLBCL, and was significant different from that in patients with the common variant (p=0.02, Table 1). Five-year overall survival rates for patients with common, giant cell-rich, polymorphic, and immunoblastic variants were 44%, 15%, 21%, and 0%, respectively (Table 1, Figure 3B). The survival curve of patients with the common variant was significantly better than that of patients with the other three variants combined (p=0.011, Figure 3C). The presence of intravascular/sinu-

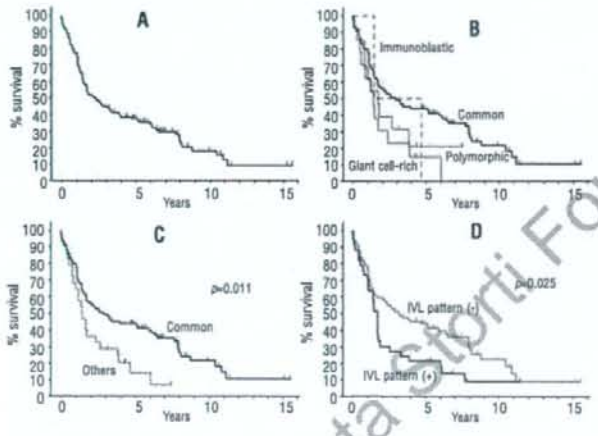

Figure 3. **Survival according to the histological features of *de novo* CD5+ diffuse large B-cell lymphoma (DLBCL). (A) Overall survival in all 120 patients with *de novo* CD5+ DLBCL.** (B) **Overall survival of patients with different histological variants of *de novo* CD5+ DLBCL.** (C) **Patients with the common variant had a better survival than those with the other three variants of *de novo* CD5+ DLBCL.** (D) **The presence of intravascular/sinusoidal infiltration had an impact on the overall survival. IVL, intravascular/sinusoidal.**

Table 2. Clinicopathological features of patients with *de novo* CD5+ diffuse large B-cell lymphoma who experienced central nervous system recurrence.

N.	Age/sex	Stage	Sites of extranodal involvement	PS >1	LDH >N	IPI score	Histological variant	IVL pattern	CR	Sites of recurrence	Period from diagnosis to CNS recurrence (months)	Survival, (months) outcome
1	62/M	IIIA	Lung, stomach, kidney, gingiva		Y	4	Common			CNS	2	8, DOD
2	77/M	IA		Y	Y	3	Polymorphic			CNS	2	4, DOD
3	76/M	IIA			Y	2	Common			CNS	3	9, DOD
4	61/F	IVB	BM	Y	Y	4	Common	Y	Y	CNS	5	9, DOD
5	67/M	IVB	Liver, BM	Y	Y	5	Common	Y	Y	CNS	6	23, DOD
6	85/M	IIIA		Y	Y	4	Common			CNS	<7	7, DOD
7	62/F	IIIA	Brain, pleura	Y	Y	5	Common	Y	Y	CNS	8	18, DOD
8	62/F	IIIB		Y	Y	4	Immunoblastic		Y	CNS, LN, liver, ascites, BM	8	18, DOD
9	38/F	IVB	BM		Y	2	Common		Y	CNS	24	72, DOD
10	66/F	III	Bone, uterus		Y	4	Common		Y	CNS (intraocular)	37	43, AWD
11	62/M	IVB	Liver, BM	Y	Y	5	Common		Y	Pelvis,CNS	39	40, DOD
12	28/F	IIA	Breast			0	Common		Y	CNS (intraocular)	57	86, AWD
13	50/M	IIIB			Y	2	Giant cell-rich	Y	Y	CNS	60	74, DOD
14	69/F	IA				1	Common		Y	CNS, etc.	71	80, DOD
15	67/F	IA			Y	2	Common	Y	Y	CNS (intraocular)	84	84, AWD
16	74/F	IA				1	Common		Y	CNS, LN	96	99, DOD

PS: performance status; LDH: lactate dehydrogenase; IVL: intravascular/sinusoidal; CR: complete response; Y: yes; BM: bone marrow; LN: lymph node; DOD: died of disease; AWD: alive with disease.

soidal infiltration also had an impact on survival (p=0.025, Figure 3D). The results of univariate and multivariate analyses to assess the impact of clinical and morphologic features on overall survival in *de novo* CD5$^+$ DLBCL patients are shown in Table 3. Univariate analysis identified the five risk factors of IPI, morphological variants, and intravascular/sinusoidal infiltration as prognostic factors important for overall survival. The presence of either *snowman-like* cells or a higher mitotic ratio (> 4/one high-power field on average) was not associated with a reduced overall survival (*data not shown*). Multivariate analysis adjusted for the five risk factors of the IPI confirmed the independent prognostic significance of histological categorization for overall survival (Table 3). Among the prognostic factors, the morphologic variant, age, performance status, and serum lactate dehydrogenase level were significantly associated with survival.

Table 3. Prognostic factors affecting overall survival of patients with *de novo* CD5$^+$ diffuse large B-cell lymphoma.

Variables	Unfavorable factor	HR	Univariate (CI)	p	HR	Multivariate (CI)	p
Comparison with risk factors							
Morphological variants	Not common	1.85	(1.14-3.01)	0.01	1.67	(1.02-2.75)	0.04
IVL pattern	Present	1.66	(1.06-2.60)	0.03		-	-
Age	>60 years	2.37	(1.44-3.92)	0.001	1.91	(1.15-3.19)	0.01
Performance status	2-4	2.81	(1.81-4.37)	<0.001	1.77	(1.11-2.85)	0.02
LDH	>Normal	3.71	(2.14-6.43)	<0.001	2.56	(1.43-4.61)	0.002
Stage	III/IV	2.34	(1.48-3.69)	<0.001		-	-
Extranodal diseases	>1 site	1.72	(1.07-2.77)	0.03		-	-
B symptoms	Present	2.09	(1.36-3.19)	<0.001		-	-
Comparison with IPI category							
Morphological variants	Not common	1.85	(1.14-3.01)	0.01	1.44	(0.87-2.36)	0.15
IPI category	HI/H	3.32	(2.14-5.15)	<0.001	3.14	(2.00-4.92)	<0.001
IVL pattern	Present	1.66	(1.06-2.60)	0.03	1.81	(1.14-2.86)	0.01
IPI category	HI/H	3.32	(2.14-5.15)	<0.001	3.46	(2.21-5.41)	<0.001

HR: hazard ratio; CI:confidence interval; HI/H: high-intermediate or high risk category of IPI; IVL: intravascular/sinusoidal; LDH, lactate dehydrogenase.

Discussion

We clarified detailed cytomorphological features of *de novo* CD5$^+$ DLBCL. A German study also documented morphological features in their series of 13 cases of *de novo* CD5$^+$ DLBCL, identifying eight centroblastic (62%), three immunoblastic (23%), and two unclassified DLBCL with irregular nuclei (15%).[13] Our findings generally appeared to be in keeping with those of the German study; however, the percentage of immunoblastic lymphoma cases (23%) was higher in the German study than in ours (2%). DLBCL developing in the setting of small lymphocytic lymphoma/chronic lymphocytic leukemia (Richter's syndrome) evidently tend to be characterized by an immunoblastic morphology and the expression of CD5.[32] In Japan, the incidence of chronic lymphocytic leukemia is one fifth of that in Western countries.[33,34] Moreover, CD5 expression was mainly examined using fresh material in the majority of studies of *de novo* CD5$^+$ DLBCL in Japan, while it was examined in paraffin-embedded material in the studies in Western countries. In Japan, the incidence of *de novo* CD5$^+$ DLBCL ranges from 4% (4/101)[35] to 10% (24/240),[36] which seems to be almost the same as that reported in Western series.[10,37] Since only two cases have been included in the current study, the clinicopathological features of the immunoblastic variant of *de novo* CD5$^+$ DLBCL remain unknown. International cooperative studies are needed to verify the hypothesis that these facts may explain the conflicting data. Since *de novo* CD5$^+$ DLBCL has various histopathological appearances, CD5 immunostaining should be performed routinely in cases of DLBCL.

In the current study, intravascular/sinusoidal patterns to various extents were observed in 38% of the cases of *de novo* CD5$^+$ DLBCL. As Murase *et al.* demonstrated recently,[21] *de novo* CD5$^+$ DLBCL with an intravascular/sinusoidal pattern showed intermediate features in terms of aggressive clinical behavior and prognosis between *de novo* CD5$^+$ DLBCL without an intravascular/sinusoidal pattern and CD5$^+$ intravascular large B-cell lymphoma, suggesting that a part of the two diseases overlaps. In the present study *snowman-like*, binucleated cells were frequently observed in *de novo* CD5$^+$ DLBCL. Further studies in CD5$^-$ DLBCL and CD5$^+$ intravascular large B-cell lymphoma are needed to evaluate their diagnostic significance in *de novo* CD5$^+$ DLBCL.

The aggressive clinical feature of *de novo* CD5$^+$ DLBCL that we previously reported[11] was confirmed by the current study and a recent study that was conducted using tumor specimens from patients with DLBCL uniformly treated with anthracycline-based chemotherapeutic regimens in a prospective, multi-center clinical trial.[37] In contrast, it has been reported that the expression of CD5 in DLBCL did not affect overall survival.[13] Recent studies revealed that patients with *de novo* CD5$^+$ DLBCL with 8p21-associated chromosomal abnormalities[10] and with 9p21 loss in comparative genomic hybridization analysis[16] have an extremely short survival. The existence of these highly aggressive subgroups of *de novo* CD5$^+$ DLBCL may explain the heterogeneity in the prognosis of this disease. The possible role of the CD5 molecule in the aggressiveness of *de novo* CD5$^+$ DLBCL remains unknown. It has been reported that CD5 supports the survival of B cells by stimulating the production of interleukin-10 and by down-regulating B-cell receptor signaling.[38] This molecular basis may explain in part why *de novo* CD5$^+$ DLBCL shows more aggressive clinical features than CD5$^-$ DLBCL.

According to the criteria established by Hans *et al.*,[30] 82% of the cases examined in the present study were non-germinal center B-cell DLBCL. Our results suggest that *de novo* CD5$^+$ DLBCL is mainly classified into the non-germinal center B-cell type, and may provide a clue to clarify the aggressiveness of such DLBCL. Our present study also revealed that *de novo* CD5$^+$ DLBCL typically shows the BCL2$^+$ BCL6$^-$ immunophenotype.

Recent clinical studies suggest that the prognosis of DLBCL expressing BCL2 protein, BCL6 protein-negative DLBCL, and DLBCL of the non-germinal center B-cell subgroup is improved by rituximab-containing chemotherapy.[39-41] In our previous study published in 2002, no patients had been treated with rituximab.[11] In the present study, some patients had been treated with rituximab as a part of salvage therapy; however, the overall survival was almost the same as that in the previous study and was not clearly improved. The therapeutic impact of adding rituximab to first-line therapy in *de novo* CD5+ DLBCL needs to be evaluated in the setting of a well-designed clinical trial.

The overall incidence of CNS recurrence in aggressive non-Hodgkin's lymphoma excluding lymphoblastic lymphoma/acute lymphoblastic leukemia and Burkitt's lymphoma is approximately 5%,[42-44] and the incidence in DLBCL seems to be less than 5%. The incidence of CNS recurrence in the present study, 13%, was marked. Most of our patients with CNS recurrence had an elevated level of serum lactate dehydrogenase, which has been reported as a potential risk factor for CNS recurrence in aggressive lymphoma.[42] In contrast, most of the patients with CNS recurrence were over 60 years old, which was reported to be a favorable factor in a study of a large number of patients.[42] To establish an optimal therapeutic strategy for CNS prophylaxis in DLBCL, the relationship between CD5 expression and CNS recurrence in DLBCL should be examined in future studies.

In conclusion, our study provides new clinicopathological information on *de novo* CD5+ DLBCL. *De novo* CD5+ DLBCL shows many unique clinicopathological and genetic features. Further studies are needed to clarify molecular mechanisms in highly aggressive subgroups of *de novo* CD5+ DLBCL.

Appendix

List of participating institutes in the CD5+ DLBCL histology project: Akita University School of Medicine, Akita Kumiai General Hospital, National Miyagi Hospital, Saka General Hospital, Tohoku University School of Medicine, Sendai City Hospital, Furukawa City Hospital, Fukushima Medical College, Iwaki General Hospital, Ohta Nishinouchi General Hospital, Takeda General Hospital, Tokyo Women's Medical University Daini Hospital, Saitama Medical School, Matsudo Municipal Hospital, Higashi Matsudo Hospital, Kameda General Hospital, Niigata University, Toyama Prefectural Central Hospital, Kanazawa University, Noto General Hospital, Nagano Municipal Hospital, Nagano Red Cross Hospital, Hamamatsu Medical Center, Inazawa Municipal Hospital, Aichi Prefectural Hospital, Toyota Memorial Hospital, Fujita Health University School of Medicine, Nishio Municipal Hospital, Toyohashi Municipal Hospital, Okazaki Municipal Hospital, Ichinomiya Municipal Hospital, Japanese Red Cross Nagoya First Hospital, Nagoya Memorial Hospital, Nagoya City University Medical School, Nagoya Ekisaikai Hospital, Aichi Cancer Center, Suzuka Chuo General Hospital, Suzuka Kaisei General Hospital, Mie University School of Medicine, Matsusaka Municipal Hospital, Matsusaka Chuo General Hospital, Matsusaka Saiseikai General Hospital, Yamada Red Cross Hospital, Ise Municipal General Hospital, Kyoto University, Kyoto Prefectural University of Medicine, Rinku General Medical Center, Okayama University Medical School, Okayama Saiseikai General Hospital, Chugoku Central Hospital of the Mutual Aid Association of Public School Teachers, Okayama Red Cross General Hospital, Fukuoka University School of Medicine, Kyushu Cancer Center, Kyushu University, and University of the Ryukyus.

Authorship and Disclosures

MY, NN, RS, TM, and SN contributed to the design of the study, provided clinical data and samples, analyzed the data, and wrote the manuscript. YK, MO, RI, TY, JS, TM, IM, KO, MN, JT, and MT provided clinical data and samples and critically reviewed the manuscript. MH, YM, RU, and HS provided clinical data and gave critical advice on the study to improve its intellectual content.

The authors reported no potential conflicts of interest.

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bjh research paper

Remission induction therapy containing rituximab markedly improved the outcome of untreated mature B cell lymphoma

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Received 29 May 2008; accepted for publication 23 July 2008

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Summary

Many controlled clinical trials have proven that rituximab improves the clinical outcome of patients with mature B cell lymphoma. This study was conducted to assess the contribution of rituximab in the actual clinical practice. Patients with newly diagnosed mature B cell lymphoma treated at 20 National Hospital Organization hospitals from January 2000 to December 2004 were consecutively registered. Rituximab was approved in September 2002 for indolent B cell lymphoma and in September 2003 for aggressive B cell lymphoma in Japan. The patients were divided into two groups depending on whether they received induction therapy containing rituximab. The endpoint was to evaluate the rituximab benefit based on 2-year progression-free survival (PFS) and 2-year overall survival (OS). A total 1126 patients received chemotherapies. Of these, 762 were diagnosed as diffuse large B cell lymphoma (DLBCL) and 215 as follicular lymphoma (FL). PFS and OS were markedly improved in the rituximab group compared with the non-rituximab group in patients with DLBCL (both $P < 0{\cdot}001$) and in patients with FL ($P < 0{\cdot}001$ and $P = 0{\cdot}003$ respectively). Rituximab, when used for remission induction therapy, significantly improved the clinical outcome of the mature B cell lymphoma patient in actual clinical practice.

Keywords: rituximab follicular lymphoma, diffuse large B cell lymphoma, clinical practice.

Non-Hodgkin lymphoma (NHL) is one of the leading causes of cancer death, and its incidence is increasing. The majority of NHL has a B cell phenotype. Almost all B cell lymphomas express CD 20 antigen on the cell surface. Rituximab, a chimeric anti-CD20 monoclonal antibody, was developed and is now widely used to treat B cell lymphoma. Many clinical

First published online 20 October 2008
doi:10.1111/j.1365-2141.2008.07390.x

studies have established the effect of rituximab against B cell lymphoma (MacLaughlin *et al*, 1998; Czuczman *et al*, 1999, 2004; Coiffier *et al*, 2002; Forstpointner *et al*, 2004; Hiddemann *et al*, 2005; Lenz *et al*, 2005; Marcus *et al*, 2005; Rivas-Vera *et al*, 2005; Habermann *et al*, 2006; van Oers *et al*, 2006; Pfreundschuh *et al*, 2006, 2008; Herold *et al*, 2007). The toxicity of rituximab has been generally graded as 1 or 2, and it occurs with the first infusion (MacLaughlin *et al*, 1998); the safety of rituximab when combined with chemotherapy has been shown to be similar to that of chemotherapy alone. Randomized phase III studies have proven the survival benefits of the addition of rituximab to multi-agent chemotherapy for patients with untreated follicular lymphoma (FL) (Hiddemann *et al*, 2005; Herold *et al*, 2007) and those with untreated diffuse large B cell lymphoma (DLBCL) (Coiffier *et al*, 2002; Pfreundschuh *et al*, 2006, 2008;). A systematic review also showed the clinical impact of rituximab for low-grade B cell lymphoma (Schulz *et al*, 2007). These data demonstrated that rituximab has an indisputable benefit for patients with untreated and relapsed/refractory B cell lymphoma who were enrolled in well controlled clinical studies. One population-based retrospective analysis by the British Columbia Cancer Registry assessed the effect of rituximab in combination with cyclophosphamide, doxorubicin, vincristine, prednisone (CHOP) for DLBCL and demonstrated improvement in treatment outcome (Sehn *et al*, 2005). This survey revealed that rituximab contributed to the management of DLBCL in clinical practice. However, the cases studied were restricted to those with DLBCL who received CHOP (with/without rituximab) with curative intent. Therefore, no study has reported the clinical benefit of rituximab in patients with B cell lymphoma in actual clinical practice. To address this point, a retrospective survey comparing patients with B cell lymphoma treated with and without rituximab was conducted. The results showed remarkable improvement in the survival of patients with FL and those with DLBCL, which account for the majority of mature B cell lymphoma patients, by the addition of rituximab in actual clinical practice.

Patients and methods

This was a retrospective cohort study that examined the clinical outcome of all untreated patients with B cell lymphoma who visited the haematological department of 20 hospitals belonging to the National Hospital Organization (NHO), a major, nationwide hospital group in Japan, from January 2000 to December 2004. This research group was founded for the purpose of creating and generalizing clinical evidence in the haematological field by NHO and is called the Clinical Hematology Group of NHO (CHG–NHO). In Japan, rituximab was approved by the Ministry of Health and Labour for the treatment of low-grade B cell lymphoma in September 2002 and for the treatment of aggressive B cell lymphoma in September 2003. The patients with B cell lymphomas were divided into two groups (the rituximab group and the non-rituximab group) based on whether they had received induction therapy containing rituximab in order to determine the benefit of rituximab as part of first remission induction therapy. This study received approval by the responsible ethics committee.

Patients

The patients included in this study were older than 15 years and were newly diagnosed as having mature B cell lymphoma with CD 20 expression by pathological or cytological examination during the period of the study. The pathological diagnosis of each institution was used. Both limited and advanced stage patients based on the Ann-Arbor classification were included (Carbone *et al*, 1971). Patients were excluded if they were human immunodeficiency virus (HIV)-positive or had central nervous system involvement at the time of presentation. All patients fitting the above criteria were serially enrolled. Final statistical analysis was performed for patients who received systemic chemotherapy, whether or not the intention was curative.

Clinical characteristics of the patients included in this survey

All patients' pathological diagnoses were done based on the WHO classification. Age, Eastern Cooperative Oncology Group (ECOG) performance status (PS), lactate dehydrogenase (LDH) levels, clinical staging (Ann-Arbor classification), number of extra-nodal lesions (0, 1 vs. ≥2) were also collected and used to calculate the International Prognostic Index (IPI) (The International Non-Hodgkin's Lymphoma Prognostic Factors Project, 1993) and the revised IPI (R-IPI; Sehn *et al*, 2007). The primary remission induction therapy regimen of all enrolled patents was determined. Usage of rituximab was the focus of this investigation. The kinds of chemotherapy were divided into two groups: those containing anthracyclin and those not containing anthracyclin.

A complete response to treatment was defined as the disappearance of all clinical evidence of disease. Progression-free survival (PFS) was defined as the interval from the diagnosis to the first recurrence of disease (progression or relapse), death from any cause, or the date of the last follow-up in patients who had no relapse. Overall survival (OS) was defined as the interval from diagnosis to death from any cause. Systemic therapy was initiated promptly after diagnosis for almost all of the patients (usually within 1 month).

Statistical analysis

The patients' clinical characteristics and treatment outcomes were compared between patient groups who received systemic chemotherapy with and without rituximab for first induction therapy. The primary endpoint of this study was to confirm the benefit of rituximab for patients with B cell lymphoma when used in remission induction by evaluating the 2-year PFS and

