Clinical Characteristics of B-cell Lymphoma-associated Hemophagocytic Syndrome (B-LAHS): Comparison of $CD5^+$ with $CD5^-$ B-LAHS

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Abstract

Objective **B-cell lymphoma-associated hemophagocytic syndrome (B-LAHS) has been increasingly reported in Asia and is regarded as a variant of intravascular lymphomatosis (IVL). Recently CD5 was reported to be expressed in some cases of diffuse large cell lymphoma and IVL. We therefore examined the expression of CD5 on lymphoma cells in B-LAHS and compared the clinical and laboratory data between $CD5^+$ and $CD5^-$ B-LAHS.**

Methods **The expression of CD5 on lymphoma cells was examined using flow cytometry and immunohistochemical analysis of paraffin sections. The clinical records were reviewed to characterize clinical features.**

Patients **Twelve patients with B-LAHS; ten men and two women, age ranging from 41 to 82 years (median, 63.5 years) were included in this study.**

Results **B-LAHS is characterized by fever and hepatosplenomegaly without lymphadenopathy at the initial presentation. Histological examination showed hemophagocytosis and infiltration of lymphoma cells in the bone marrow, and in some cases intravascular proliferation of lymphoid cells characteristic of IVL. All patients showed increased levels of lactate dehydrogenase, C-reactive protein, ferritin and soluble interleukin-2 receptor. In eight of the twelve patients, lymphoma cells were positive for CD5. But no differences were observed in the clinical or laboratory findings between $CD5^+$ B-LAHS and $CD5^-$ B-LAHS.**

Conclusion **No clinical differences were observed between $CD5^+$ B-LAHS and $CD5^-$ B-LAHS. Further studies are required to elucidate the differences in pathogenesis between these two subgroups of B-LAHS.**
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Key words: intravascular lymphomatosis, hemophagocytic syndrome

Introduction

Recently, B-cell lymphoma-associated hemophagocytic syndrome (LAHS) has been increasingly reported in Asian countries, especially in Japan (1, 2). A survey of 142 Japanese LAHS cases recently conducted by Takahashi et al (3) demonstrated that half (68 cases) were B-LAHS. We previously reported the clinical, immunological and cytogenetic features of seven cases of B-LAHS (2). These seven cases were characterized by fever, hepatosplenomegaly without lymphadenopathy at initial presentation, bone marrow involvement of lymphoma cells with hemophagocytosis, CD5 expression on lymphoma cells and abnormalities of chromosomes 8p21 and 19q13. Histologically, lymph nodes were diffusely invaded with large atypical lymphoid cells, compatible with a diagnosis of diffuse large cell lymphoma (DLCL) (4). In addition, intravascular proliferation of lymphoid cells characteristic of intravascular lymphomatosis (IVL) was reported (1).

CD5 is expressed in most cases of chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL) (5, 6). However, de novo $CD5^+$ diffuse large B-cell lymphoma was recently reported as a distinct subgroup of DLCL, which is derived from a subset of B1-cells (7, 8).

In the present study, we examined the expression of CD5 of lymphoma cells using flow cytometry and immunohistochemical analysis of paraffin sections, and compared the clinical and laboratory data between $CD5^+$ and $CD5^-$ B-LAHS.

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Materials and Methods

Patients

Twelve patients with B-LAHS were included in this study. The clinical records were reviewed to characterize clinical features.

Morphology

The bone marrow smears were stained with Wright-Giemsa. The bone marrow, lymph node and splenectomized samples were subjected to histological examination.

Immunophenotyping

The surface immunophenotyping of lymphoma cells was performed by flow cytometry using a broad panel of monoclonal antibodies as described previously (9). Mononuclear cells were separated from heparinized bone marrow or lymph node samples using Ficoll-Hypaque centrifugation. Cells were stained with the following antibodies against B-cell-associated markers: fluorescein isothiocyanate (FITC)-conjugated anti-CD5 (UCHT2; Pharmingen, San Diego), CD10 (J5; Coulter, Hialeah), and CD20 (B9E9; Immunotech, Marseilles, France) and anti-immunoglobulin. After incubation with these antibodies, cells within the 'blast window' were analyzed using an EPICS profile flow cytometer (Coulter). Seven of the twelve patients (cases 2, 3, 4, 6, 7, 8 and 10) had low percentages of lymphoid cells in bone marrow. We therefore considered a marker to be positive when more than 30% of the cells analyzed were positive, after subtracting negative controls stained with matched mouse isotypic-irrelevant monoclonal antibodies. For formalin-fixed, paraffin-embedded tissue sections, antigen retrieval by heating was performed before staining with anti-CD5 monoclonal antibody (M7194, Dako, Glostrup). Catalyzed signal amplification (CSA) system (Dako) was used for qualitative identification of antigens by light microscopy.

Statistical analysis

Student's *t*-test was used to compare the clinical and laboratory data between $CD5^+$ and $CD5^-$ B-LAHS. $p<0.05$ was considered statistically significant.

Results

Clinical features

The clinical features of the twelve patients are summarized in Table 1. The patients consisted of ten men and two women; age ranging from 41 to 82 years, with a median of 63.5 years. At the time of initial presentation, all of the patients had fever and eleven of the twelve patients had splenomegaly. Nine patients also had hepatomegaly. Only two of the twelve patients had lymphadenopathy and three of them had lung lesions. None of the patients had skin lesions nor neurological symptoms. Hepatomegaly was observed in five of the eight patients with $CD5^+$ B-cell lymphoma, while all four $CD5^-$ lymphoma patients had hepatomegaly, but this difference was not significant (Table 1). No differences were observed in age, sex or other clinical features between $CD5^+$ and $CD5^-$ B-cell lymphoma.

Laboratory findings

The laboratory findings at initial presentation are summarized in Table 2. Eleven of the twelve patients showed anemia, four showed leukocytopenia, and ten patients showed thrombocytopenia. All of the patients showed hemophagocytosis with various percentages of lymphoma cell infiltration in the bone marrow (BM) (0 to 47.7%). An increased level of alanine aminotransferase (ALT) was observed in six patients, total bilirubin in one, and D-dimer in four patients. Other findings of interest were increased levels of lactate dehydrogenase (LDH), C-reactive protein (CRP), ferritin, and soluble interleukin-2 receptor (sIL-2R) in all patients examined. CD5 expression in lymphoma cells was examined by flow cytometry or immunohistochemical analyses; eight patients were positive and four were negative. In $CD5^+$ B-cell lymphoma, higher levels of LDH (2,071 IU/*l*) and lower levels of ferritin (1,132 ng/ml) were observed as compared with $CD5^-$ B-cell lymphoma, but these differences were not significant. No significant differences were observed in other laboratory parameters (Table 3).

Morphology

The Wright-Giemsa-stained bone marrow smears in all patients showed normocellularity with mature benign-looking

Table 1. Patient Characteristics

	$CD5^+$ (n=8)	$CD5^-$ (n=4)	Total (n=12)
Age (years) (median)	62.5 (41–73)	67.5 (54–82)	63.5 (41–82)
Sex (male)	7	3	10
Fever	8	4	12
Hepatomegaly	5	4	9
Splenomegaly	7	4	11
Lymphadenopathy	1	1	2
Lung involvement	2	1	3
Skin lesion	0	0	0
Neurological signs	0	0	0

Table 2. Laboratory Data and Clinical Outcome

	Case											
	1	2	3	4	5	6	7	8	9	10	11	12
Hb (g/dl)	14.3	9.9	10.3	9.8	9.2	9	7.1	11.6	10.1	10.9	9.8	10.2
WBC ($\times 10^9/l$)	10.2	6.2	8.6	6	7.7	4.2	3.6	4.8	2.2	2.8	3.8	9
Plt ($\times 10^9/l$)	35	109	124	44	20	76	34	48	23	141	41	41
LC in BM (%)	28	0.8	0.8	6.8	21	<0.5	6.8	0	47.7	1.6	12.6	9.8
LDH (IU/*l*)	1,143	873	1,497	5,085	503	640	1,246	1,401	1,424	2,175	1,936	2,684
ALT (IU/*l*)	108	17	10	183	47	28	29	93	36	135	19	87
T. Bilirubin (mg/dl)	15.9	0.7	0.5	1.1	1	0.5	0.7	1.2	0.6	0.5	0.9	1.1
Creatinine (mg/dl)	0.6	0.9	1	0.8	1	0.5	0.7	0.9	1.1	1.3	0.8	1.4
CRP (mg/dl)	17.9	20.3	9.6	6.6	6.6	7.9	18.4	11.5	5.4	2.4	9	14.3
D-dimer (μg/ml)	0.5	n.d.	5	n.d.	n.d.	<0.2	<0.2	7.3	n.d.	<1	2.7	62.1
Ferritin (ng/ml)	981	1,164	322	891	3,968	1,548	971	1,895	337	450	n.d.	3,128
sIL-2R (U/ml)	19,300	9,360	5,120	16,265	49,533	5,480	10,380	10,100	8,400	1,809	n.d.	n.d.
CD5 expression in LC	pos	pos	neg	pos	neg	neg	pos	neg	pos	pos	pos	pos
Biopsy, autopsy	n.d.	D, L (Sp)	n.d.	D, L, IVL	n.d.	n.d.	D, L (LN)	D, L (LN)	n.d.	n.d.	n.d.	n.d.
Initial therapy	CHOP	CHOP	CHOP	CHOP	CHOP	PSL	CHOP	CHOP	CPM/PSL	PSL	VEPA	CHOP
Survival (months)	2	11	36	16	1	11	32+	34+	1	24	11	11

LC: lymphoma cells, pos: positive, neg: negative, n.d.: not done, D, L: difuse, large cell type, Sp: spleen, IVL: intravascular lymphomatosis, LN: lymph node, CHOP: cyclophosphamide, doxorubicin, vincristine, prednisolone, PSL: prednisolone, CPM: cyclophosphamide, VEPA: vincristine, cyclophosphamide, prednisolone, doxorubicin.

Table 3. Laboratory Findings

	$CD5^+$ (n=8)	$CD5^-$ (n=4)	p value
Hb (g/dl)	10.3 (7.1–14.3)	10 (9–11.6)	0.83
WBC ($\times 10^9/l$)	5.5 (2.2–10.2)	6.3 (4.2–8.6)	0.62
Platelets ($\times 10^9/l$)	58.5 (34–141)	67 (20–124)	0.75
Lymphoma cells in BM (%)	14.3 (0.8–47.7)	5.6 (0–21)	0.35
LDH (IU/*l*)	2,071 (873–5,085)	1,010 (503–1,497)	0.17
ALT (IU/*l*)	77 (17–183)	45 (10–93)	0.36
T. Bilirubin (mg/dl)	3.3 (0.5–15.9)	0.8 (0.5–1.2)	0.35
Creatinine (mg/dl)	1.0 (0.6–1.4)	0.9 (0.5–1)	0.56
CRP (mg/dl)	11.8 (2.4–20.3)	8.9 (6.6–11.5)	0.44
Ferritin (ng/ml)	1,132 (337–3,128)	1,933 (322–3,968)	0.30
sIL-2R (U/ml)	10,919 (1,809–19,300)	17,558 (5,120–49,533)	0.59

BM: bone marrow.

histiocytes phagocyting erythroblasts, granulocytes and platelets, suggesting a diagnosis of hemophagocytic syndrome (HPS). In addition, BM was infiltrated with various percentages of atypical large-sized lymphoid cells with fine chromatin, sometimes nuclear irregularity, and a moderate amount of basophilic cytoplasm, frequently with several vacuoles. Autopsy was performed in one patient (case 4), and tumor cells were found to have infiltrated the spleen, sinusoid and portal areas of the liver, the lumina of the small vessels in the lungs and kidneys. These findings indicated a diagnosis of IVL.

Immunophenotypic and genotypic analyses

The diagnosis of B-cell lymphoma was made based on the expression of B-cell antigen (CD20 and surface immunoglobulin) determined by flow cytometric analysis of lymphoma cells in all except one patient (case 10), who was diagnosed by the rearrangement of immunoglobulin heavy chain (IgH) gene. With regard to other B-cell antigens, all eleven patients examined were negative for CD10, while eight of the twelve patients (case 1, 2, 4, 7, 9, 10, 11 and 12) were positive for CD5, which was detected by flow cytometry and immunohistochemical analysis (Fig. 1).

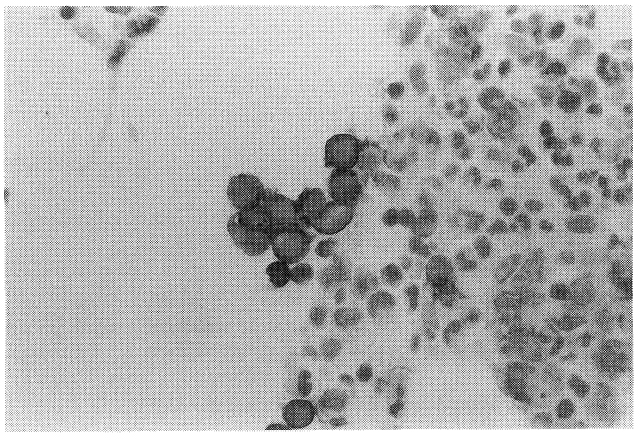


Figure 1. Immunohistochemical staining of lymphoma cells in bone marrow (case 10). Large lymphoma cells were positive for CD5 (×400).

Clinical course

The prognosis of B-LAHS was poor, with a median survival of 11 months in our series. All except four patients (cases 6, 9, 10 and 11) received the CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone) regimen as the initial therapy. Four patients (cases 3, 4, 7 and 8) underwent peripheral blood stem cell transplantation (PBSCT) after induction therapy with CHOP, and one of these patients continued into complete remission. No significant differences were observed in the mean survival period between $CD5^+$ and $CD5^-$ B-LAHS (13.5 versus 20.5 months, p=0.40).

Discussion

B-LAHS is extremely rare in Western countries, but has recently been increasingly reported in Asian countries (1–4). It is characterized by fever and hepatosplenomegaly without lymphadenopathy at initial presentation. Histological examination in some cases showed the invasion of tumor cells in the vessels, suggesting a diagnosis of IVL (1). Murase et al (1) suggested that B-LAHS might fall within the framework of IVL and proposed the name Asian variant of IVL (AIVL) for this peculiar disease because the majority of cases have been reported in Asia.

In most of the reported Japanese cases, the diagnosis of B-cell lymphoma was made immunohistochemically using bone marrow samples. We previously examined the immunophenotype of lymphoma cells using flow cytometry, and demonstrated that they were positive for CD5 in four of the seven patients examined (2). The expression of CD5 can be studied on formalin-fixed paraffin-embedded samples using antigen retrieval by heating. In the present study, eight of the twelve patients examined were shown to express CD5. CD5 is expressed in most cases of chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL) and in some cases of DLCL (5–8). Taniguchi et al (7) recently demonstrated that de novo $CD5^+$ diffuse large cell B-cell lymphoma is derived from a subset of B-1 cells, distinct from other $CD5^+$ B-cell lymphomas such as the CLLs and MCLs, and showed the involvement of various extranodal sites with lymphadenopathy. Therefore, we compared $CD5^+$ B-LAHS with $CD5^-$ B-LAHS. Although a higher level of LDH and lower level of ferritin were observed in $CD5^+$ B-LAHS as compared with $CD5^-$ B-LAHS, no significant differences were observed in patients' age, sex, clinical or laboratory features.

Kanda et al (10) and Khalidi et al (11) reported that three of four and five of ten patients with IVL were positive for CD5, respectively, suggesting that IVL is heterogenous based on the expression of CD5. In fact, IVL was reported to be present simultaneously with DLCL involving either nodal or extranodal extravascular sites (12–14). Estalilla et al (15) reported that IVL could be divided into three groups immunophenotypically; $CD20^+CD5^+$, $CD20^+CD5^-CD10^+$, and $CD20^+CD5^-CD10^-$. However, no differences were observed in the histological findings between $CD5^+$ IVL and $CD5^-$ IVL. Recently Murase et al (16) reported that CD5 is not a marker to identify a distinct subtype of IVL. These observations were compatible with those of the present study demonstrating no differences between $CD5^+$ B-LAHS and $CD5^-$ B-LAHS.

The prognosis of B-LAHS is poor, with a median survival of 11 months in our series. This was in accordance with the report of a Japanese survey with a median survival of 242 days (3). Although the treatment outcome for patients with de novo $CD5^+$ DLCL was very poor with frequent relapse as compared with $CD5^-$ DLCL (8), our data indicated no differences in the mean survival between $CD5^+$ and $CD5^-$ B-LAHS. In our series, one of four patients who underwent PBSCT continued into complete remission. More aggressive treatment modalities might be required to improve the survival in this disease.

In conclusion, we found no differences in the clinical or laboratory features between $CD5^+$ and $CD5^-$ B-LAHS although the number of patients examined was small. Further studies are required to elucidate the differences in the clinicopathological features between these two subgroups of B-LAHS.

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