Virchows Arch (2008) 453:501–509
DOI 10.1007/s00428-008-0673-y

ORIGINAL ARTICLE

Primary splenic diffuse large B-cell lymphoma manifesting in red pulp

Makoto Kashimura · Masahiro Noro · Bunshiro Akikusa · Atsushi Okuhara · Shuji Momose · Ikuo Miura · Masaru Kojima · Jun-ichi Tamaru

Received: 9 April 2008 / Revised: 4 September 2008 / Accepted: 5 September 2008 / Published online: 26 September 2008

Abstract We evaluated six cases of diffuse large B-cell lymphoma (DLBCL) involving the red pulp of the spleen. All had B symptoms and an aggressive clinical course. The lymphoma cells proliferated diffusely and non-cohesively in the cords of the red pulp. The lymphoma involved the bone marrow in four of the five patients and the liver in all four of the patients examined. However, lymph node (LN) involvement was rare at presentation, and systemic LN involvement was not observed even in the terminal phase. The lymphoma cells infiltrated the intrasinusoidal/intravascular and interstitial spaces of the involved tissues and were detected in the peripheral blood in two of the six patients. CD5-expressing lymphoma cells were detected in four of the five patients examined. Because these cases had some unique clinical features and occurred in distinct splenic sites, we proposed that primary splenic DLBCL manifesting in red pulp is a distinct clinicopathological entity.

Keywords Large B-cell lymphoma · Splenic red pulp · Intrasinusoidal lymphomatous infiltration · Bone marrow · Liver

M. Kashimura (✉)
Department of Hematology, Matsudo City Hospital,
4005 Kamihongo Matsudo,
Chiba 271-8511, Japan
e-mail: kasimura@mvi.biglobe.ne.jp

M. Noro · B. Akikusa
Department of Pathology, Matsudo City Hospital,
Matsudo, Japan

A. Okuhara
Laboratory of Hematology, Matsudo City Hospital,
Matsudo, Japan

S. Momose · J.-i. Tamaru
Department of Pathology, Saitama Medical Center,
Saitama Medical University,
Kawagoe, Japan

I. Miura
Department of Hematology and Oncology,
St Marianna University School of Medicine,
Kawasaki, Japan

M. Kojima
Department of Pathology and Clinical Laboratories,
Gunma Cancer Center Hospital,
Ohta, Japan

Introduction

Extranodal diffuse large B-cell lymphomas (DLBCLs) have specific clinicopathologic features that are dependent on the organ of origin [1]. Primary splenic DLBCL is a rare type of lymphoma. The majority of the DLBCLs are derived from the white pulp of the spleen and form one large, or multiple nodules [2]. Before the advent of immunohistological examination, the absence of mass formation and the diffuse infiltration of large neoplastic cells into the red pulp might have been diagnosed as malignant histiocytosis [3]. Kuratsune et al. [4] reported the first case of DLBCL that non-cohesively proliferated into the splenic red pulp and demonstrated clinicopathological features of malignant histiocytosis. Since their initial report, only 18 cases of DLBCLs non-cohesively infiltrating the splenic red pulp have been reported [4–13]. These reports support the existence of DLBCL manifesting in the splenic red pulp (DLBCLRP). Kroft et al. [8] identified two cases of DLBCLRP involving the bone marrow (BM) and the liver, and Morice et al. [10] reported two cases of DLBCLRP with prominent BM intravascular/intrasinusoidal lymphomatous infiltration.

Because there have been few case reports of DLBCLRP, there is no comprehensive list of its clinicopathological features. To clarify the features of this rare lymphoma, we describe six new cases of DLBCLRP and present an analysis of the clinicopathological features of an additional 18 previously reported cases [4–13]. After reviewing the data, we developed seven characteristic features of DLBCLRP. Based on these features, we propose that DLBCLRP is a distinct clinicopathologic entity. Moreover, we discuss the relationship between DLBCLRP and an Asian variant of intravascular lymphomatosis (IVL) with splenomegaly [14].

Materials and methods

Retrospective analyses were conducted for six patients clinicopathologically diagnosed with primary splenic large B-cell lymphoma manifesting in red pulp in our hospital from 2000 to 2007. Two hundred and ninety two patients were diagnosed with malignant lymphoma, including 228 with B-cell lymphomas (DLBCL 129, follicular lymphoma 58, marginal zone lymphoma 19, mantle cell lymphoma 6, etc), 45 with T-cell lymphomas, and 12 with Hodgkin's lymphomas during this 8-year period. Thirteen patients were diagnosed with primary splenic lymphoma, two with splenic marginal zone lymphoma, one with follicular lymphoma, and ten with DLBCL. Two patients were diagnosed as having DLBCL with a micronodular pattern. Eight patients were clinically diagnosed with DLBCLRP; however, two were excluded based on the unavailability of splenic tissues. All procedures were performed with informed consent of the patients.

Tissue specimens obtained from surgery, biopsy, or necropsy were fixed in formalin and embedded in paraffin. The paraffin-embedded sections were dewaxed with xylene. The sections were stained with hematoxylin–eosin (HE) for light microscopic examination, and by the streptavidin–biotin–peroxidase method (Nichirei Co., Tokyo, Japan) for immunohistochemical analysis. The panel of primary antibodies included L26 (CD20), JCB117 (CD79a), F7.2.38 (CD3), UCHL-1 (CD45RO), 1F8 (CD21), 124 (Bcl-2), PG-B6p (Bcl-6), MIB-1 (Ki-67), MUM1p (MUM1), IgM polyclonal (DAKO A/S, Glostrup, Denmark), MT1 (CD43), 4C7 (CD5), 56C6 (CD10), 1B12 (CD23), 1G12 (CD30), IgD polyclonal (Novocastra, Newcastle upon Tyne, UK), and SP4 (Cyclin D1, Lab Vision, Fremont, CA, USA). The primary antibodies were replaced with mouse or rabbit serum as a negative control.

Epstein–Barr virus (EBV)-encoded RNA (EBER) was detected by in situ hybridization (ISH). ISH was performed using the BioGenex Automated Staining System (i6000; BioGenex; San Ramon, CA, USA). The paraffin-embedded sections were dewaxed with xylene, treated with proteinase K, and hybridized with a fluorescein-conjugated oligonucleotide EBER probe (PR005-10X, BioGenex). To visualize the bound probe, a Super Sensitive polymer-HRP ISH Detection System (DF300-YCX, BioGenex) was used according to the manufacturer's instructions.

Karyotypes were obtained at the time of diagnosis from all six patients from spleen ($n=5$), and lymph node (LN; $n=1$) samples, as previously described [15]. Chromosomal abnormalities were described according to ISCN [16].

Serum samples were analyzed with an immunofluorescence kit using anti-EBV capsid antigen (VCA), early antigen, and EBV-encoded nuclear antigen (TFB Inc., Tokyo, Japan). Antibodies against the human immunodeficiency virus (HIV) and HTLV-1 were examined using chemiluminescent enzyme immunoassay kits (Abbot Japan and Fuji Rebio Inc., Tokyo, Japan).

We characterized the degree of extramedullary hematopoiesis in the splenic samples as erythropoietic (grade 1), granulopoietic (grade 2), and trilineage hematopoietic (grade 3). Hemophagocytosis of normal splenic histiocytes was characterized as grade 1 (<10) and grade 2 (>10) erythrophagocytes in 10 high power fields. In bone marrow samples, grade 1 indicated thrombophagocytosis and grade 2 indicated leukoerythrophagocytosis.

Results

Clinical features

In this series, five of the six patients were men (age, 64–81 years; median, 69 years). At presentation, all patients were febrile. Four patients (nos. 1, 3–5) had splenomegaly without lymphadenopathy. One patient (no. 2) had localized LN swelling at presentation and a second patient (no. 6) had localized LN swelling at the time of surgery, which suggested the presence of a primary splenic lymphoma. All patients had high levels of lactate dehydrogenase (LDH), soluble interleukin-2 receptor, hypoalbuminemia, and moderately elevated C-reactive protein (Table 1). Differential blood counts revealed the following: Marked lymphocytopenia was detected in all patients except 1 (no. 5), thrombocytopenia was detected in five patients (nos. 1–5), and anemia was detected in four patients (nos. 2, 4–6). In two patients (nos. 1 and 4), lymphoma cells were detected in the peripheral blood (Table 2). Cholestasis and hepatocellular damage with hepatomegaly was observed in four patients (nos. 1, 2, 5, and 6), suggesting hepatic involvement.

Characteristic clinical features of DLBCLRP (Table 2) included BM involvement in four of the five patients (nos. 1, 2, 5, and 6; determined at admission), and hepatic

Table 1 Laboratory data, clinical stage, and international prognostic index upon admission

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age (years)/sex	75 M	68 M	70 M	81 M	64 F	65 M
White blood cell count, $\times 10^9$/L	2.4	3.9	7.6	7.4	2.3	2.9
Neutrophils, $\times 0^9$/L	1.8	3.3	6.6	4.4	0.9	2.2
Lymphocytes, $\times 10^9$/L	0.4	0.2	0.4	0.7	1.1	0.5
Hemoglobin level, g/L	140	116	133	100	87	91
Platelet count, $\times 10^9$/L	40	83	99	116	38	137
LDH	1,045	4,312	1,822	2,690	460	404
ALP	842	1,677	228	193	2,102	1,168
γ-GTP	489	374	120	26	191	153
LAP	131	225		80	161	138
AST	217	380	55	67	51	49
ALT	34	211	37	11	36	58
CRP	165	122	105	93	58	25
TP	56	53	65	56	60	49
Albumin	27	26	30	29	28	25
sIL-2R	4,050	5,060	5,250	7,790	19,500	15,800
Ferritin	847	na	710	na	276	2,110
Hemophagocytosis in BM[a]	+	+	+	na	+	++
Clinical stage	4B	4B	4B	4B	4B	4B
IPI	H	H	H	H	H	H

Abbreviation and normal level (in parentheses): *M* male, *F* female, *ALP* alkaline phosphatase (130–350 in cases 1, 4, 5, and 6; 66–220 in cases 2 and 3), *LDH* lactate dehydrogenase (80–200 in cases 1, 4, 5, and 6; 255–434 in cases 2 and 3), *LAP* leucin aminopeptidase (M 11–64, F 8–45), *AST* aspirate aminotransferase (0–31), *ALT* alanine aminotransferase (0–41), *CRP* C-reactive protein (less 3 mg/L), *sIL-2R* soluble interleukin-2 receptor (220–530 U/mL), *TP* total protein (67–83 g/L), albumin (38–53 g/L), ferritin (M 27–320, F 3.4–89 ng/mL), *BM* bone marrow, *na* not available, *IPI* international prognostic index, *H* high risk

[a] Grading of hemophagocytosis of normal histiocytes in bone marrow as grade + and ++ indicating thrombophagocytosis and leukoerythrophagocytosis

infiltration in four patients (nos. 1, 5, and 6, and no. 2 determined at biopsy and necropsy, respectively). Enlarged LN were rare and limited to localized areas. Systemic lymphadenopathy was not observed even during the terminal stage of the disease. Hemophagocytosis by normal histiocytes was observed in the spleen and BM of all six patients.

We assessed two treatment groups: (a) three patients (nos. 2–4) were treated with six courses of CHOP (combination of doxorubicin, vincristine, cyclophosphamide, and prednisolone), and (b) the remaining three patients (nos. 1, 5, and 6) were treated with six courses of rituximab and CHOP followed by two courses of rituximab, etoposide, and nimustine hydrochloride with intrathecal administration of methotrexate, Ara-C, and prednisolone for prophylaxis of central nervous system lymphoma.

Follow-up (1–75 months; median, 38 months) information was obtained for all six patients. Two patients (nos. 4 and 2) of

Table 2 Clinicopathological features of cases with primary splenic LBC lymphoma diffusely infiltrating the red pulp

			Spleen	Red pulp		HPC[a]	EMH[b]	BM	ISI	II	Liver	ISI	Portal	Lymph node		Blood
			Weight	Cord	Sinus									Splenic H	Syst	
Patient 1	75	M	525	+	+	++	+++	+	+	+	+	+	+	−	−	8%
Patient 2	68	M	na	+[c]	+[c]	+	+	+	−	+	+[c]	+[c]	+[c]	na	Inguinal	−
Patient 3	70	M	900	+	+	++	+	−	−	−	na	na	na	+	−	−
Patient 4	81	M	546	+	+	+	+	nd	na	na	na	na	na	−	−	16%
Patient 5	64	F	675	+	+	+	+++	+	−	+	+	+	+	+	−	−
Patient 6	65	M	220	+	+	+	+	+	−	+	+	+	+	−	Abdominal	−

ISI intrasinusoidal infiltration, *II* interstitial infiltration, *HPC* hemophagocytosis, *EMH* extramedullary hematopoiesis

[a] Grading of hemophagocytosis of normal histiocytes in spleens as + and ++ indicating less than ten and more than ten erythrophagocytes in 10 high power fields

[b] Grading of extramedullary hematopoiesis in spleens as +, ++, and +++ indicating erythropoiesis, granulopoiesis, and trilineage hematopoiesis, respectively

[c] Necropsy

the former group died of lymphoma, within 1 and 5 months of the onset of the disease, respectively. All three patients of the latter group survived following first complete remission.

All patients were negative for HIV and HTLV-1. Antibodies to EBV indicated an old infection. M-protein and autoantibodies, including Coombs' test, were not detected in any of the patients.

Pathologic features

Spleen size and weight were abnormally high in all informative cases (nos. 1, 3, 4, 5, and 6). In one patient (no. 2), spleen weight was not available (Table 2). The weight of the spleen, which is 80 to 120 g in healthy Japanese adults, was 220 to 900 g (median, 546 g) in the patients. Cut surfaces of the spleens showed beef red color and a lack of mass formation. In all six patients, the splenic red pulp was expanded and diffusely infiltrated by non-cohesive large lymphoma cells (nos. 1, 3, 4, 5, and 6 before therapy, and no. 2 at necropsy). Lymphoma cells were present in the cord and had infiltrated the sinuses to various degrees (Fig. 1a–c). In one patient (no. 5), a small number of large lymphoma cells were scattered among numerous T cells/histiocytes, and the patient was diagnosed with T-cell/histiocyte-rich B-cell lymphoma. All patients had extramedullary hematopoiesis of erythroblasts. Trilineage extramedullary hematopoiesis was observed in two patients (nos. 1 and 5). In all six patients, there were an abnormally high number of histiocytes, which were engaged in hemophagocytosis. In two (nos. 1 and 3) of these patients, there was intensive hemophagocytosis.

In one patient (no. 1), CD20 and CD34 immunohistochemistry revealed large lymphoma cells that were visible in the sinusoids and a few were present in the cords (Fig. 2a,b). In the remaining patients, the lymphoma cells had diffusely infiltrated the interstitial spaces (Fig. 2e). It is possible that the intravascular lymphomatous infiltration of this lymphoma disappeared due to the destruction of endothelial cells by lymphoma cells in the significantly infiltrated lesions. Thrombophagocytosis in the BM was observed in all patients, and leukoerythrophagocytosis was detected in one of the five patients examined (no. 6).

In four patients examined (nos. 1, 5, and 6 at biopsy, and no. 2 at necropsy), lymphoma cells had infiltrated into the sinusoidal and portal areas of the liver (Fig. 2c).

In one patient (no. 2), the lymph nodes were replaced completely by lymphoma cells. Intravascular infiltration of lymphoma cells was not visible in the HE-stained samples. However, diffuse infiltration into the vascular spaces was clearly detected by immunostaining for CD34 (Fig. 2d). Furthermore, in one patient (no. 4), intravascular infiltration of lymphoma cells was prominent in the adipose tissue of the splenic hilus (Fig. 2f), and in another patient (no. 1) infiltration into the subcutaneous vessels was observed. In three patients (nos. 2–4), lymphoma cells had infiltrated below the endothelial cells or into the vascular wall (Fig. 1d).

Immunohistochemistry of lymphoma cells

All patients had lymphoma cells positive for CD20 and Bcl-2 and negative for EBER, Cyclin D1, and CD23 (Table 3). The cells in two patients (nos. 3 and 6) were positive for CD10. Of the remaining four patients with CD10-negative lymphoma cells, the cells were negative for bcl-6 in three patients (nos.1, 4, and 5), and positive for bcl-6 and MUM1 in one patient (no. 2). CD5-positive cells were found in four of five patients by flow cytometry and three of five patients by immunohistochemistry. Cells of five patients examined were positive for surface membrane (sm) IgM and negative for smIgD (Table 3). Positive rate of Ki67 varied from 10% to 80%.

Chromosomal analysis

Chromosomes were analyzed in all six patients. Two patients (nos. 5 and 6) had normal karyotypes. The remaining four patients (nos. 1–4) were CD5-positive and carried complex chromosomal aberrations. One of these patients (no. 4) showed the 11q13 aberrations, whereas another (no. 2) showed the 3q27 abnormality (Table 4).

Discussion

Here, we have reported six cases of DLBCLRP. To further clarify the clinicopathological characteristics of DLBCLRP, we analyzed a total of 24 cases (Tables 5 and 6) of DLBCLRP, including six cases encountered by one of the authors (M. Kashimura). In the present series, 18 of the 24 patients were men and the age range of all the patients was 40–81 years (median, 64.5 years).

The size and weight of the spleens that were examined had increased in all informative cases. Spleen weight data were not available for five of the 24 patients. The weight of the spleen, which is 80–120 g and 110–170 g in healthy adults of Japan and western countries, respectively, was 220 to 2,600 g (median, 1,300 g) in the present cases.

We found that DLBCLRP has seven characteristic clinicopathological features as shown in Table 7 and a pattern of infiltration of the spleen. Lymphoma cells were present in the cord in all but one case of the 24 cases of splenic infiltration. In the remaining patient, the lymphoma cells were restricted to the sinusoids [7].

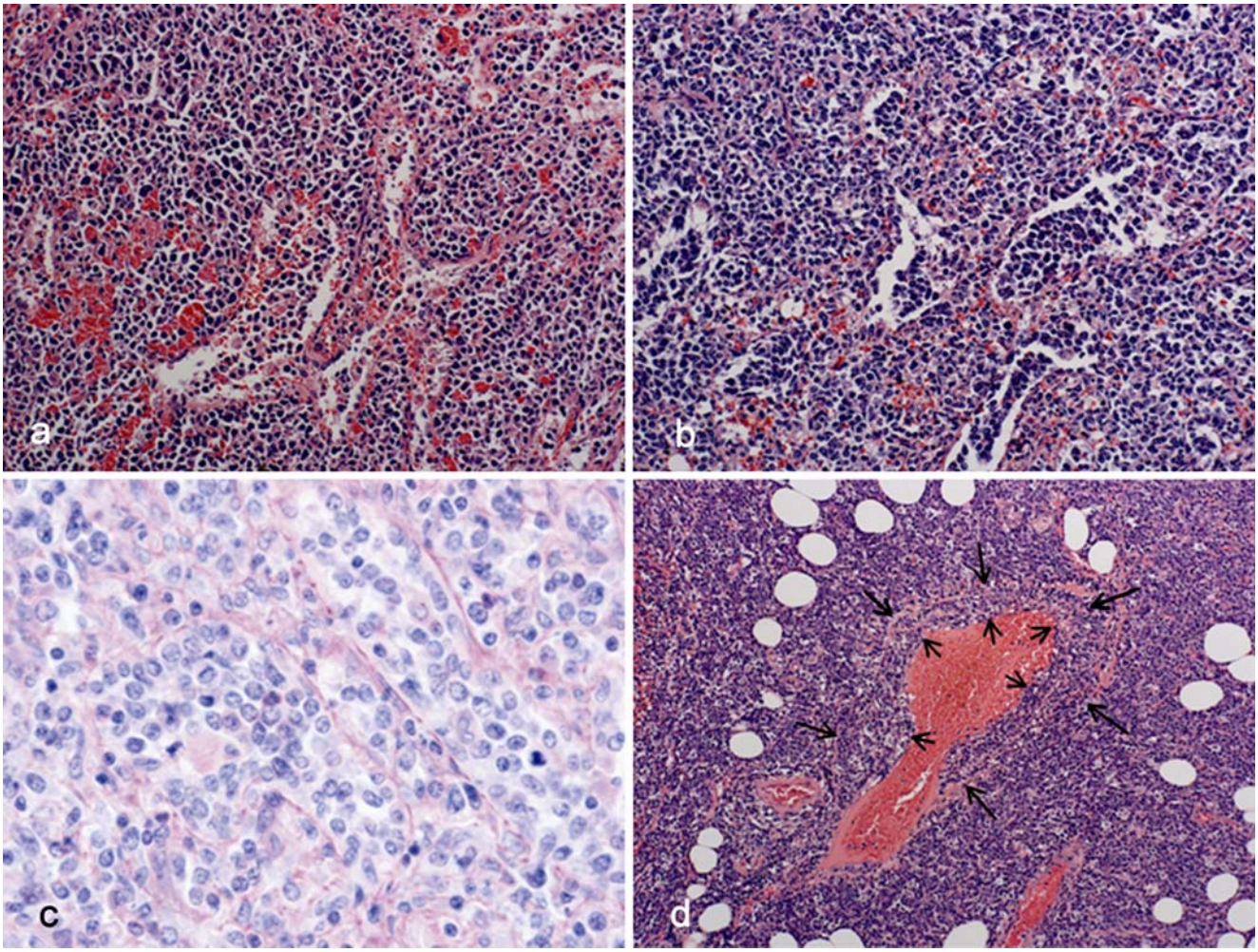

Fig. 1 Lymphoma cells infiltrate the Billroth's cords and sinuses of the splenic red pulp and the vascular wall. **a** Medium power field of the affected spleen. The tumor cells have diffusely infiltrated the Billroth's cords of red pulp, case 3 HE staining. Original magnification, ×100. **b** Medium power field of the affected spleen. Tumor cells have diffusely infiltrated the Billroth's cords as well as the sinusoid, case 4 HE staining. Original magnification, ×100. **c** High power magnification of PAS-stained sections showing the basement membrane of the sinusoid. Note the intrasinusoidal and extrasinusoidal lymphoma cell infiltrations, case 1. Original magnification, ×350. **d** Monomorphous lymphoma cell infiltration in the vascular wall. *Short arrows* indicate the vascular endothelial cells and *long arrows* indicate the arterial adventitia. Case 3 HE staining. Original magnification, ×40

At presentation, lymphoma cells were frequently found in the BM (13/20, 65%) and the liver (11/17, 65%). However, LN involvement was rare and limited to a localized area (10/21, 48%), and LN swelling was not significant. Systemic LN swelling was not observed, even during the terminal phase of the disease.

Another characteristic of DLBCLRP is an intrasinusoidal infiltration of lymphoma cells in the BM (3/7, 43%) and the liver (8/8, 100%) as well as interstitial infiltration of lymphoma cells in the BM (10/11, 91%) and the liver (7/8, 88%). Furthermore, we observed intravascular infiltration of lymphoma cells in the LN (no. 2) and the adipose tissue (nos. 1 and 4), and the presence of lymphoma cells in the peripheral blood (10/23, 43%). This rate appears higher than that observed for nodal DLBCL.

Moreover, we observed lymphoma cell surface expression of the CD5 antigen in eight of the ten patients (80%) [8, 10, 12], including four of our original six patients. Low intensity of the lymphoma cell CD5 antigen might not be detectable by immunohistochemistry (Tables 3 and 6).

None of the patients had a history of chronic lymphocytic leukemia. Transformation of splenic marginal zone lymphoma (SMZL) into DLBCL [17, 18], which might be associated with CD5-positive transformation, must be ruled out in order to diagnose DLBCLRP as the de novo DLBCL. Despite a careful study, we were unable to find proliferative lesions of medium-sized lymphoma cells in any of our cases, which is usually observed when DLBCL transforms from SMZL. Lymphoma cells of all five patients examined were positive for smIgM; however, they were

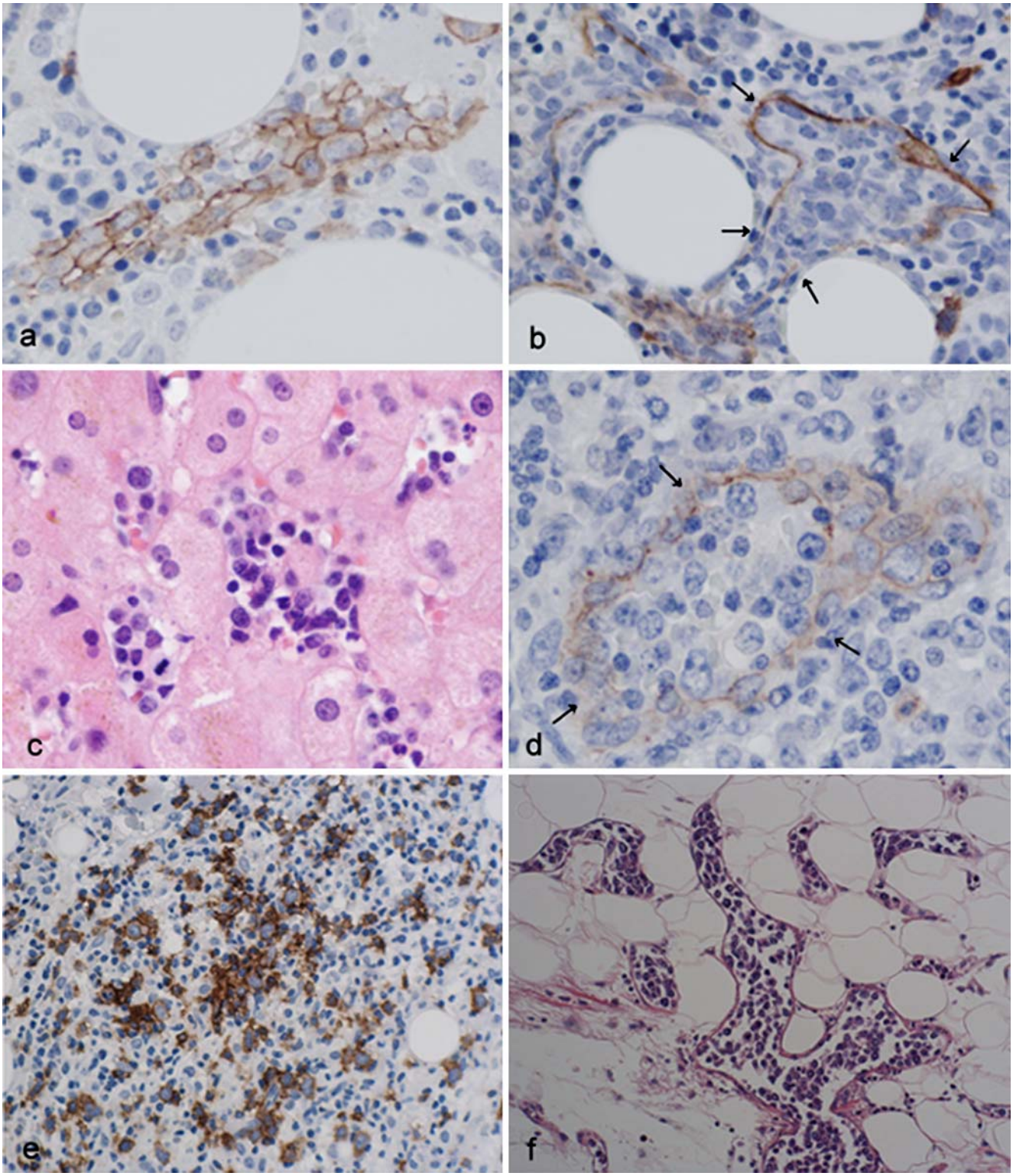

Fig. 2 Intrasinusoidal/intravascular lymphoma cell infiltration in the bone marrow, liver, lymph node, and adipose tissue. **a** CD20 immunostaining showing the predominantly intrasinusoidal pattern of marrow infiltration by large B-cell lymphoma, case 1. Original magnification, ×450. **b** CD34 immunoperoxidase staining of bone marrow sinusoidal cell lining (*arrow*). Large lymphoma cells are present in a sinus, case 1. Original magnification, ×350. **c** High power field of affected liver. Note an intrasinusoidal infiltration of large lymphoma cells, case 1 HE staining. Original magnification, ×350. **d** CD34 immunoperoxidase staining highlights the intravascular lymphoma cells from diffuse lymphomatous infiltration of lymph node parenchyma. *Arrows* show sinusoidal cell lining, case 2. Original magnification, ×400. **e** Medium power field of the affected BM. The tumor cells diffusely infiltrated in the cords of BM, case 6. Original magnification, ×100. **f** Perisplenic adipose tissue. Note the intravascular infiltrate of large lymphoma cells, case 4 HE staining. Original magnification, ×150

Table 3 Immunophenotype of primary splenic LBC lymphoma diffusely infiltrating the red pulp

	CD20	CD5: FCM	CD5: IHC	CD10	Cyclin D1	bcl-2	CD23	MUM1	Ki67 (%)	bcl-6	EBER-ISH	smIg FCM	IgM IHC	IgD IHC
Patient 1	+	+	Weak+	−	−	+	−	−	20	−	−	M-κ	+	−
Patient 2	+	+	+	−	−	+	−	+	20	+	−	κ	na	na
Patient 3	+	+	−	+	−	+	−	+	30	−	−	λ	+	−
Patient 4	+	+	+	−	−	+	−	−	10	−	−	M-λ	+	−
Patient 5	+	na	−	−	−	+	−	−	80	−	−	na	+	−
Patient 6	+	−	−	+	−	+	−	−	20	+	−	na	+	−

FCM flow cytometry, *IHC* immunohistochemistry, *ISH* in situ hybridization, *smIg* surface membrane immunoglobulin

negative for smIgD, which is unusual for SMZL cells [19, 20]. Although four of the six patients (nos. 1–4) showed complex chromosomal aberrations, none of them had trisomy 3 or allelic loss of 7q21-32, which is usually observed with SMZL [21, 22]. Clinically, autoimmune phenomena and the presence of M-protein have been frequently observed in SMZL patients [17, 20, 23–25], but these were not observed in our cases. Therefore, our cases are not likely to be associated with SMZL transformation.

Therefore, these four patients (nos. 1–4) were diagnosed with de novo CD5-positive DLBCL [26]. Two of these patients with complex chromosomal aberration had chromosome abnormalities involving 3q27 (no. 2) and 11q13 (no. 4), which have been previously reported in CD5-positive DLBCL [27]. Yamagichi et al. [28] recently reported four morphological variants of de novo CD5-positive DLBCL. Using the molecular classification system by Hans et al. [29], lymphoma cells of 82% of the patients were of the non-germinal center B-cell type and the others were germinal center B-cell type. Interestingly, intravascular and/or intrasinusoidal lymphomatous infiltration was observed in 38% of the cells. Although data were not shown, the bone marrow, liver, and spleen were reported to be the most frequently involved anatomical sites. De novo CD5-positive DLBCL is heterogeneous in morphological, molecular, and clinical aspects. A potion of the cases with de novo CD5-positive DLBCL with splenomegaly may be DLBCLRP. While lymphoma cells of two other patients (nos. 5 and 6) in this study were CD5 negative, clinicopathological features other than CD5 were identical in these six patients (Table 7).

This high rate of CD5-positive lymphoma cells may be an aberrant expression of the aggressive lymphoma cells [30–32]. In previous genetic studies, it was suggested that de novo CD5-positive DLBCL originates from somatically mutated CD5 progenitor B cells [33, 34]. The mechanism of expression of CD5 in this lymphoma remains unsolved.

The lymphoma cells positive for CD10 in two patients (nos. 3 and 6) were considered germinal center B-cell type. Of the remaining four patients that were negative for CD10, three were negative for bcl-6 (nos. 1, 4, and 5) and one was positive for bcl-6 and MUM1 (no. 2). Therefore, later these were considered non-germinal center B-cell type (Table 3) [29]. Clinicopathological features were not clearly distinguishable among patients with germinal center B-cell-like DLBCL and those with non-germinal center B-cell-type DLBCL, except for the appearance of the lymphoma cells in the peripheral blood. However, our limited sample size makes it difficult to regard this feature as significant.

Table 4 Chromosomal analysis of DLBCLRP

Patient 1	47, XY, der(1)del(1)(p?)dup(1)(q25q32), add(6)(q21), der(9)add(9)(p13)add(9)(q34), add(10)(q26),+16,−19,+r	20/20
Patient 2	44, XY, t(3;14)(q27;q32),−4, add(5)(p11),−9, −10,add(12)(p13),−13,−15, add(18)(p11), add(21)(p11), +der(?)t(?;13)(?;q12), der(?)t(?;15)(?;q11); +mar	5/20
	46, XY	15/20
Patient 3	48,X, −Y, add(2)(p23), del(6)(q?), +add(7)(q11), add(7)(q22)x2, +11, +add(18)(q21)	20/20
Patient 4	46, X, -Y, der(2)add(2)(p13)t(2;11)(q37;q13),add(4)(q35),del(6)(q?), add(8)(p11), add(11)(q13), +18	4/4
Patient 5	46,XX	20/20
Patient 6	46,XY, inv(9)(p12q13)	20/20

Table 5 Summary of the clinical findings DLBCLRP including our cases [4–13]

Age distribution (years)	40–81 (median 64.5)
Male/female	18:6
B symptom	14/15 (93%)
Weight of the spleen (g) (19 cases)	220–2,600 (median 1,300)
Appearance of tumor cells in peripheral blood	10/23 (43%)
Bone marrow involvement	13/20 (65%)
Liver involvement	11/17 (65%)
Lymph node involvement	
Splenic hilus	5/16 (31%)
Abdominal	6/21 (29%)
Other	4/21 (19%)
Systemic lymph node involvement	0/21 (0%)

Table 7 Clinicopathological characteristics of DLBCLRP

Lymphoma cells infiltrate diffusely and non-cohesively in the cords of splenic red pulp
Lymphoma cells frequently involve the bone marrow and the liver at presentation
Lymph node involvement is rare and limited to the local area, and lymph node swelling is not significant. Systemic lymph node swelling is not observed even in the terminal phase of the disease.
Intrasinusoidal as well as interstitial lymphomatous infiltration is observed in involved tissues
Lymphoma cells are often found in the peripheral blood
Lymphoma cells frequently express surface CD5 antigen
Hemophagocytosis of normal histiocytes is observed in the bone marrow and the splenic red pulp

DLBCLRP is a clinically highly aggressive lymphoma compared with conventional CD5-negative DLBCL and de novo CD5-positive DLBCL. The patients with de novo CD5-positive DLBCL showed more aggressive clinical features and parameters (LDH level, clinical stage, and international prognostic index (IPI) score) than those with CD5-negative DLBCL [26]. All six patients in this study had fever, high LDH levels, clinical stage 4B, and a high-risk IPI score.

Another characteristic feature of DLBCLRP is hemophagocytosis of normal histiocytes observed in all the BMs (intensive cases; 1/5, 20%) and spleens (intensive cases; 2/6, 33%). This might be due to the high cytokine concentrations produced by these aggressive lymphoma cells [35]. But none fulfilled the criteria for adult hemophagocytic syndrome [36–38]. Moreover, the frequent presence of B symptoms (14/15) was a characteristic of DLBCLRP. On the basis of these clinicopathological features, we propose that DLBCLRP is a distinct clinicopathological entity.

Table 6 Summary of pathological and phenotypic findings [4–13]

Pattern of splenic infiltration	
Splenic sinus	14/19 (74%)
Splenic cord	23/24 (96%)
Pattern of liver infiltration	
Intrasinusoidal	8/8 (100%)
Portal area	7/8 (88%)
Pattern of bone marrow infiltration	
Intrasinusoidal	3/7 (43%)
Interstitial	10/11 (91%)
Hemophagocytosis in spleen	11/12 (92%)
Hemophagocytosis in bone marrow	4/5 (80%)
Extramedullary hematopoiesis in spleen	7/10 (70%)
Tumor cell phenotype	
B-cell marker 24/24	24/24 (100%)
CD5 (FCM)	8/10 (80%)
CD5 (IHC)	3/12 (25%)
CD10 (IHC)	4/14 (29%)

The clinicopathological features of the cases of the Asian variant of IVL (AIVL) with splenomegaly are similar to those of DLBCLRP. Splenectomy samples from two patients with AIVL had cut surfaces that were beef red in color and there was a diffuse, large, B-cell lymphoma cell infiltrate in the red pulp [14]. However, the international consensus meeting of intravascular large B-cell lymphoma (IVLBCL) proposed a new definition of IVLBCL, which included cases from both western and Asian countries [39]. They added the criterion of a concomitant minimal extravascular location of the neoplastic cells [40] to the WHO criteria, which states that the neoplastic lymphocytes might only be present in the lumina of the small vessels, such as capillaries [41]. DLBCLRP produced interstitial infiltration as well as intrasinusoidal lymphomatous infiltration in the BM, liver, and LN (Fig. 2d and Table 5). The infiltration of DLBCLRP lymphoma cells into the liver and BM was a more characteristic feature of the SMZL than AIVL. However, further studies are required to clarify these relationships.

Briefly, DLBCLRP is an aggressive lymphoma with B symptoms. During the early phase, it spreads to the liver and/or BM via intrasinusoidal and/or interstitial infiltration. Because our characterization of DLBCLRP was based on the observations of a limited number of patients, further studies are required to confirm that DLBCLRP is a distinct clinicopathologic entity.

Conflict of interest statement The authors declare no competing financial interests.

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