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

Motoko Yamaguchi,[1] Naoya Nakamura,[2] Ritsuro Suzuki,[3] Yoshitoyo Kagami,[4] Masataka Okamoto,[5] Ryo Ichinohasama,[6] Tadashi Yoshino,[7] Junji Suzumiya,[8] Takuhei Murase,[9] Ikuo Miura,[10] Koichi Ohshima,[11] Momoko Nishikori,[12] Jun-ichi Tamaru,[13] Masafumi Taniwaki,[14] Masami Hirano,[5,15] Yasuo Morishima,[4] Ryuzo Ueda,[16] Hiroshi Shiku,[1] and Shigeo Nakamura[1]

[1]Mie University Graduate School of Medicine, Tsu; [2]Tokai University, Isehara; [3]Nagoya University Graduate School of Medicine, Nagoya; [4]Aichi Cancer Center, Nagoya; [5]Fujita Health University School of Medicine, Toyoake; [6]Tohoku University Postgraduate School of Medicine, Sendai; [7]Okayama University Graduate School of Medicine and Dentistry, Okayama; [8]Fukuoka University Chikushi Hospital, Fukuoka; [9]Nishio Municipal Hospital, Nishio; [10]St. Marianna Medical University, Kawasaki; [11]Kurume University School of Medicine, Kurume; [12]Kyoto University, Kyoto; [13]Saitama Medical Center, Kawagoe; [14]Kyoto Prefectural University of Medicine, Kyoto; [15]Meijo University, Nagoya, and [16]Nagoya City University Medical School, Nagoya, Japan

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Correspondence:
Motoko Yamaguchi, M.D., Ph.D., Department of Hematology and Oncology, Mie University Graduate School of Medicine, 2-174 Edobashi, Tsu, Mie 514-8507, Japan.
E-mail: waniwani@clin.medic.mie-u.ac.jp

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.

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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,

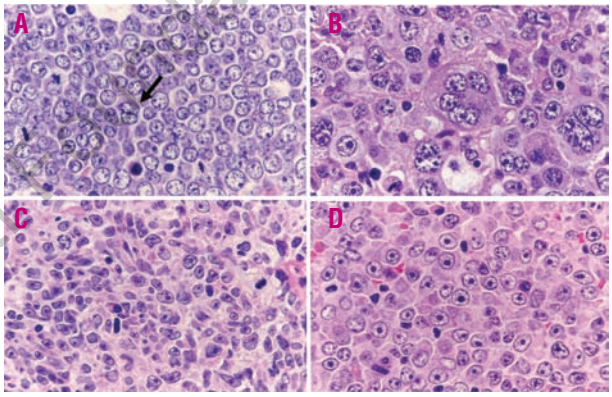

Figure 1. Cytomorphologic features of four variants of *de novo* CD5+ DLBCL. The cells, varying from medium to large in size, are uniform, with a pale basophilic or amphophilic cytoplasm. (A) Common variant, which can be described as the monomorphic or centroblastic variant. *Snowman-like*, bi-nucleated 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.

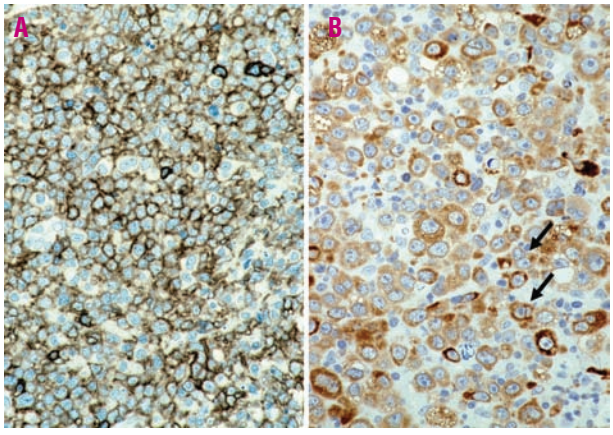

Figure 2. Immunohistochemical features of *de novo* CD5+ DLBCL. Lymphoma cells are positive for CD5 (A) and BCL2 (B). *Snowman-like*, bi-nucleated cells can be seen (arrow).

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-

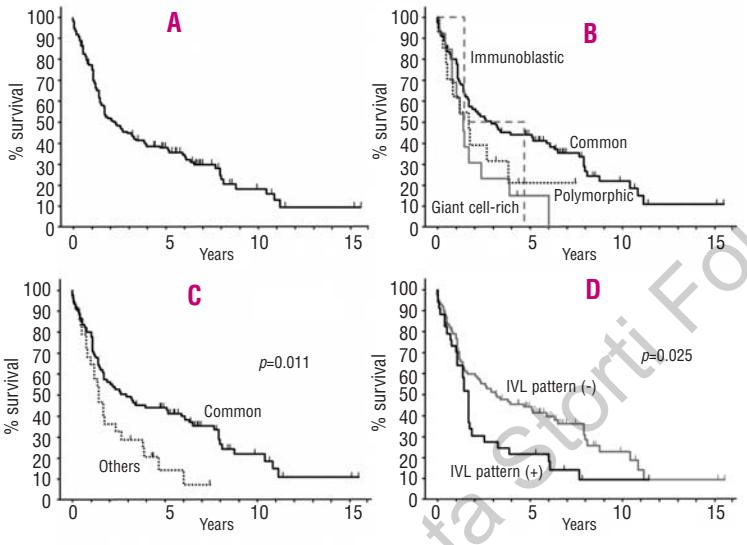

Figure 3. Survival according to the histological features of *de novo* CD5+ diffuse large B-cell lymphoma (DLBCL). (A) Overall survival in all 120 patients with *de novo* CD5+ DLBCL. (B) Overall survival of patients with different histological variants of *de novo* CD5+ DLBCL. (C) Patients with the common 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	Univariate HR	Univariate (CI)	Univariate p	Multivariate HR	Multivariate (CI)	Multivariate 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[18] 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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