CD5-Positve Diffuse Large B-Cell Lymphoma Presenting in Bone Marrow without Lymphadenopathy

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In this study, we reported five cases of $CD5^+$ diffuse large B-cell lymphoma (DLBCL) which initially presented as bone marrow involvement without lymphadenopathy. The cases involved two males and three females, with an average age of 73.8 years. The patients had fever and showed general fatigue. Three of the five cases presented hepatosplenomegaly. Laboratory examination highlighted anemia, thrombocytopenia, presence or absence of leukopenia, hypoalbuminemia as well as a considerable elevation of serum lactate dehydrogenase, soluble IL-2R and ferritin. Bone marrow aspiration smears showed large-sized lymphoma cells, which were found to aggregate, and clot sections showed many clusters of lymphoma cells, which had a large and round or indented nucleus with vesicular chromatin and occasional small nucleoli. Mitotic rate was high. In the bone marrow clot section, whereas three cases exhibited a diffuse infiltration of the lymphoma cells (diffuse type), two cases exhibited a sinusoidal proliferation (intravascular type). Cytogenetic analysis showed many and complex abnormalities in all 3 cases examined. The lymphoma cells were positive for CD5, CD19, CD20, CD38, IgM, bcl-2 and bcl-6, while they were negative for CD3, CD10, CD23, CD30, cyclinD1 and TdT. In four cases, after a semi-nested PCR amplification of the immunoglobulin heavy chain (IgH) gene, a discrete band could be detected. All the cases exhibited an in-frame sequence and the frequency of somatic mutations ranged from 2.1 to 11.1% with no intraclonal diversity. These DLBCL may have derived from the same counterpart as $CD5^+$ B-CLL. There were no differences in laboratory, immunological and molecular examination between the diffuse type and the intravascular type. Recurrences in the bone marrow and an aggressive clinical course were also observed in both types.

Key words CD5, bone marrow, diffuse large B-cell lymphoma, immunoglobulin heavy chain gene

INTRODUCTION

CD5 antigen is a T-cell associated marker which is also expressed in the case of B cells in chronic lymphocytic leukemia (B-CLL) and in several types of non-Hodgkin's lymphoma (NHL) with the B-cell phenotype[1-4]. The CD5 antigen is expressed in most cases of B-CLL and mantle cell lymphoma (MCL), in some cases of hairy cell leukemia (HCL) and in 5-10% of diffuse large B-cell lymphoma (DLBCL)[1-5]. B-CLL is a neoplastic disease characterized by the accumulation of small mature-appearing lymphocytes with a B-cell phenotype in blood, bone marrow and lymphoid tissues. B-CLL is phenotypically characterized by the presence of CD5 and CD23, and low levels of surface IgM and IgD[3,4]. Cytogenetic abnormalities situated in chromosomes 12 (trisomy 12), 13 (13q14), and 14 (14q+) are known to occur in B-CLL[4]. B-CLL may arise from $CD5^+$ B cells (B1 cells), which are exposed to a self-antigen, and produce auto-reactive antibodies[6]. MCL is considered to be derived from B cells in the mantle zone of secondary follicles[7]. It is described as a B-cell neoplasm with translocation of the bcl-1 gene (11q23) and immunoglobulin

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heavy chain (IgH) gene (14q32), and overexpression of cyclin D1[8,9]. Whereas CD5 expression is one of the major characteristics of B-CLL and MCL, CD5+ DLBCL constitutes a minor population of DLBCL[7–10]. CD5+ DLBCL is reported to frequently involve extranodal organs and show aggressive clinical behavior in comparison to CD5− DLBCL[5]. Somatic mutation analysis of the immunoglobulin heavy chain (IgH) gene variable region (VH gene) shows that most cases of CD5+ MCL derive from a germline or have low mutation rates, while both of CD5+ B-CLL and CD5+ DLBCL derive from a germline and comprise somatic mutations[10]. Most of CD5− DLBCL show somatic hypermutation and are derived from germinal center (GC) or post-GC B cells of nodal and extranodal organs[11–13].

Although DLBCL with bone marrow involvement is found in advanced stages of the disease, DLBCL with bone marrow involvement in the absence of lymphadenopathy as initial presentation has been rarely reported[14,15]. These patients frequently have fever of unknown origin at the time of presentation, and have a poor prognosis with survival being measured in days to weeks for most reported cases. However, it is not known whether the expression of the CD5 antigen occurs under such circumstances.

We retrieved from our CD5+ DLBCL files, five cases of a peculiar form of CD5+ DLBCL where the initial diagnoses were bone marrow involvement in the absence of lymphadenopathy. These cases exhibited peculiar profiles in terms of clinicopathological, immunological and genetic aspects. The five cases could be grouped with respect to histology into three classes, i. e. one showing a diffuse infiltration of the lymphoma cells, which constitutes the diffuse type, and two showing a sinusoidal proliferation, which constitute the intravascular type. However, our data on clinicopathological, immunological and genetic examination reveal that there are no differences between the two types.

MATERIALS AND METHODS

Aspiration smears of the bone marrow were evaluated by May-Giemsa stain. Aspiration clots of the bone marrows were fixed in 20% buffered formalin and embedded in paraffin for the routine histological, immunohistochemical and molecular studies. The histological diagnosis was established according to the WHO classification[13]. The immuno-peroxidase staining was performed by the streptavidin-biotin complex technique[16]. Monoclonal and polyclonal antibodies used in this study were: CD3 [CD3, polyclonal antibody against CD3, DAKOPATTS (DAKO), Denmark], CD5 [CD5, Novocastra Laboratories (NOVO), UK], CD10 (CD10, NOVO), CD20 (FB1, Pharmingen, USA), CD23 (CD23, The binding site Ltd., UK), CD30 (CD30, DAKO), cyclin D1 (cyclin D1, NOVO), TdT (TdT, DAKO) bcl-2 (DAKO), bcl-6 (Santa Cruze Technology, USA) and immunoglobulin (μ, δ, γ, α, κ and λ, DAKO). DNA was extracted from the paraffin-embedded sections by a treatment with proteinase K (PCR grade, Roche diagnostics, Germany). The variable regions (CDR2 and FW3) and the VDJ region (CDR3) of the IgH gene were amplified by semi-nested PCR, using primers for FR2A, LJH and VLJH, according to a previously described method[17]. CDR2 and FW3 of the VH gene were then analyzed. The presence of intraclonal diversity was examined according to a previously described method[10].

RESULTS

The clinical findings are summarized in Table 1. There were two males and three females, ranging in age from 68 to 82 years with an average age of 73.8 years. The patients had fever and presented general fatigue. Hepatosplenomegaly was found in three cases. Case 2 showed a slight swelling of the spleen in the abdominal echogram. Lymphadenopathy was absent during the entire course of each case. Laboratory examination exhibited anemia, thrombocytopenia, with or without leukopenia, hypoalbuminemia as well as a considerable elevation of serum lactate dehydrogenase (LDH), soluble IL-2R and ferritin (Table 2). In cases 1 and 2, ^{67}Ga scintillation revealed no abnormal accumulation except in the bone marrow (cases 1 and 2) and uterus (case 1). A preceding low grade B-cell leukemia/ lymphoma was not found in any cases. All patients except case 4 received a combined chemotherapy. Cases 1, 2, 4 and 5 died of the lymphoma (Table 1). Cases 1, 2 and 5 had a relapse in the bone marrow and systemic dissemination. Case 5 had systemic relapse with central nervous system involvement following hematological recurrence.

Table 1. Clinical summary of CD5+ diffuse large B-cell lymphoma

No.	Age	Sex	Initial symptoms	Bone marrow involvement	Hepato-megaly	Spleno-megaly	Lymph node enlargement	Others	Therapy	Clinical outcome
Case 1	71	F	General fatigue and generalized edema	+	+	+	−		CY ADM VCR PSL	Dead for 3rd relapse 23 months from the intial diagnosis
Case 2	73	F	Fever	+	−	*±	−		ADM VCR PSL MTX ETP	Dead for 2nd relapse 17 months from the intial diagnosis
Case 3	75	M	Noncontributory	+	+	+	−		CY ADM VCR PSL	Alive, 4 months CR
Case 4	82	M	Fever and general fatigue	+	+	+	−	Pleural and ascitic effusion	no	Dead within 1 month
Case 5	68	F	General fatigue fever and loss of appetite	+	−	−	−		ADM VCR PSL MTX	Dead for 2nd relapse with CNS invasion 20 months from the intial diagnosis

Abbreviations : CY, cyclophosphamide ; ADM, adriamycin ; VCR, vincristine ; PSL, prednisolone ; MTX, methotraxisate ; ETP, etoposide ; CR, complete remission ; CNS, central nervous system.
*, slight swelling in echogram

An autopsy was performed in case 2. A diffuse infiltration of the lymphoma cells was found in the bone marrow, spleen, and portal area and sinus of the liver. The lymphoma cells were located in generalized vessels.

Table 2. Laboratory findings in CD5+ diffuse large B-cell lymphoma

		Case 1	Case 2	Case 3	Case 4	Case 5
Blood serum						
Leukocytes	(/μl)	12,600	1,900	2,700	6,910	5,900
Red blood cells	(x10³/μl)	3,300	2,390		3,380	3,330
Hemoglobin	(g/dl)	9.3	7.3	11.3	12.1	9.2
Platelets	(x10³/μl)	125	129	44	39	111
LDH	(u/l)	8,248	3,174	1,742	2,297	1,429
Total protein	(g/dl)	5.2	4.9		5.8	5.6
Albumin	(g/dl)	2.1	2.6		2.5	2.6
BUN	(mg/dl)	23.8	11		16.5	15.7
Creatinine	(mg/dl)	1.5	0.6		0.8	0.6
Uric acid	(mg/dl)	12	3.2		4.4	4.5
Ferritin	(mg/ml)	282				460
IL-2R	(u/ml)	28,546	9,010	4,050	3,040	
IgG	(mg/dl)	1,403			2,501	
IgA	(mg/dl)	153			592	
IgM	(mg/dl)	122			94	
Bone marrow						
NCC		275,500	173,000			112,000
Megakaryocytes		180	111			
Abnormal cells	(%)	40.5		39.6		60.0
Cell marker by flow-cytometry		CD5, CD20, HLA-DR, SmIgM, λ	CD5, CD19, CD20 CD38, HLA-DR, SmIgM,	CD5, CD19, CD20 CD38, HLA-DR, Smλ	NT	NT
Chromosomal abnormality		48 XX del(2) del(6) +11 +18 −19 +r : (16/20)	89 XXXX add(1)(p13) add(6)(q11) add(8)(p11) add(9)(p11) add(11)(q23) : (15/20)		NT	48 XX del(1)t(1;3)(q32:p11), del(8)(q22) add(12)(q24) add(15)(p1) +18 +18 −19 +r : (11/20)

A small number of atypical lymphoid cells were detected in the peripheral blood smears in a few cases.

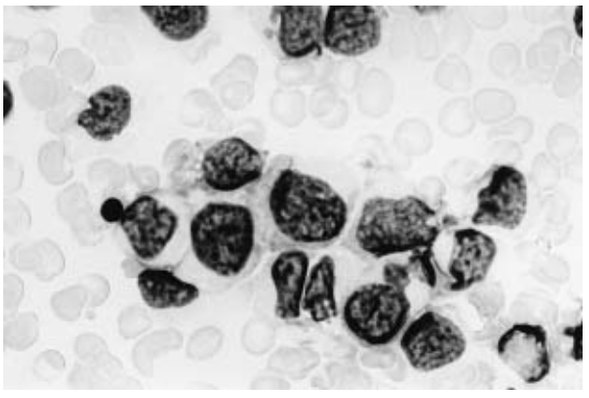

Fig. 1. Aspiration smear of the bone marrow of case 1 (May-Giemsa staining).
Aggregated lymphoma cells are present. A large, irregular-shaped nuclei can be observed.

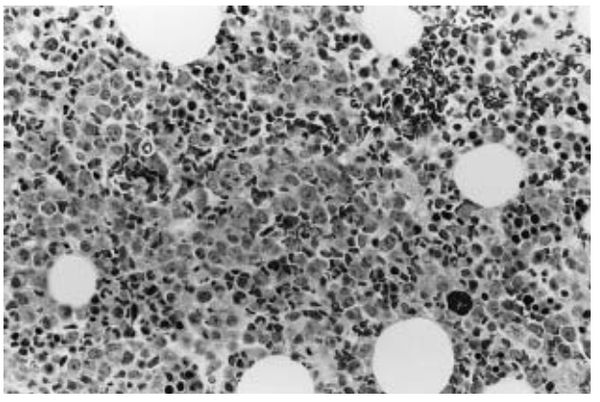

Fig. 2. Clot section of the bone marrow aspiration of case 1 (Hematoxylin-eosin staining).
A diffuse proliferation of the lymphoma cells can be seen. The lymphoma cells have a round or indented nucleus with vesicular chromatin and occasional small nucleoli.

The lymphoma cells accounted for 39.6% to 60% in NCC 112,000 to 275,500/μl of aspiration smears of the bone marrows. There was no evidence of B-CLL or MCL in examined bone marrows. The aggregated lymphoma cells which were uniformly large in size had basophilic cytoplasm with a round or slightly irregular nucleus (Fig. 1). The lymphoma cells in case 2 were twice as large as in the other cases. One or more small sized-vacuoles were occasionally seen in the cytoplasm. In only case 2, a small number of macrophages was seen and occasionally showed phagocytes.

The clot sections of the bone marrow aspiration materials showed normo- or hypercellularity with many clusters of lymphoma cells, which had a round or indented nucleus with vesicular chromatin and occasional small nucleoli (Fig. 2). The mitotic rate was high. A diffuse proliferation of the lymphoma cells was seen in cases 1 through 3 (diffuse type), whereas a sinusoidal proliferation of the lymphoma cells was observed in cases 4 and 5, showing a intravascular proliferation pattern (intravascular type). Hemophagocytosis was not found.

In flow cytometric analysis of the cases 1 trough 3, the lymphoma cells were positive for CD5, CD19, CD20, CD38, surface membrane (Sm) IgM andλ, but not for CD3, CD4, CD8, CD10, CD21, CD23, CD25 or CD30 (Table 2). The immunoperoxidase staining of the paraffin embedded sections exhibited CD5, CD20 & bcl-2 in all 5 cases, bcl-6 in 4 out of the 5 cases and Ig in 3 out of the 5 cases. (Table 3 and Fig. 3). All the cases were negative for CD3, CD10, CD23, CD30, cyclinD1 and TdT. Many gains and losses of whole chromosomes and parts of chromosomes were identified in 3 cases examined. No common abnormalities were present (Table 2). Southern-blot analysis in case 2 showed a rearrangement band with the IgH gene probe and the germline band with the TCRβprobe. Semi-nested PCR of the IgH gene amplified a discrete band in cases 1, 2, 4 and 5, as well as a smear in case 3. Nucleotide sequences of CDR2 and FW3 were compared to the closest germline sequence in the database. All four cases exhibited an in-frame sequence. The frequency of somatic mutations ranged between 2.1 and 11.1% with no intraclonal diversity (Table 4). Mutation frequency of four cases is shown in Table 4. Intraclonal diversity of the IgH gene of case 2 was not found.

An autopsy was performed in case 2. A diffuse lymphoma infiltrate was found in the bone marrows, the spleen, portal areas and sinuses of the liver. Also slightly swelling lymph nodes of mesenterium, hepatic hilar and retroperitoneum were observed. The lymphoma cells were also identified in vessels of various organs.

DISCUSSION

In this report, we have described five cases of $CD5^+$ DLBCL where the initial findings were bone marrow involvements in the absence of lymphadenopathy.

DLBCL presenting bone marrow involvements in the absence of lymphadenopathy is rare.

Table 3. Histology and immunohistology of CD5+ diffuse large B-cell lymphoma

No.	Histology			Immunohistology (Paraffin embedded tissue)									
	Cell-morphology	Intravascular pattern	Hemephagocytosis	CD3	CD5	CD10	CD20	CD23	CD30	Ig	TdT	bcl-2	bcl-6
Case 1	Centroblastic	−	−	−	+	−	+	−	−	μδλ	−	+	+
Case 2	Centroblastic	−	+/−*	−	+	−	+	−	−	μκ	−	+	+
Case 3	Centroblastic	−	−	−	+	−	+	−	−	−	−	+	−
Case 4	Centroblastic	+	−	−	+	−	+	−	−	μλ	−	+	+
Case 5	Centroblastic	+	−	−	+	−	+	−	−	−	−	+	+

*, few macrophages with red blood cell-hemophagocytosis were observed.

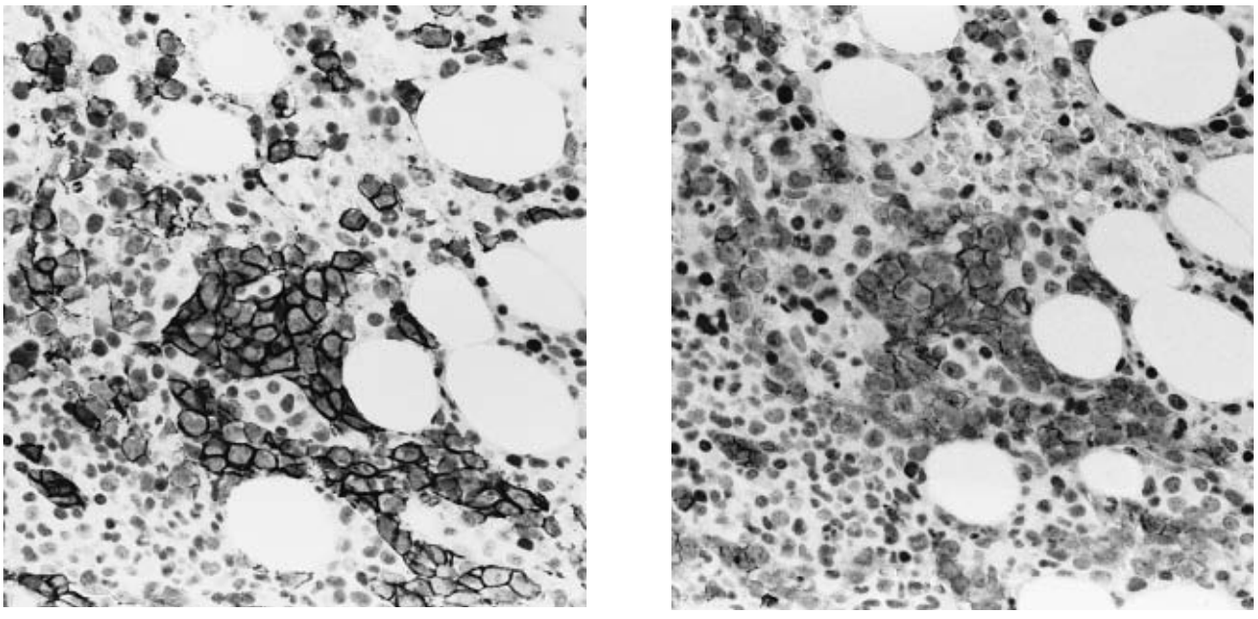

Fig. 3. Immunoperoxidase staining on case 1.
Both of CD20 (left) and CD5 (right) react with the lymphoma cells.

Table 4. Somatic mutation analysis of the VH gene in CD5+ diffuse large B-cell lymphoma

	R/S (CDR2)	R/S (FW3)	Number of mutations/ Total number of observations (%)	VH family	Intraclonal diversity
Case 1	0/0	6/2	8/144(5.6%)	4	
Case 2	2/0	2/0	4/147(2.7%)	3	No
Case 3	ND				
Case 4	1/1	8/6	16/144(11.1%)	4	
Case 5	0/0	1/2	3/144(2.1%)	4	

Abbreviations: R/S, number of replacement mutation/ number of silent mutation.
ND, no data, because the PCR of the IgH gene only lead to smeary bands in this case.
A part of data was published elsewhere[10].

Wong *et al*., in 1992, reported 14 cases of large cell lymphoma initially presenting bone marrow involvements in the absence of peripheral lymphadenopathy[14]. These patients frequently have fever of unknown origin at the time of presentation and have a poor prognosis, with survival being measured in days to weeks for most patients reported. Wong's series included three cases of T-cell phenotype and four cases of B-cell phenotype, however, it is not known whether CD5+ DLBCL cases were included or not. Cytogenetic and molecular data are also

unavailable. Bain *et al*. reported on the cases of 12 patients with leukemia as a manifestation of a *de novo* DLBCL. This series includes CD5-positive and CD5-negative cases as well as 11 cases with lymphadenopathy[15].

In our experience, presentation of DLBCL in the bone marrow without lymphadenopathy accounts for less than 1% of all DLBCL cases. We have only seven cases of presentation of DLBCL in bone marrow without lymphadenopathy, which consisted of these five $CD5^+$ cases and two CD5- cases. High proportion of CD5-positivity in DLBCL presenting in the bone marrow without lymphadenopathy is presumed.

Intravascular large B-cell lymphoma (IV-LBCL) is known to occur without lymphadenopathy and the bone marrows are frequently involved[18-21]. Estalilla *et al*. reported five cases of intravascular large B-cell lymphoma (IV-LBCL) diagnosed by bone marrow biopsy[20]. Three $CD5^+$ cases were included. Kanda *et al*. also reported that four out of five cases of IV-BCL examined were positive for CD5[21]. Therefore, DLBCL presenting bone marrow involvement in the absence of lymphadenopathy consists of $CD5^+$ and CD5- cases. Among them, IV-BCL shows mainly $CD5^+$ phenotype.

In our series, whereas three cases exhibited a diffuse infiltration of the lymphoma cells (diffuse type), two cases exhibited a sinusoidal proliferation (intravascular type). The latter cases, the intravascular type, were regarded as being IV-LBCL. Peculiar profiles with respect to clinical, immunohistochemical and genetic aspects were identified in both types. There were no differences between the two types. In both types, hepatosplenomegaly, an abnormal elevation of serum LDH and soluble IL-2 receptor, and hypoalbuminemia were prominent. Recurrences in the bone marrow and an aggressive clinical course were also observed in both types. The cause of hypoalbuminemia could not be determined properly. However the lymphoma cells may infiltrate the liver, or a cytokine produced by the lymphoma cells may be related to this phenomenon.

Murase *et al*. first reported five cases of DLBCL with hemophagocytic syndrome (HS), which constitute a distinct variant of intravascular lymphomatosis (IVL), and proposed the term "Asian variant of IVL"[22]. They subsequently analyzed 25 cases of DLBCL-HS and concluded that DLBCL-HS is the equivalent of the Asian variant of IVL[23]. According to their criteria of DLBCL-HS, all cases but one, in our five case series, are regarded as DLBCL-HS. Each of the four cases fulfills at least three of the following criteria : (1) hemophagocytosis in the hematopoietic system ; (2) hepatomegaly and/or splenomegaly, (3) bone marrow invasion of the lymphoma cells ; and (4) a lack of overt lymphadenopathy and tumor formation. In our four cases, however, only one case revealed intravascular lymphoma in the bone marrow sample, whereas the other three cases did not. Case 2 initially had a diffuse proliferation of large lymphoma cells in the bone marrow, and an autopsy of this case revealed that the lymphoma cells could be widely observed in vessels of multiple organs such as lungs and kidneys. Intravascular proliferation of the lymphoma cells in this case is closely related to a hematogenous spread of DLBCL. This case is different from intravascular lymphoma. Murase et al. documented that the intravascular pattern was not seen frequently in the spleen and marrow of their series[23]. These suggest that DLBCL which occurred in the spleen or the bone marrow have a tendency of producing hematogenous spread, resulting in the Asian variant of IVL. Murase's series included only five $CD5^+$ cases (29%). This frequency of CD5 positivity is considered low. It is not comparable with our bone marrow cases.

The lymphoma cells of both types expressed CD5, IgM, IgM and IgD, bcl-2 and bcl-6, as well as CD20. $CD5^+$ DLBCL are usually positive for IgM, but negative for CD23 and CD10[10]. $CD5^+$ IV-LBCL is also reported to be CD23-negative[22]. The chromosomal abnormality was interesting : many gains and losses of whole chromosomes, and of parts of chromosomes were identified, but no cases with alteration of 3q27 were found in either type. Matolcsy *et al*. reported that bcl-6 proto-oncogene rearrangement, which is involved in chromosome band 3q27 aberrations, was found in four among nine cases of *de novo* $CD5^+$ DLBCL[2]. We have performed somatic mutation analysis of the VH gene. Although data reported on somatic mutations of various B-cell neoplasms clearly corresponds to those of their normal counterparts[23,24], $CD5^+$ B-CLL and $CD5^+$ DLBCL are exceptional. Both of $CD5^+$ B-CLL

and CD5+ DLBCL are composed of germline cases and somatically mutated cases[10]. We demonstrated that VH gene of four cases in this series exhibited an in-frame sequence and somatic mutation frequencies of diffuse type and intravascular type were 2.7% and 5.6%, on one hand, and 2.1% and 11.1%, on the other, respectively. These data suggest that CD5+ DLBCL where the initial findings were involvement of the bone marrow in the absence of lymphadenopathy, may be derived from a same counterpart as CD5+ B-CLL.

In conclusion, we reported five cases of CD5+ DLBCL, which initially presented bone marrow involvement without lymphadenopathy. These cases could be separated into an diffuse type and an intravascular type (IV-BCL) in terms of histology. Clinicopathological, immunological, cytogenetic and molecular data suggest no differences between the two types. Recurrences in the bone marrow and an aggressive clinical course were observed in both types.

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