Clinicopathological features of lymphoma/leukemia patients carrying both *BCL2* and *MYC* translocations

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ABSTRACT

Background

Lymphoid neoplasm with 18q21.3/*BCL2* and 8q24/*MYC* translocation to immunoglobulin (*IG*) genes as dual-hit lymphoma/leukemia is very rare and known to have a poor clinical outcome.

Design and Methods

To clarify the clinicopathological characteristics of this malignancy, we analyzed 27 cases of cytogenetically proven dual-hit lymphoma/leukemia.

Results

Dual-hit lymphoma/leukemia was diagnosed at presentation in 22 cases and at relapse or disease progression in 5 cases. At the time of diagnosis of dual-hit lymphoma/leukemia, extranodal involvement was found in 25 cases (93%) and central nervous system involvement occurred in 15 cases (56%). The median survival and 1-year survival rate of the 27 cases were only 6 months and 22%, respectively, after diagnosis of the dual-hit lymphoma/leukemia. Seven cases of triple-hit lymphoma/leukemia (dual-hit lymphoma/leukemia with 3q27/*BCL6* translocation) were included; the median survival of these patients was only 4 months from the diagnosis of the dual-hit lymphoma/leukemia. The duration of survival of the patients with a triple-hit malignancy was shorter than that of the other 20 cases of dual-hit lymphoma/leukemia (p=0.02). The translocation partner of *MYC* subdivided the dual-hit cases into two groups; 14 cases of *IGH* and 13 cases of *IGK/L*. The MIB-1 index was investigated in 14 cases with aggressive B-cell lymphoma, and was higher in the group with *MYC-IGH* translocation (n=7) than in the *MYC-IGK/L* group (n=7) (p=0.02). Overall survival was not different between the *MYC-IGH* translocation group (n=14) and the *MYC-IGK* or *MYC-IGL* translocation group (n=13).

Conclusions

Dual-hit lymphoma/leukemia is a rare but distinct mature B-cell neoplasm with an extremely poor prognosis characterized by frequent extranodal involvement and central nervous system progression with either of the translocation partners of *MYC*.

Key words: *BCL2*, *MYC*, dual-hit lymphoma/leukemia.

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Introduction

Translocation of the *BCL2* gene on chromosome band 18q21.3 results in consistent expression of the apoptosis inhibitor of the Bcl2 protein.[1] *BCL2* usually translocates to the immunoglobulin heavy chain (*IGH*) gene as t(14;18)(q32;q21.3) and rarely to *IG* light chain (*IGK*, *IGL*) loci as t(2;18)(p11;q21.3) or t(18;22)(q21.3;q11).[2] The t(14;18) is observed in 70% to 95% of cases of follicular lymphoma (FL)[3,4] and 20% to 30% of cases of diffuse large B-cell lymphoma.[5,6] The *MYC* gene on chromosome band 8q24 acts as an accelerator of cell proliferation.[7] *MYC* translocates to 14q32/*IGH* as t(8;14)(q24;q32) or less commonly to 2p11/*IGK* as t(2;8)(p11;q24) or 22q11/*IGL* as t(8;22)(q24;q11).[2] The 8q24/*MYC* translocation is detected in most cases of Burkitt's lymphoma and up to 10% of cases of diffuse large B-cell lymphoma.[8]

The World Health Organization (WHO) classification[2] translates the fruit of work in immunology and molecular biology to morphology. Lymphoid neoplasms are classified into two groups based on B or T/NK cell origin and further classified into precursor or mature cell types. Most mature B-lymphoid neoplasms show disease-specific chromosome abnormalities. Lymphoma/leukemia cases with both 18q21.3/*BCL2* and 8q24/*MYC* translocations to *IG* genes are rarely identified and most of them are classified as B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt's lymphoma (IL). These lymphomas are termed as *BCL2/MYC* dual-hit lymphoma/leukemia (DHL). Although DHL has been reported to have a poor prognosis,[2] the clinicopathological characteristics of this conditions have not been sufficiently studied thus far.

Here, we report the clinicopathologic and genetic features of 27 cases with translocations of both 18q21.3/*BCL2* and 8q24/*MYC* identified by chromosome analysis. We examined differences between cases with translocation of *MYC-IGH* and cases with translocation of *MYC-IGK/L*, and also studied the prognosis of patients with triple-hit lymphoma/leukemia (THL) involving 18q21.3/*BCL2*, 8q24/*MYC*, and 3q27/*BCL6*.

Design and Methods

We identified cases of mature B-cell neoplasms (lymphoma/leukemia) in which the translocations of both 18q21.3/*BCL2* and 8q24/*MYC* were found in identical cells by chromosome analysis. Clinical data relating to 27 cases of DHL were collected from 20 institutions. Pathological specimens from 20 cases were reviewed by three pathologists (*NN, KT,* and *JK*). Cases in which the two translocations were observed separately by fluorescent *in situ* hybridization analysis were not included in this study. Clinical data such as gender, International Prognostic Index (IPI),[9] age, serum lactate dehydrogenase (LDH) levels, performance status, clinical stage, number and site of extranodal involvements, B symptoms, and presence of a bulky mass (defined as a tumor with a minimum diameter of at least 10 cm or one-third the transverse thoracic diameter) were analyzed. Histological diagnosis involved hematoxylin-eosin staining and immunohistochemistry. The data collection procedure was submitted to the ethical committee or institutional review board of each institute. The procedures of this study were conducted in accordance with the Helsinki Declaration.

Statistical analysis

Fisher's exact probability test and the Mann-Whitney test were used to determine statistically significant differences between groups. A survival curve was constructed using the Kaplan-Meier method. p values less than 0.05 were considered to indicate statistical significance.

Results

Clinical data review

The characteristics of the 27 patients with DHL are shown in Table 1. All data were obtained at the onset of DHL. The median age (range) of the patients at the onset of DHL was 51 years (36-79 years). There were 23 cases of B-lymphoma (lymphoma-type DHL) and four of B-leukemia (leukemia-type DHL). All 11 tested cases were negative for human immunodeficiency virus. Most (25/27) patients had elevated serum LDH. Patients with leukemia-type DHL had higher serum LDH levels than those with lymphoma-type DHL (p=0.01). Among the 23 lymphoma-type DHL patients, 22 were at an advanced clinical stage and 15 patients had involvement of two or more extranodal sites. Only two patients had no extranodal involvement. In the 23 cases of lymphoma-type DHL, bone marrow (65%) was the most frequent site of extranodal involvement at the onset of DHL, followed by peripheral blood (30%) and pleural effusion (30%), as shown in Table 2. The IPI was high or high-intermediate in 87% (20/23) of the patients with lymphoma-type DHL.

The cases were divided into two subgroups depending on the timing of the diagnosis of DHL, as shown in Table 1. Twenty-two cases (18 with lymphoma-type DHL and 4 with leukemia-type DHL) who presented with DHL initially were classified as DHL-1. The other five cases (all with lymphoma-type DHL) who exhibited DHL at relapse or disease progression after treatment of the initial lymphoma/leukemia were classified as DHL-2. The clinical data of the DHL-2 cases refer to the onset of DHL, instead of the initial lymphoma/leukemia. In the DHL-2 group, four of the initial lymphoma/leukemia cases were diagnosed as FL and one as IL. The time from the diagnosis of the initial lymphoma/leukemia to the onset of DHL in the five DHL-2 cases was 3, 3, 3, 25, and 31 months.

All 27 patients received systemic chemotherapy. Multi-agent induction chemotherapy such as CHOP, CODOX-M/IVAC, or HyperCVAD, with (n=14) or without (n=8) rituximab, was given to most (22/23) patients with lymphoma-type DHL. Patients with leukemia-type DHL underwent combination

Table 1. Clinical features and karyotype of patients with dual-hit lymphoma/leukemia.

		DHL	MYC-IGH type	MYC-IGK/L type	*p*	Double hit only	Triple hit	*p*
Number		27	14	13		20	7	
Gender	Male / Female	12/15	7/7	5/8	NS	8/12	4/3	NS
Age	Median (Range) ≤ 60/>60	51 (36-79) 16/11	57 (42-77) 7/7	50 (36-79) 9/4	 NS	51 (36-79) 13/7	61 (44-71) 3/4	 NS
LDH	Normal / Elevated	2/25	1/13	1/12	NS	2/18	0/7	NS
Performance status	0-1/2-4	12/15	5/9	7/6	NS	11/9	1/6	NS
Clinical Stage[1)]	I, II/III, IV	1/22	0/13	1/9	NS	0/17	1/5	NS
Extranodal involvement[1)]	0-1/2-4	8/15	2/11	6/4	0.04	5/12	3/3	NS
IPI[1)]	L, LI/HI, H	3/20	1/12	2/8	NS	3/14	0/6	NS
Bone marrow involvement[1)]	Absent/Present	7/16	3/10	4/6	NS	5/12	2/4	NS
B symptoms[1)]	Absent /Present[2)]	12/10	5/7	7/3	NS	9/7	3/3	NS
Bulky mass	Absent/Present[2)]	21/5	11/2	10/3	NS	15/4	6/1	NS
Clinical entity	B-ALL B-ML	4 23	1 13	3 10	NS	3 17	1 6	NS
DHL type	DHL-1 DHL-2	22 5	13 1	9 4	NS	17 3	5 2	NS
Karyotype	t(14;18) + t(8;14) t(14;18) + t(8;22) t(14;18) + t(2;8) t(2;18) + t(8;14) t(8;14;18)	11 9 4 1 2	11 0 0 1 2	0 9 4 0 0		9 6 3 0 1	2 3 1 1 1	

Clinical data at the time of diagnosis of DHL are shown. [1)]Leukemia cases are not covered because these items are usually applied only to lymphoma. [2)]Data unknown in one case. DHL-1 means dual hit lesion at presentation. DHL-2 means metachronous dual hit lesion after precursor lymphoma.

chemotherapy for acute lymphocytic leukemia. Complete remission or complete remission-uncertain was observed in six of the 23 patients with lymphoma-type DHL and two of the four patients with leukemia-type DHL. However, seven (5 lymphoma-type and 2 leukemia-type) of the eight patients who achieved a complete remission (confirmed or uncertain) relapsed. No patients received up-front autologous or allogeneic transplantation at first remission. The overall survival curve is depicted in Figure 1A. The median survival and 1-year survival rate were only 6 months and 22%, respectively. Involvement of the central nervous system (CNS) was observed in up to 56% of patients (15/27) including two patients at the onset of DHL-1. The cause of death was progression of DHL in 87% of the patients who died.

Table 2. Extranodal sites of involvement among 23 cases of lymphoma-type dual-hit lymphoma/leukemia.

Involved site	Number (%)	Involved site	Number (%)
Bone marrow	15 (65)	Soft tissue	2 (9)
Peripheral blood	7 (30)	Breast	1 (4)
Pleural effusion	7 (30)	Gingiva	1 (4)
Stomach	3 (13)	Kidney	1 (4)
Ascites	3 (13)	Liver	1 (4)
Bone	2 (9)	Lung	1 (4)
Central nervous system	2 (9)	Ovary/uterus	1 (4)
Small intestine	2 (9)	Pancreas	1 (4)
Muscle	2 (9)	Pericardial effusion	1 (4)

Chromosomal data review

The results of chromosome analysis are summarized in Table 1 and listed in detail in Table 3. All cases were analyzed by examining the G-banding after trypsin exposure, except UPN21, for whom Q-banding was used. UPN23, bearing a complex translocation, was analyzed by G-banding and spectral karyotyping. Chromosome analysis revealed *BCL2-IGH* and *MYC-IG* translocations in all but one case (UPN13) in which *BCL2-IGK* and *MYC-IG* translocations were detected. The translocations detected in 11 cases were t(14;18) and t(8;14); in nine cases, t(14;18) and t(8;22); in four cases, t(14;18) and t(2;8); in one case, t(2;18) and t(8;14); and in two cases (UPN12, UPN23), a t(8;14;18) 3-way translocation.[10-12] Trisomy (or tetrasomy) 7 and trisomy

Table 3. Chromosomal analysis.

UPN	Type	Abnormal karyotype	Cells	Sample	Ref
1	DHL-1	46,XY,add(1)(q21),add(1)(q32),add(3)(q21),add(6)(q21),t(8;14)(q24;q32),t(14;18)(q32;q21) 48,idem,+add(6),+10	6/20 2/20	BM	13
2	DHL-1	46,XX,t(8;14)(q24;q32),t(14;18)(q32;q21),del(15)(q11;q14),-22,+mar2	17/20	BM	
3	DHL-1	45-49,XY,-1,del(2)(p21),del(3)(q11),-4,+5,-6,+7,t(8;14)(q24;q32),+add(9)(p24),+11,-13,t(14;18)(q32;q21), +der(14)t(14;18),+14,-19,-21,+mar 74-89?4N?,XXY,del(1)(p12),-1,del(2)(p21),-3,add(3)(p26),-4,-4,-5,-6,del(6)(q21),t(8;14)(q24;q32), t(8;14)(q24;q32),+8,+8,del(10)(q23),-12,-13,-13,t(14;18)(q32;q21),t(14;18)(q32;q21),-19,+mar3,dms	 2/20 18/20	BM	
4	DHL-1	49,XX,+5,add(8)(p21),t(8;14)(q24;q32),+10,+add(12)(q13),t(14;18)(q32;q21),del(15)(q?) 50,idem,+add(8)	19/20 1/20	LN	14
5	DHL-1	49,XY,t(8;14)(q24;q32),+12,+13,t(14;18)(q32;q21),-15,add(17)(q21),+2mar	3/7	AS	
6	DHL-1	46,XX,add(3)(q21),t(8;14)(q24;q32),t(14;18)(q32;q21) 49,idem,+add(1)(p11),+21,+der(21)t(12;21)(q13;q22)	4/16 12/16	BM	15
7	DHL-1	46,XY,t(8;14)(q24;q32),t(14;18)(q32;q21)	19/20	BM	
8-1 -2	DHL-1	48,XX,+8,t(8;14)(q24;q32),+12,t(14;18)(q32;q21) 48,XX,+8,t(8;14)(q24;q32),+12,t(14;18)(q32;q21) 48,idem,1~2dmin 49,idem,+mar	3/20 4/8 2/8 2/8	BM PE	
9	DHL-1	47,XY,+X,add(2)(p11.2),der(3)t(3;14)(q27;q32)t(14;18)(q32;q21),+6,der(6)t(6;22)(q13;q11.2),add(6)(q13), der(7)t(7;11)(p22;q13),t(8;14)(q24;q32),-11,ins(12;?5)(q13;?q11q35),der(13)t(13;17)(q32;q21), der(14)t(3;14)t(14;18),del(15)(q13q15),der(18)t(14;18),del(22)(q11.2) 48,idem,+mar	 7/8 1/8	LN	16
10	DHL-1	47,XX,add(1)(p11),t(2;15)(p11.1;q11.2),add(3)(q21),add(6)(p21),+7,t(8;14)(q24;q32),t(14;18)(q32;q21) 47,XX,add(1)(p11),t(2;15)(p11.1;q11.2),add(3)(q21),add(5)(q13),add(6)(p21),+7, t(8;14)(q24;q32),t(14;18)(q32;q21) 47,XX,add(1)(p13),t(2;15)(p11.1;q11.2),add(3)(q21),+7,t(8;14)(q24;q32),t(14;18)(q32;q21) 47,XX,add(1)(p13),t(2;15)(p11.1;q11.2),add(3)(q21),add(5)(q13),+7,t(8;14)(q24;q32),t(14;18)(q32;q21) 47,XX,add(1)(p11),t(2;15)(p11.1;q11.2),add(3)(q21),add(6)(p21),+7,t(8;14)(q24;q32), add(13)(q22),t(14;18)(q32;q21)	6/16 4/16 4/16 1/16 1/16	PE	
11	DHL-1	48,XX,+7,+8,t(8;14)(q24;q32),t(14;18)(q32;q21) 48,idem,add(1)(q32) 48,idem,add(22)(q13)	3/13 7/13 3/13	BM	
12	DHL-1	46,XX,add(1)(p36),add(2)(p11),der(3)t(1;3)(q12;p25),add(5)(q31),t(5;12)(q22;q22),add(7)(p13), t(8;14;18)(q24;q32;q21),der(9)add(9)(p11)add(9)(q22),add(13)(q14),del(13)(q?),add(15)(q22)	 15/20	BM	
13	DHL-1	46,XY,i(1)(q10),t(2;18)(p11;q21),t(3;22)(q27;q11),t(8;14)(q24;q32),der(14)t(8;14)	20/20	BM	
14	DHL-1	49,XY,+Y,t(8;22)(q24;q11),+12,t(14;18)(q32;q21),+mar1 49,idem,add(9)(p11)	8/13 5/13	LN	
15	DHL-1	48,XX,add(6)(p21),+7,t(8;22)(q24;q11),+12,t(14;18)(q32;q21),add(19)(p13) 48,idem,del(1)(p?)	3/20 4/20	BM	
16	DHL-1	47,X,+X,-Y,del(6)(q?), der(8)del(8)(p11)?t(8;22)(q24;q11), t(14;18)(q32;q21), der(22)?t(8;22), +der(?)t(?;1)(?;q11)	 18/20	BM	
17	DHL-1	50,X,+X,-Y,+7,t(8;22)(q24;q11.2),+9,+12,+der(14)t(14;18)(q32;q21),t(14;18)(q32;q21)	18/20	LN	
18	DHL-1	46~50,XX,+del(X)(p22),+del(1)(p22),t(1;13)(p36;q22),del(5)(q33),del(6)(q13),+7,t(8;22)(q32;q11), dup(12)(q13q24),t(14;18)(q32;q21),+mar	 10/10	LN	
19	DHL-1	46,XX,t(8;22)(q24;q11),t(14;18)(q32;q21)	18/20	BM	
20	DHL-1	48,XX,t(3;14)(q27;q32),del(6)(q?),t(8;22)(q24;q11),-10,+der(12)t(1;12)(q21;q24),t(14;18)(q32;q21),+17,+mar1 49,idem,+8	13/20 2/20	BM	
21	DHL-1	49,XY,+X,add(1)t(1;8)(p36;p11),t(2;3)(p11;q27),t(2;8)(p11;q24),add(9)(p21),+12,t(14;18)(q32q21),+mar	18/20	M	
22	DHL-1	45~46,XX,t(2;8)(p11.2;q?24.1),t(14;18)(q32;q21),dup(17)(q21q25),inc	4/20	BM	17
23	DHL-2	45,X,-Y,t(3;14)(q27;q32),t(8;14;18)(q24;q32;q21),der(17)t(8;17)(q13;p11.2)t(8;18)(q24;q21) 46,idem,+mar1 47,idem,+Y,+8,del(13)(q?),+17,-der(17)t(8;17)t(8;18)	2/12 2/12 2/12	BM	

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24	DHL-2	45,X,-X,der(1),t(2;3)(p12;q27),del(6),t(8;22)(q24;q11),add(14)(q22),t(14;18)(q32;q21),-17,add(21)(p11),+mar1 45,idem,del(5)(q) 45,idem,add(2)(q31),+14,-add(14)	1/20 1/20 1/20	BM	18
25	DHL-2	70,XX,-X,i(1)(q10),t(3;4)(q27;p12),-4,+5,+6,i(6)(p10)×2,+7,+7,t(8;22)(q24;q11)×2,-11,+12,+13, t(14;18)(q32;q21)×2,-15,-18,+20,-21,-22,+mar1	1/1	PE	
26	DHL-2	54,XY,dup(1)(q23q42),t(2;8)(p12;q24),add(3)(p21),+4,+7,+der(8)t(2;8),+12,+13,del(13)(q?)?2, t(14;18)(q32;q21),+18,+der(18)t(14;18),+20 54,idem,-12,+mar 54,idem,add(4)(p11),add(7)(q22),-del(13),+mar 55,idem,-del(13),+der(14)t(14;18),+mar	1/4 1/4 1/4 1/4	LN	
27	DHL-2	49,XX,+der(1)t(1;7)(p13;p13),t(1;7)(p13;p13),t(2;8)(p12;q24),+7,+der(8)t(2;8),add(9)(p11),ins(12;?)(q13:?), t(14;18)(q32;q21),add(21)(p11) 48,idem,add(1)(p32),add(6)(p11),-7,t(8;11)(q22;q13)	5/18 12/18	BM	

BM; bone marrow, LN; lymph node, AS; ascites, PE; pleural effusion, M; muscle, Dual translocations are underlined.

12 were detected in nine and eight cases, respectively. An additional translocation involving 3q27/*BCL6* was detected in seven cases (UPN9, UPN13, UPN20, UPN21, UPN23, UPN24, UPN25), and among them, the *BCL6-IG* translocation was identified in five cases. These seven cases were regarded as having THL. Of our 27 cases, six have been previously reported.[13-18]

Pathological review

The results of the pathological review are summarized in Table 4. A reviewed diagnosis of the 20 DHL cases revealed 1 case of grade 3a FL (UPN10), one case of grade 3b FL (UPN17), 15 cases of IL, and three cases of composite lymphoma that showed different lymphoma histologies synchronously in identical lymph nodes (UPN1, UPN23) or at different sites (UPN16). UPN1, belonging to the DHL-1 group, exhibited FL and IL in identical lymph nodes synchronously. UPN16, belonging to the DHL-1 group, exhibited FL in a lymph node and IL in the bone marrow synchronously. UPN23, belonging to the DHL-2 group, exhibited FL as the initial lymphoma/leukemia and FL and IL in identical lymph nodes at relapse, as shown in Figure 2A. The typical histology of DHL is shown in Figures 2B-D. Pathological diagnoses were made according to the WHO classification.[2] No cases showed the typical phenotype of Burkitt's lymphoma/Burkitt's leukemia ($CD10^+$, $Bcl6^+$, $Bcl2^-$, MIB-1 index >95%).[19] CD20 immunostaining was positive in 18 of 19 cases, with the degree of positivity varying from partial (positive in less than 30% of tumor cells) to diffuse (positive in more than 30% of tumor cells). UPN9 was CD20-negative by immunostaining but CD20-positive by flow cytometry. Other immunohistochemical analyses revealed positive/negative results of 18/1 for CD10 (negative in UPN7), 19/1 for Bcl2 (negative in UPN27), 11/4 for Bcl6, and 2/16 for MUM-1. The MIB-1 index was lower than 90% in 13 of 14 tested cases (except for follicular lesions in composite lymphomas).

According to the criteria of Hans *et al.*,[20] 17 of the 19 tested cases showed diffuse CD10 positivity (more than 30% of tumor cells) on immunostaining and were classified as the germinal center B cell-like (GCB) subtype. UPN18 showed partial CD10 positivity (less than 30% of tumor cells), Bcl6 positivity, and MUM-1 negativity and was also classified as the GCB subtype. Only UPN7 was classified as the non-GCB subtype as this patient was negative for CD10, Bcl6, and MUM-1. CD10 immunostaining was not performed in the case of UPN9.

Partner of the MYC translocation

The translocation partner of *MYC* consisted of *IGH* in 14 cases and *IGK/L* in 13. Cases with more than one

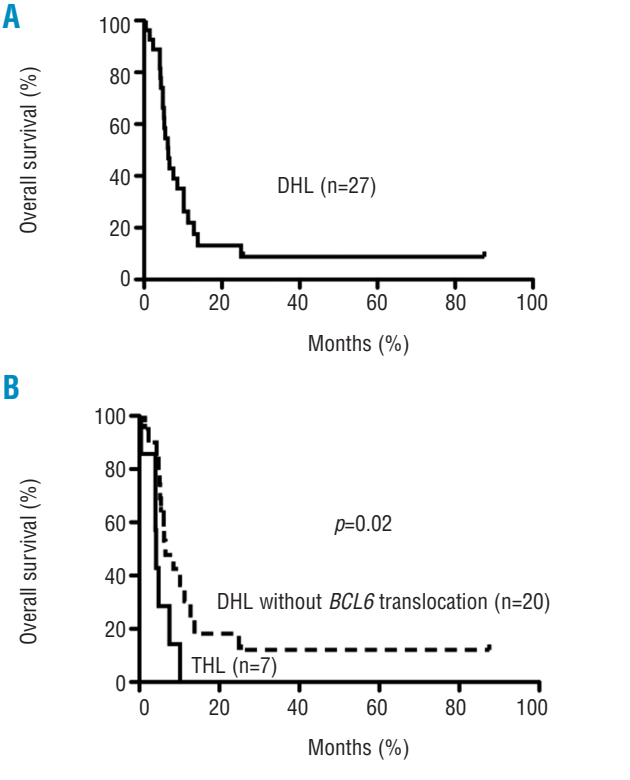

Figure 1. Overall survival of 27 cases of DHL. (A) The 1-year survival rate is only 22%. In the five type-2 DHL cases, the survival duration is measured from the diagnosis of DHL. The two patients with the longest survival were those without extranodal involvement at the diagnosis of DHL. (B) The survival duration of patients with THL (DHL with *BCL6* translocation) was shorter than that of the other 20 DHL cases (p=0.02).

Table 4. Pathological review at the diagnosis of dual-hit lymphoma/leukemia.

UPN	Tpe	Site	DHL site	Reviewed site	Reviewed diagnosis	MYC/IG	Immunostaining						MIB-1 (%)
							SSA	CD20	CD10	BCL-2	BCL-6	MUM-1	
1	DHL-1	Different	BM	LN	Composite (IL + FL, grade 2)	MYC/IGH	+	+/-	+	+ (w)	ND	-	84.1
2	DHL-1	Identical	BM	BM	IL	MYC/IGH	+	+	+	+	+	-	57.1
3	DHL-1	Different	BM	RT	IL	MYC/IGH	-	+/-	+	+	+	ND	ND
5	DHL-1	Different	AS	ST	IL	MYC/IGH	-	+	+	+	+	-	88.6
6	DHL-1	Identical	BM	BM	IL	MYC/IGH	-	+	+ (w)	+	-	-	82.6
7	DHL-1	Identical	BM	BM	IL	MYC/IGH	-	+	-	+	-	-	80.5
8	DHL-1	Different	BM, PE	ST	IL	MYC/IGH	-	+	+	+	ND	ND	90.8
9	DHL-1	Identical	LN	LN	IL	MYC/IGH	+	-	ND	+	-	-	ND
10	DHL-1	Different	PE	LN	FL, grade 3a	MYC/IGH	-	+	+	+	+	-	ND
12	DHL-1	Identical	BM	BM	IL	MYC/IGH	-	+	+	+	ND	-	ND
13	DHL-1	Different	BM	Gingiva	IL	MYC/IGH	-	+	+	+	+	-	75.0
23	DHL-2	Different	PB	LN	Composite (IL + FL, grade 1)	MYC/IGH	+	+	+	+	+	+	ND
14	DHL-1	Identical	LN	LN	IL	MYC/IGL	+	+	+	+ (w)	+	-	62.9
15	DHL-1	Identical	LN	LN	IL	MYC/IGL	+	+/-	+ (w)	+	-	+ (w)	52.9
16	DHL-1	Identical	BM	BM	Composite (IL + FL, grade 2)	MYC/IGL	-	+/-	+	+	+	+	81.2
17	DHL-1	Identical	BM,LN	LN	FL, grade 3b	MYC/IGL	-	+	+	+	+	-	ND
18	DHL-1	Identical	LN	LN	IL	MYC/IGL	+	+	+/-	+	+	-	47.1
19	DHL-1	Identical	BM	BM	IL	MYC/IGL	+	+/-	+	+	ND	-	64.7
21	DHL-1	Identical	M	M	IL	MYC/IGK	-	+	+	+	+	-	54.9
27	DHL-2	Identical	BM	BM	IL	MYC/IGK	-	ND	+	-	ND	-	58.7

Identical: the reviewed specimen was obtained from the site of chromosomal dual translocation; different: the reviewed specimen was obtained from another site of chromosomal dual translocation. SAA: starry sky appearance; BM: bone marrow; AS: ascites; PE: pleural effusion; LN: lymph node; M: muscle; PB: peripheral blood; RT: retroperitoneum; ST: stomach; T: tonsil; ND: not done. +: diffuse positive (>30%); + (w): diffuse but weakly positive (>30%); +/-: partially positive (<30%); -: negative. Composite lymphoma cases showed different lymphoma histologies synchronously in identical lymph nodes (UPN1, UPN23) or at different sites (UPN16). The MIB-1 index was measured in an area of diffuse lymphoma in cases of composite lymphoma.

extranodal site involved were more common in the *MYC-IGH* group (p=0.04). The other factors were similarly distributed. The MIB-1 index was investigated in 14 cases among the 20 pathologically reviewed cases shown in Table 4. It was higher in the *MYC-IGH* translocation group (n=7) than in the *MYC-IGK/L* group (n=7) (p=0.02). Overall survival was not different between patients with *MYC-IGH* translocation (n=14) and those with the *MYC-IGK/*L translocation (n=13) (*data not shown*).

Triple-hit lymphoma/leukemia

There were seven cases of THL. All of them died and their median survival was only 4 months from the diagnosis of DHL. Variables of clinical factors were similarly distributed in DHL without *BCL6* translocation and THL, as shown in Table 1. The duration of survival of patients with THL was shorter than that of the other 20 DHL cases, with a median survival of 6 months (p=0.02), as shown in Figure 1B.

Discussion

We accessed chromosomally proven DHL cases having both 18q21.3/*BCL2* and 8q24/*MYC* translocations to analyze their clinicopathological features. This report is the largest case-series study conducted.[21-29] *BCL2/MYC* DHL account for most of the *double hit lymphomas*, which contain a *MYC* breakpoint in combination with a *BCL2* and/or *BCL6* breakpoint, as defined in the WHO classification.[2]

Whether the *BCL2-IG* translocation and *MYC-IG* translocation arise concurrently or separately has not yet been determined. In the DHL-2 group (UPN23-27), the initial lymphoma/leukemia was FL in four cases and IL in one case (UPN24). At least in four of these cases the *BCL2-IG* translocation definitely preceded the *MYC-IG* translocation. The interval from the onset of the initial lymphoma/leukemia to the onset of DHL ranged from 3 to 31 months. In the DHL-1 group, there were two cases of composite lymphoma with FL and IL (UPN1 and UPN16). These two cases suggest the transformation of FL into diffuse lymphoma. In UPN1, detailed examination using fluorescent *in situ* hybridization analysis of paraffin-embedded tissue and DNA sequencing in the immunoglobulin heavy chain gene by the microdissection technique[30] confirmed that the *BCL2-IG* translocation occurred before the *MYC-IG* translocation.[13] These data indicate that the *BCL2-IG* translocation occurs first and is followed by the *MYC-IG* translocation at least in some cases of DHL. It is also possible that an additional

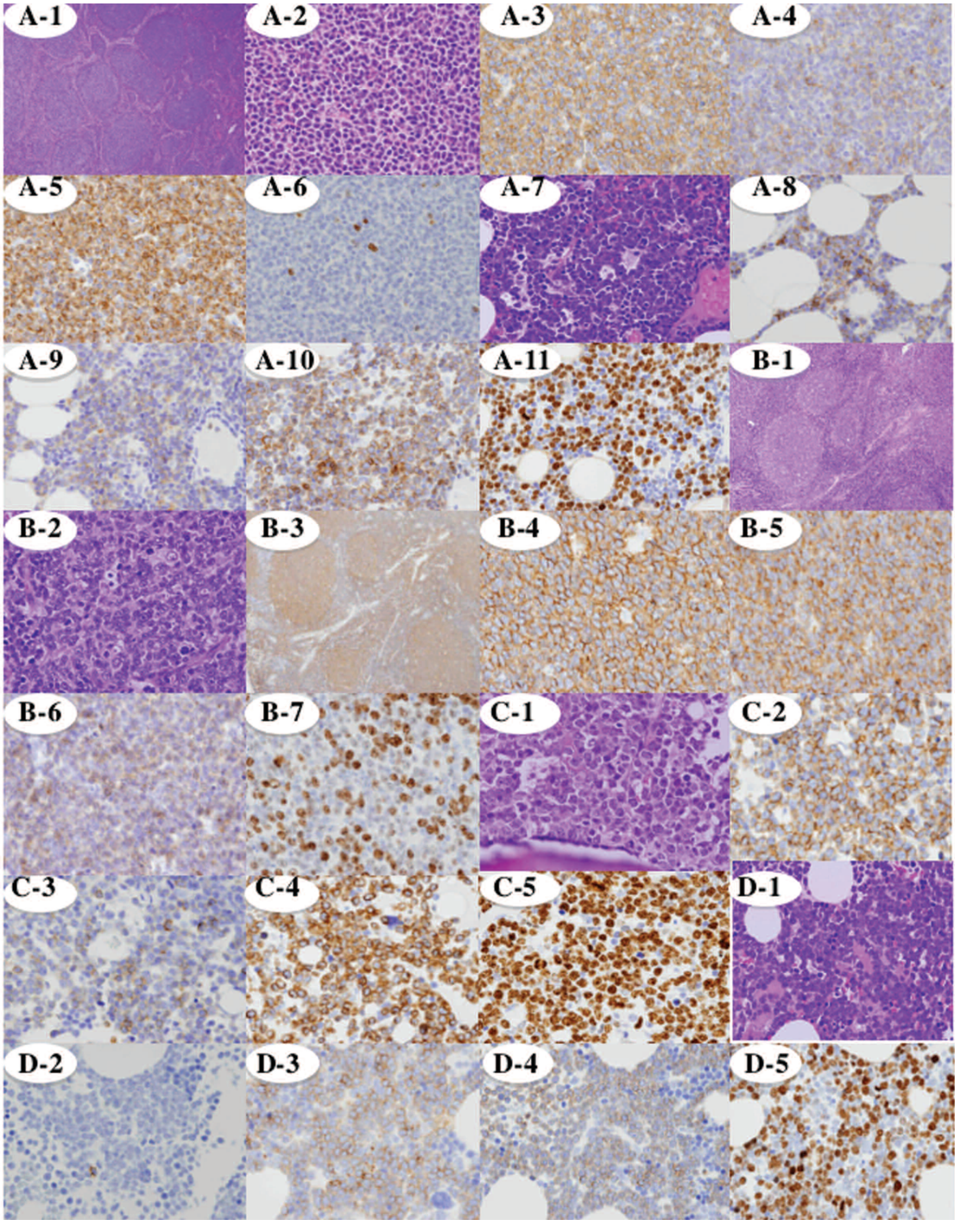

Figure 2. Pathological review: hematoxylin-eosin staining or peroxidase immunostaining (antigen addressed). Original magnification was ×400 in all panels unless otherwise stated. (A) Composite lymphoma of grade 1 follicular lymphoma and B-cell lymphoma, unclassifiable with features intermediate between diffuse large B-cell lymphoma and Burkitts' lymphoma (IL) (UPN23). The resected lymph node is effaced by nodular proliferation of the lymphoma cells (A-1, original magnification ×40), which exhibits a small-cleaved nucleus (A-2), indicating follicular lymphoma, grade 1. They are CD20-positive (A-3), CD10-positive (A-4), and BCL2-positive (A-5). The ki-67 index of the follicular lymphoma cells is about 10% (A-6). In the adipose tissue around the lymph node, there is diffuse proliferation of the lymphoma cells with medium to large-sized nuclei with scattered starry sky macrophages, indicating IL (A-7). The lymphoma cells are positive for CD20 (A-8), CD10 (A-9), and Bcl2 (A-10). The ki-67 index of the IL cells is approximately 60% (A-11). (B) Follicular lymphoma, grade 3b (UPN17). The lymph node shows a nodular and diffuse proliferation of the lymphoma cells (B-1, original magnification ×100). The lymphoma cells have a large-sized round nucleus with a prominent nucleolus (B-2). They are positive for CD20 (B-3, original magnification ×100, B-4), CD10 (B-5), and Bcl2 (B-6). The ki-67 index of the lymphoma cells is approximately 50% (B-7). (C) IL (UPN6). The bone marrow biopsy specimen shows diffuse proliferation of the lymphoma cells that have a large-sized round or cleaved nucleus with prominent nucleoli and vesicular chromatin (C-1). The lymphoma cells are positive for CD20 (C-2), CD10 with weak staining (C-3), and Bcl2 (C-4). The ki-67 index of the lymphoma cells is 82.6% (C-5). The translocation partner of *MYC* is *IGH*. (D) IL (UPN19). The bone marrow biopsy specimen shows diffuse proliferation of the leukemia cells with medium to large-sized nuclei (D-1). The lymphoma cells are partially CD20 positive (D-2), CD10 positive (D-3), and Bcl2 positive (D-4). The ki-67 index of the leukemia cells is 64.7% (D-5). The translocation partner of *MYC* is *IGL*.

8q24/*MYC* translocation occurs in non-neoplastic circulating B-cells with the t(14;18),[31,32] resulting in DHL-1 features. As for the patterns of 8q24/*MYC* translocation, 13 cases (48%) showed translocation to *IG* light chain gene. This is different from the frequency observed in usual Burkitt's lymphoma in which up to 85% of the cases show 8q24/*MYC* translocation to an *IGH* gene, resulting in t(8;14). This is a characteristic of DHL. It might be attributable to the fact that only one *IGH* gene would remain as the partner of *MYC* in the presence of the *BCL2-IGH* translocation.

Typical Burkitt's lymphoma histology was not observed in any of the 20 pathologically reviewed cases. The MIB-1 index reflects the cell ratio in the cell cycle and might fluctuate depending on the degree of cell proliferation in the presence of *MYC* overexpression in DHL. In most cases of DHL, the index was below 90%. This differs considerably from typical Burkitts' lymphoma, in which it is almost 100%. The group with the *MYC-IGH* translocation showed a higher MIB-1 index than the group with the *MYC-IGK/L* translocation. In DHL, the proliferation potential might differ according to the translocation partner of the *MYC*, although its impact on survival is not apparent. It is reported that in most cases of *MYC-IGH* translocation, the breakpoints of *MYC* are located 5' of the coding region, either in the first intron, within the first exon, or 5' of the first exon, while in cases with *MYC-IGK/L* translocation, they may be at considerable distances centromeric or telomeric from the *MYC* coding exons.[33] The breakpoint of *MYC* might have a role in determining the proliferation potential in DHL.

The prognosis of DHL is extremely poor. Most patients died within 1 year of the diagnosis of DHL despite chemotherapy. Extranodal involvement, a transient response to chemotherapy, repeated relapses, highly aggressive disease, and frequent CNS progression were characteristic of DHL. High-dose chemotherapy followed by stem cell transplantation should be indicated for DHL. In seven of our 27 cases of DHL, the 3q27/*BCL6* translocation was also detected. Because of the very short survival of patients with this translocation, one should pay attention to the presence or absence of the 3q27/*BCL6* translocation during the diagnosis of DHL. In our series, the longest surviving (beyond 7 years) patient was UPN4.[14] UPN4 initially had DHL with t(14;18) and t(8;14) as diffuse large B-cell lymphoma and reached complete remission with CHOP chemotherapy. After 55 months, relapse occurred only as FL with a t(14;18) chromosome abnormality. This suggests the mechanism of additional acquisition of t(8;14) to the original clone with t(14;18) and disappearance of the clone carrying both t(14;18) and t(8;14) as a result of chemotherapy. The second longest survival (beyond 2 years) was noted in the case of UPN18. UPN18 initially had DHL also with t(14;18) and t(8;14) as diffuse large B-cell lymphoma and achieved complete remission with CHOP and MACOP-B chemotherapy with rituximab. UPN18 is still in first complete remission beyond 2 years. Interestingly, they were the only two patients without initial extranodal involvement at the onset of DHL among our 27 patients. This might suggest the possible mechanism of controlling nodal DHL.

In this study, DHL was defined as a lymphoma/leukemia with chromosome translocations of both *BCL2-IG* and *MYC-IG*. DHL is a rare but distinct subgroup among the mature B-cell neoplasms; it is characterized by extranodal involvement and CNS progression with an extremely poor prognosis.

Authorship and Disclosures

NT: designed the research, collected and analyzed data and wrote the paper; MT: performed the pathological research; NN: designed the research, analyzed and interpreted data, and performed the pathological review; KT and JK: performed the pathological review; SM, KM AK, RH, YY, YM, SF and TH: collected data and performed the clinical research; YI: finally approved the submission; MI: critically reviewed the manuscript and gave an important intellectual contribution. All authors gave their approval to the manuscript submission.

The authors report no potential conflicts of interest.

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