J Clin Exp Hematopathol
Vol. 51, No. 1, May 2011

Case Study

Double-Hit Lymphoma at Second Relapse of Burkitt-Like Lymphoma : A Case Report

Hiroaki Tanaka,[1)] Shinichiro Hashimoto,[1)] Daijiro Abe,[1,2)] Shio Sakai,[1,2)] and Toshiyuki Takagi[1,3)]

Double-hit lymphoma (DHL) is a rare and extremely unfavorable type of lymphoma with concurrent chromosomal translocations of *BCL2* and *MYC*. It is considered that *BCL2* translocation precedes *MYC* events in lymphomagenesis of DHL. In fact, most cases of DHL arise *de novo* or following FL. We describe a very rare case of DHL arising from Burkitt-like lymphoma according to the revised European-American classification of lymphoid neoplasms. A 67-year-old Japanese male presented with persistent fever. [^{18}F]-fluorodeoxyglucose positron emission tomography revealed multiple abnormal accumulations in the bone marrow, pancreas, and periphery of the left kidney. The patient was diagnosed with Burkitt-like lymphoma according to a bone marrow biopsy. At the disease onset and the first relapse, chemotherapy was effective and the patient experienced sustained and complete remission. At the second relapse, however, the clinical presentation and morphology of lymphoma cells were nearly identical, but a high level of chemoresistance was acquired, and the patient succumbed almost 1 month after hospitalization. Chromosomal analyses revealed a complex karyotype with concurrent t(14;18) and t(8;22) translocations, which have not been previously detected. It is therefore important to note that DHL cannot be diagnosed without chromosomal analysis. Cytogenetic analyses should thus be performed for patients with high-grade B-cell lymphoma and who experience a recurrence of this lymphoma. 〔*J Clin Exp Hematopathol 51(1)* : *43-47, 2011*〕

Keywords: double-hit lymphoma, Burkitt-like lymphoma, *BCL2*, *MYC*

INTRODUCTION

The chromosomal translocations of *BCL2* and *MYC* are characteristic translocations of follicular lymphoma (FL) and Burkitt lymphoma, respectively. Lymphoid neoplasms with concurrent *BCL2* and *MYC* translocations are rare, but several case series concerning this neoplasm have been published.[1-5] In the 2008 World Health Organization (WHO) criteria for the classification of lymphoma, this neoplasm was newly classified as a double-hit lymphoma (DHL) in the category of B-cell lymphoma unclassifiable with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma.[6]

DHL is a rare type of lymphoma with aggressive progression, extremely poor prognosis, and high resistance to intensive chemotherapy, including high-dose chemotherapy with stem cell transplantation. It is considered that *BCL2* translocation precedes *MYC* translocation events in the lymphomagenesis of DHL. In fact, most cases of DHL arise *de novo* or following a history of FL.[2-4]

We herein describe a rare patient with DHL arising from Burkitt-like lymphoma according to the revised European-American classification of lymphoid neoplasms (REAL classification), in whom concurrent *IgH-BCL2* and *IgL-MYC* translocations were detected at the second relapse of the disease.

CASE REPORT

In September 2006, a 67-year-old Japanese male patient who had been receiving dietetic treatment for diabetes mellitus was referred to our hospital because of a persistent fever. Since August 2006, the patient had suffered from this persistent fever and visited his family doctor. The patient was administered antibiotics with no improvement, and was admitted to our hospital for close examination. At the first presentation, the laboratory findings revealed elevated lactate dehydrogenase (LDH) and C-reactive protein (CRP) levels, and [^{18}F]-fluorodeoxyglucose positron emission tomography (FDG-PET) revealed multiple abnormal accumulations in the bone marrow, pancreas, and at the periphery of the left kid-

Received : May 22, 2010
Revised : July 24, 2010
Accepted : August 20, 2010
[1)]Department of Hematology, Oami Hospital, Chiba, Japan
[2)]Department of Hematology, Chiba University Hospital, Chiba, Japan
[3)]Division of Hematology-Oncology, Chiba Cancer Center Hospital, Chiba, Japan
Address correspondence and reprint request to : Hiroaki Tanaka, M.D., Ph.D.
Department of Hematology, Oami Hospital
884-1, Tomita, Oamishirasato-machi, Sanbu-gun, Chiba, 299-3221, Japan
E-mail : hiroakitanaka@oami-hp.jp

ney. Malignant lymphoma was suspected and the patient was hospitalized.

No particular abnormalities were detected by a physical examination. Superficial lymph nodes, liver, and spleen were not palpable. In addition, no skin lesions were observed.

Using enhanced computed tomography (CT), no lymph node swelling or organomegaly was identified, but enhanced lesions in the periphery of the pancreas were recognized. Abnormal laboratory findings (Table 1) included albumin 2.6 g/dL ; glucose 265 mg/dL ; LDH 2,889 IU/L ; CRP 17.20 mg/dL ; and soluble interleukin-2 receptor (sIL-2R) 3,720 U/mL. Bone marrow examination revealed 40.6% of large abnormal lymphocytes with some vacuoles and basophilic cytoplasm (Fig. 1a), which were positive for CD10, CD19, and CD20 and had a laterality of lambda chain. Chromosomal analyses of the bone marrow specimens using G-banding staining showed a deleted Y chromosome (Table 2a).

Bone marrow biopsy specimens (Fig. 2) revealed the interstitial or intravascular proliferation of medium- or large-sized nuclear cells (Fig. 2a, 2b), which were positive for LCA and L26 (Fig. 2c), and 80% MIB-1 (Fig. 2d), using immunohistochemical staining. The bone marrow structure was not destroyed.

The clinical presentation and tumor cell morphology met the criteria for Burkitt-like lymphoma according to the REAL classification, and CHOP therapy [doxorubicin (ADM) 60 mg, cyclophosphamide 900 mg, vincristine 1.6 mg, and prednisolone (PSL) 100 mg] was begun on September 20, 2006. Soon after the first course of CHOP therapy, the clinical findings were improved, and the second course of chemotherapy followed, in which 500 mg of Rituximab (Ritux) was added (R-CHOP therapy). After eight courses of R-CHOP therapy, the patient achieved complete remission (CR) on March 15, 2007.

In February of 2008, the patient suddenly suffered from edema and pain of his right leg (first relapse). Laboratory examinations revealed elevated LDH, CRP, and sIL-2R levels, and enhanced CT examination showed identical abnormal findings as found at the beginning of disease onset. CHOP therapy was begun on February 15, 2008. The clinical findings improved quickly, and subsequently, the patient underwent four courses of R-modified ICE therapy (Ritux : 500 mg on day 1, ifosfamide : 1,500 mg on days 2 to 6, carboplatin : 500 mg on day 2, etoposide : 100 mg on days 2 to 6), and achieved CR on June 24, 2008. High-dose chemotherapy followed by autologous stem cell transplantation was next

Table 1. Laboratory examination at the first admission

Peripheral blood		Blood chemistry		Serology	
WBC	6,100/μL	TP	6.8 g/dL	CRP	17.20 mg/dL
Myelo	1%	Alb	2.6 g/dL	IgG	1,241 mg/mL
Meta	2%	GOT	60 IU/L	IgA	439 mg/mL
Stab	11%	GPT	41 IU/L	IgM	80 mg/mL
Seg	68%	LDH	2,889 IU/L	sIL 2 R	3,720 U/mL
Mo	6%	AlP	628 IU/L	Antibody against EBV	
Ly	11%	GTP	43 IU/L	VCA-IgG	× 640
Eo	1%	AMY	70 mg/dL	VCA-IgM	< × 10
Hb	12.3 g/dL	Cre	1.0 mg/dL	EA-IgG	× 10
Plt	27.1 × 10^4/μL	Glu	265 mg/dL	EA-IgG	× 160

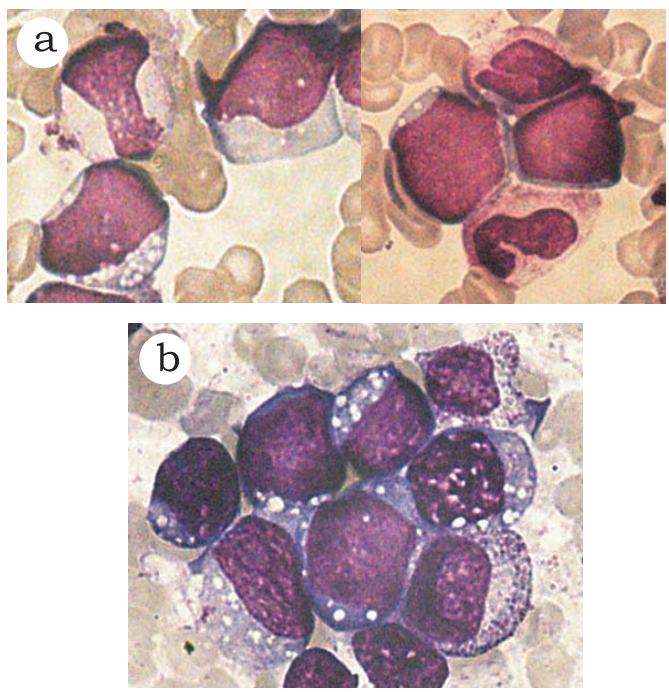

Fig. 1. Lymphoma cells at the disease onset (*1a*) and the second relapse (*1b*). (***1a***) Bone marrow examination revealed 40.6% of large abnormal lymphocytes with some vacuoles and basophilic cytoplasm. (***1b***) There were massive lymphoma cells that had almost the same morphologic features as at the disease onset. Stamp smear of bone marrow biopsy specimen : (*1a*) & (*1b*) May-Giemsa stain, × 400.

Table 2. Chromosomal analysis by G-banding staining at the onset and the second relapse

(a)	At the disease onset	
	45, X, -Y	[3/20]
	46, XY	[17/20]
(b)	At the second relapse	
	56, XY, +X, +7, +7, +7, +8, t(8;22)(q24;q11.2) × 2, +11, +13, del(13)(q?) × 2, add(14)(q22), t(14;18)(q32;q21), der(18)t(14;18), +20, +20, +mar1	[1/20]
	54, XY, +X, +1, +7, +7, +8, t(8;22)(q24;q 11.2) × 2, +11, +13, del(13)(q?) × 2, add(14)(q22), der(14)add(14)(p11.2) t(14;18)(q32;q21), der(18)t(14;18) × 2, +20	[10/20]
	46, XY	[9/20]

planned, but it could not be performed because the patient did not give his consent. The patient was watched carefully and thereafter did not receive any further treatment.

In November of 2008, the patient suffered from severe bilateral pain of his legs, lower abdomen, and lumbar region, and was immediately hospitalized (second relapse). The laboratory findings revealed elevated LDH, CRP, and sIL-2R levels, and the bone marrow examination revealed massive lymphoma cells, which had almost identical morphologic features to those at the disease onset (Fig. 1b). Chromosomal analyses of the bone marrow revealed that these cells had complex karyotypes with concurrent t(14;18) and t(8;22) translocations (Table 2b). The enhanced CT examination showed few abnormalities, but FDG-PET examination revealed multiple abnormal accumulations at bone marrow, fundus of stomach, head of pancreas, and the periphery of the right kidney. The patient underwent salvage chemotherapy, which consisted of Ritux, irinotecan, ADM, and PSL (Ritux : 500 mg on day 1, irinotecan : 35 mg on days 2 and 3, ADM : 50 mg on day 4, and PSL : 80 mg on days 1 to 4), but the effects were minimal and transient. The patient suffered from urodynia and abdominal distension. Plain CT examinations revealed high-density diffuse lesions spreading extensively from the right upper abdomen to the right retroperitoneal region, hypertrophy of the urinary bladder wall, bilateral hydronephropathy, and ascites. Urinary cytology revealed lymphoma cells that were identical to those in the bone marrow. High-dose cytarabine therapy (cytarabine 1,000 mg two times per day on days 1 and 2) was therefore administered. The combination administration of high-dose methotrexate was not performed because of the patient's ascites. The patient thereafter continued to deteriorate with gross hematuria and died of lymphoma on December 27, 2008.

Cytogenetic analyses by fluorescence *in situ* hybridization (FISH) were retrospectively performed using formalin-fixed bone marrow specimens collected at the disease onset or at the second relapse. Both *MYC* and *BCL2* translocations (Fig. 3) were detected in the specimen at the second relapse, but they were not evaluable in the initial onset specimen mainly

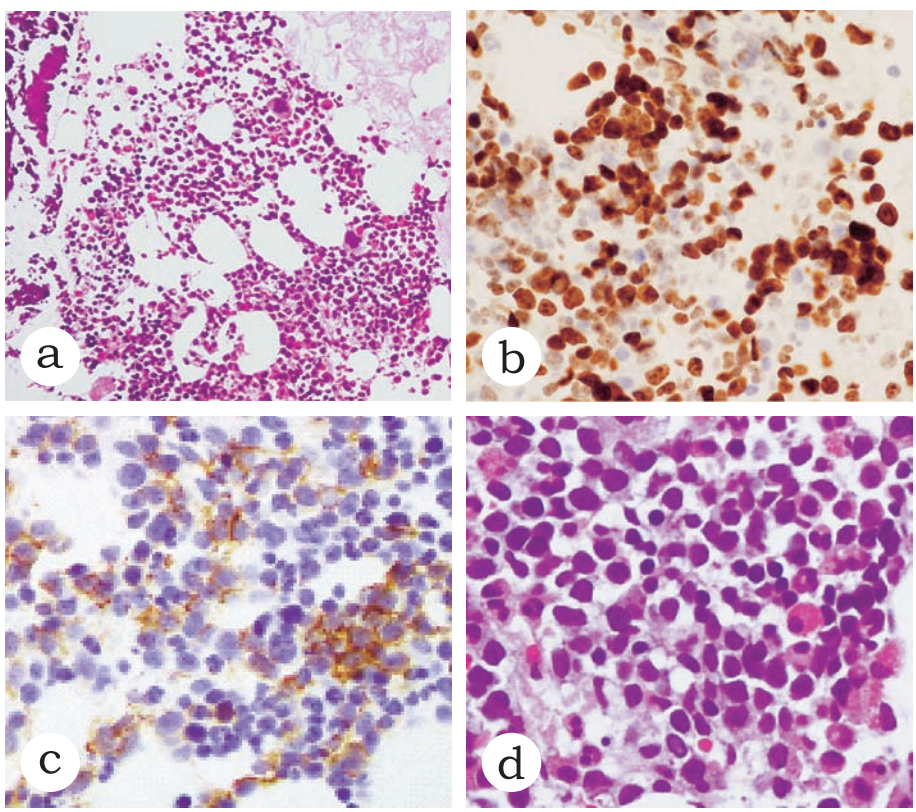

Fig. 2. Bone marrow biopsy at the disease onset. (***2a & 2b***) There was interstitial or intravascular proliferation of medium- or large-sized cells. Bone marrow structure was not destroyed. (***2c***) Lymphoma cells were positively stained by CD20 (L26) immunohistochemistry. (***2d***) Eighty percent of lymphoma cells were positively stained by MIB-1 immunohistochemistry. (*2a*) & (*2b*) H&E stain, (*2a*) × 100, (*2b*) × 400 ; (*2c*) & (*2d*) Counterstained with hematoxylin, × 400.

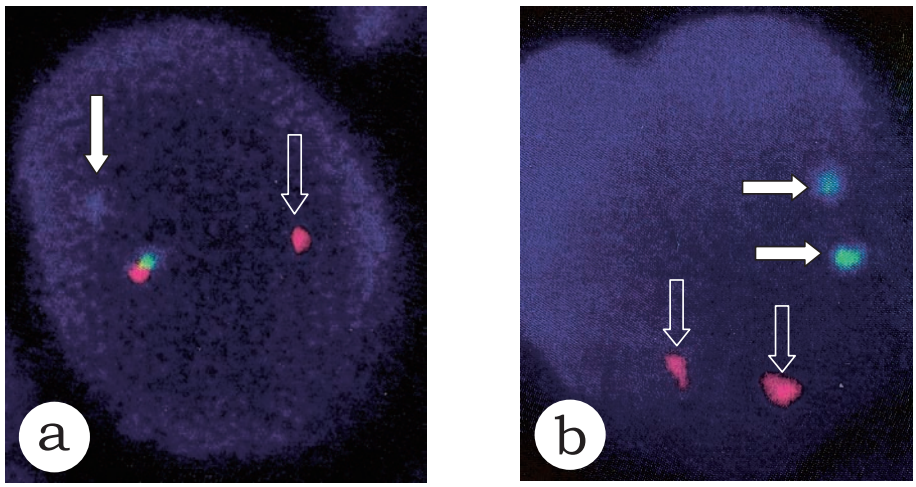

Fig. 3. Fluorescence *in situ* hybridization analysis for *BCL2* and *MYC* in bone marrow at the second relapse. Green-labeled DNA probe (*white arrow*) and red-labeled DNA probe (*blank arrow*) bind to both sides of *MYC* and *BCL2*, respectively. Translocation event of these genes shows split signals with two colors. (***3a***) Split signal by *MYC* translocation. (***3b***) Split signal by *BCL2* translocation.

due to technical problems following strong auto-fluorescence effects.

DISCUSSION

DHL is an emerging disease concept in which concurrent *BCL2* and *MYC* translocations are characteristic genetic features. DHL is now described as a well-characterized group in the category of B-cell lymphoma unclassifiable with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma in the WHO classification 2008.[6] Moreover, DHL is thus considered to warrant a separate category due to the clinical and molecular cytogenetic features that distinguish it from other high-grade B-cell lymphomas in future classifications.[7]

DHL progresses to extranodal lesions such as in the bone marrow, central nervous system, and gastrointestinal tract.[1-5] Lymphoma cells are generally positive for CD10, 19, and 20, consistent with the germinal center phenotype.[1,3,5] DHL has a high progression ability. MIB-1-positive cells are reported in less than 95% of lesions, which is less than that observed in Burkitt lymphoma, which demonstrates over 95% of MIB-1-positive cells.[3,5,7] A complex karyotype including both *BCL2* and *MYC* translocations is frequently recognized.[1,4]

BCL2 deregulation caused by BCL2 translocation is known to increase cell survival by preventing apoptosis. BCL2 translocation, by prolonging cell survival, predisposes the cell to the acquisition of secondary chromosomal aberrations. *MYC* translocation can occur as a secondary event and DHL arises. This lymphomagenesis has been demonstrated *in vivo* in transgenic mouse models.[8,9] In fact, there are several cases of patients with a known history of FL, who had a preexisting BCL2 translocation prior to the diagnosis of DHL, and most cases of secondary DHL are transformed from FL.[2-4]

In our case, at the time of disease onset, the clinical presentation and the tumor cell morphology met the Burkitt-like lymphoma criteria according to the REAL classifications.[10,11] Interestingly enough, the clinical presentation and morphology were almost the same, through the time of disease onset to the second relapse. However, chemosensitivity was completely different from that observed at either the disease onset or the first relapse, and the patient died of DHL almost 1 month after the second relapse of the disease. It is not clear when the patient developed the *BCL2* and *MYC* translocations because of a lack of FISH analysis data at the disease onset. Naturally, the possibility that the double hit was already present at onset but not detectable by G-banding assay cannot be refuted. However, chemosensitivity was clearly different between the disease onset and the second relapse. In addition, it is difficult to believe that the chromosomal abnormality of these rapidly proliferative lymphoma cells was not detected by G-banding assay. Presumably, double hit could have been completed during the clinical course, just prior to the second relapse.

DHL has a high resistance to intensive chemotherapy, including high-dose chemotherapy followed by stem cell transplantation.[3,4] Patient prognosis is poor, and over half of all DHL patients die within 1 year.[1-5,12] The high chemoresistance in DHL is considered to be due to the apoptotic inhibition of the deregulation of BCL2 and the enhanced proliferation of the deregulation of *MYC*. Parker *et al*. re-

ported two cases of DHL successfully treated with an aggressive immunochemotherapy regimen, autologous stem cell transplantation, and radiation therapy : No recurrence of the disease was seen in the radiation field in the first patient, and no recurrence in the second patient was observed after total body irradiation (TBI), suggesting that radiation therapy may be beneficial for patients with DHL, and that up-front TBI-based hematopoietic stem cell transplantation could potentially produce sustained complete remission for DHL.[13]

Early recognition of DHL is important for considering up-front TBI-based hematopoietic stem cell transplantation. However, it is important to note that DHL cannot be diagnosed without chromosomal analysis. Close examination, including chromosomal analysis, is therefore desirable for all patients with high-grade B-cell lymphoma and recurrence of this lymphoma.

ACKNOWLEDGMENTS

The authors would like to thank Prof. K. Oshima of Kurume University for providing valuable data and cytogenetic analyses by FISH (*BCL2* and *MYC*), immunohistochemical staining for MIB-1 analysis.

REFERENCES

1 Kanungo A, Medeiros LJ, Abruzzo LV, Lin P: Lymphoid neoplasms associated with concurrent t(14;18) and 8q24/c-*MYC* translocation generally have a poor prognosis. Mod Pathol 19:25-33, 2006

2 Lin P, Medeiros LJ: High-grade B-cell lymphoma/leukemia associated with t(14;18) and 8q24/*MYC* rearrangement: a neoplasm of germinal center immunophenotype with poor prognosis. Haematologica 92:1297-1301, 2007

3 Johnson NA, Savage KJ, Ludkovski O, Ben-Neriah S, Woods R, *et al.*: Lymphomas with concurrent *BCL2* and *MYC* translocations: the critical factors associated with survival. Blood 114:2273-2279, 2009

4 Tomita N, Tokunaka M, Nakamura N, Takeuchi K, Koike J, *et al.*: Clinicopathological features of lymphoma/leukemia patients carrying both *BCL2* and *MYC* translocations. Haematologica 94:935-943, 2009

5 Niitsu N, Okamoto M, Miura I, Hirano M: Clinical features and prognosis of *de novo* diffuse large B-cell lymphoma with t(14;18) and 8q24/*c-MYC* translocation. Leukemia 23:777-783, 2009

6 Kluin PM, Harris NL, Stein H, Leoncini L, Raphael M, *et al.*: B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma. In: Swerdlow SH, Campo E, Harris NL Jaffe ES, Pileri SA, *et al* (Eds): WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed, Lyon, IARC, pp. 265-266, 2008

7 de Jong D: Novel lymphoid neoplasms -the borderland between diffuse large B-cell lymphoma and Burkitt's lymphoma. Haematologica 94:894-896, 2009

8 Marin MC, Hsu B, Stephens LC, Brisbay S, McDonnell TJ: The functional basis of *c-MYC* and *bcl-2* complementation during multistep lymphomagenesis *in vivo*. Exp Cell Res 217:240-247, 1995

9 McDonnell TJ, Korsmeyer SJ: Progression from lymphoid hyperplasia to high-grade malignant lymphoma in mice transgenic for the t(14;18). Nature 349:254-256, 1991

10 Harris NL, Jaffe ES, Stein H, Banks PM, Chan JK, *et al.*: A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood 84:1361-1392, 1994

11 Armitage JO, Weisenburger DD: New approach to classifying non-Hodgkin's lymphomas: clinical features of the major histologic subtypes. Non-Hodgkin's Lymphoma Classification Project. J Clin Oncol 16:2780-2795, 1998

12 Mead GM, Barrans SL, Qian W, Walewski J, Radford JA, *et al.*: A prospective clinicopathologic study of dose-modified CODOX-M/IVAC in patients with sporadic Burkitt lymphoma defined using cytogenetic and immunophenotypic criteria (MRC/NCRI LY10 trial). Blood 112:2248-2260, 2008

13 Parker SM, Olteanu H, Vantuinen P, Lawton CA, Schultz CJ, *et al.*: Follicular lymphoma transformation to dual translocated Burkitt-like lymphoma: improved disease control associated with radiation therapy. Int J Hematol 90:616-622, 2009