Case Study

Double-Hit Lymphoma with a Feature of Follicular Lymphoma Concurrent with Clonally Related B Lymphoblastic Leukemia : A Preference of Transformation for the Bone Marrow

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We describe a 65-year-old woman with follicular lymphoma (FL), grade 1, stage IV, which occurred concurrently with B lymphoblastic leukemia/lymphoma. Through the evaluation of FL, the cells that were morphologically suspected of having undergone transformation were found in the bone marrow, and flow cytometric and cytogenetic analyses detected the transformed population that suggested concomitant t(8;22) with typical t(14;18) FL cells. Repeated analyses of the lymph nodes demonstrated the typical morphological, phenotypic, and cytogenetic features of FL. The patient received several multiagent chemotherapy regimens, but the disease gradually became resistant, and the patient died of leukemic progression. In B-cell malignancies, cases involving both *BCL2* and *MYC* translocations simultaneously, so-called "double-hit leukemia/ lymphoma (DHL)", have occasionally been reported. Patients with this type of translocation have a very poor clinical outcome, and no standard therapy has been established. In our case, FL was supposed to have transformed into B lymphoblastic leukemia via Burkitt's lymphoma-like phase. Our case is unique in that the transformed DHL cells, derived from clonally related FL cells, showed ongoing transformation from Burkitt-like feature to B lymphoblastic leukemia exclusively in the bone marrow, which suggests that the bone marrow may provide a preferable milieu for malignant transformation. Similar cases should be accumulated and analyzed carefully. 〔*J Clin Exp Hematopathol* 52(2) : 113-119, 2012〕

Keywords: *BCL2*, *MYC*, follicular lymphoma, B lymphoblastic leukemia, double-hit lymphoma

INTRODUCTION

Lymphomas with concurrent *BCL2* and *MYC* translocations, so-called double-hit lymphomas (DHLs), are rare and have been reported to have an aggressive clinical course and a poor prognosis.[1,2] In the WHO Classification of Tumors 4th edition, DHL was included as "B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma (DLBCL) and Burkitt's lymphoma (BL)".[3] Although the majority of cases have morphological features that are intermediate between DLBCL and BL, some transformed follicular lymphoma (FL) may fall into this category. This is morphologically and immunophenotypically heterogeneous and is not considered a distinct disease entity ; in rare cases that are positive for terminal deoxynucleotidyl transferase (TdT), the diagnosis of B lymphoblastic leukemia/lymphoma may be preferred.[3] Nine cases of histological transformation from FL to B lymphoblastic leukemia/lymphoma have been reported in the literature, and 8 of these cases were shown to have both *BCL2* and *MYC* rearrangements.[4–9]

Here, we describe a somewhat complicated case of typical nodal FL, grade 1, which presented with transformation accompanied by concurrent *BCL2* and *MYC* translocations in the bone marrow. The unique clinical features and suspected pathogenesis of this case are discussed.

CASE REPORT

A 65-year-old woman was admitted to another hospital in May 2008 complaining of abdominal distension and profuse night sweats. Computed tomography (CT) disclosed cervical, axillary, mediastinal, mesenteric, para-aortic, iliac, and inguinal lymphadenopathy associated with massive pleural effusion and ascites. A biopsy specimen of the left inguinal lymph node revealed FL, grade 1 (Fig. 1). She was referred

to our hospital on the sixth hospital day.

She appeared to be badly ill, was suffering from respiratory distress, and complained of a persistent low-grade fever. A physical examination revealed decreased respiratory sounds in the left lung, a markedly distended abdomen with massive ascites, and swelling of numerous superficial lymph nodes. Peripheral blood analysis revealed a white blood cell count of 8.8×10^9/L, hemoglobin level of 132 g/L, and platelet count of 192×10^9/L. Her lactate dehydrogenase (LDH) and solu-

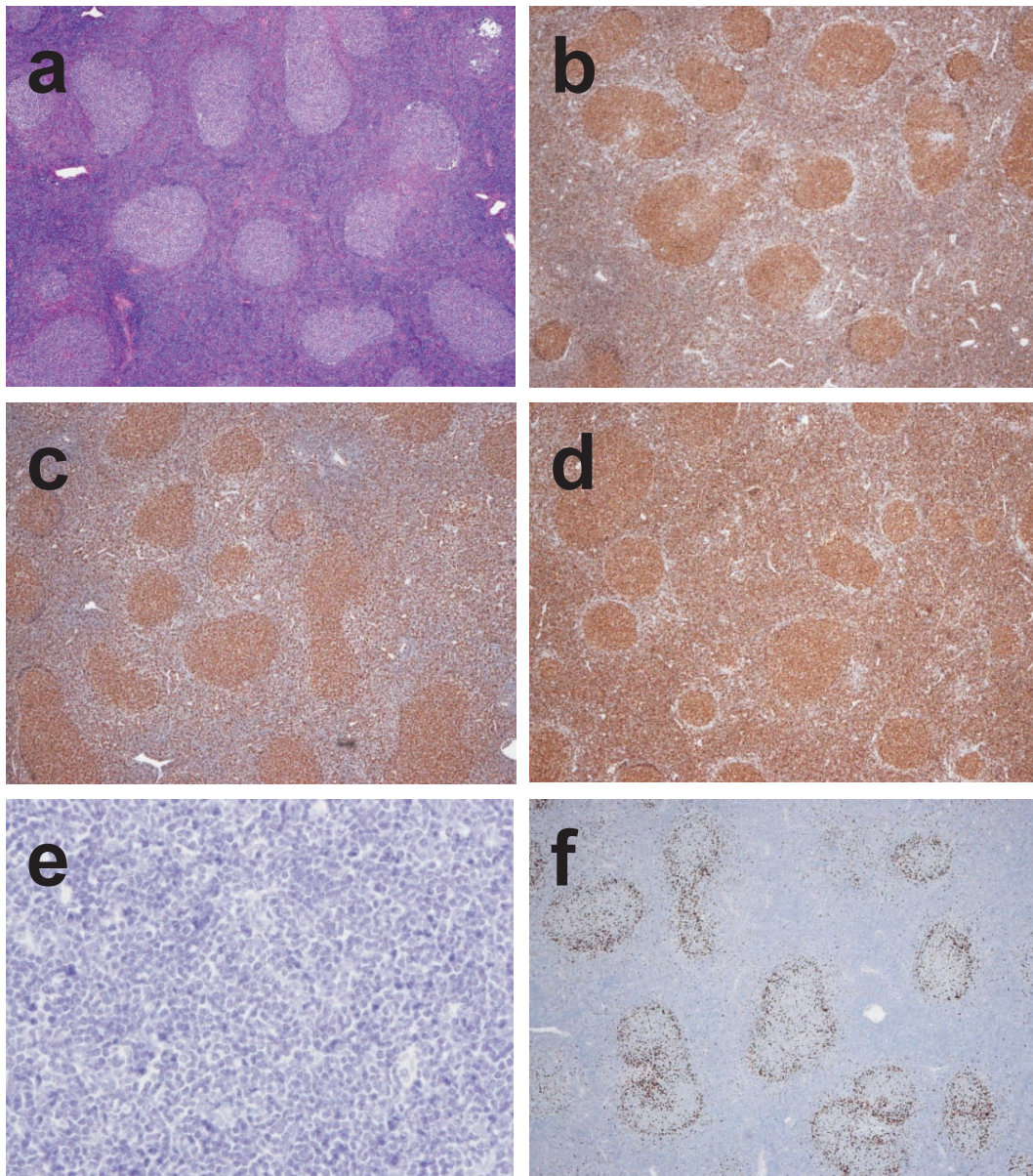**Fig. 1.** Microscopic examination of the inguinal lymph node before the therapy. (*1a*) Scattered neoplastic follicles with attenuated mantle zones (H&E stain). (*1b*, *1c*, *1d*, & *1e*) The neoplastic cells were positive for CD10 (*1b*), CD20 (*1c*), and *BCL2* (*1d*) and negative for terminal deoxynucleotidyl transferase (*1e*). (*2f*) The MIB-1 (anti-Ki-67) index was estimated to be 15-20% (*1a-1f*; original magnification, × 40).

ble interleukin-2 receptor levels were 1,038 IU/L and 3,400 U/mL, respectively. Her bone marrow was hypercellular (782 × 10⁹/L). Of the bone marrow cells, 64.8% were small to medium-sized abnormal lymphocytes, and the few large immature cells were scattered. Flow cytometric analysis (FCM) revealed two distinct populations. The smaller population was recognized in the lymphoid gate representing FL cells, and the other was larger and was detected in the blast gate. However, these populations were commonly positive for CD10, CD19, CD20, and Igκ (Fig. 2). Chromosomal analysis of the bone marrow aspirate showed both t(14;18) and t(8;22) in the same cells as follows : 48, XX, +X, t(8;22) (q24;q11.2), der (9) (p?), +12, t(14;18) (q32;q21) in 6 out of the 20 mitotic cells analyzed, which was confirmed after the initiation of chemotherapy.

Under a provisional diagnosis of FL, grade 1, stage IV, multiagent chemotherapy including rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP) was immediately initiated, paying full attention to the scarcely identified large cells. After the second course, the bone marrow was normalized morphologically and phenotypically with normal karyotype. After the fourth course of R-CHOP, partial remission was achieved. However, her left inguinal lymph node did not respond well to the therapy and was biopsied in September, which confirmed the presence of FL, grade 1. FCM showed that the lymphoma cells were positive for CD10, CD19, CD20, Igκ, and HLA-DR and negative for TdT.

Soon after the completion of the sixth course of chemotherapy, rapidly elevated serum levels of LDH were noted in
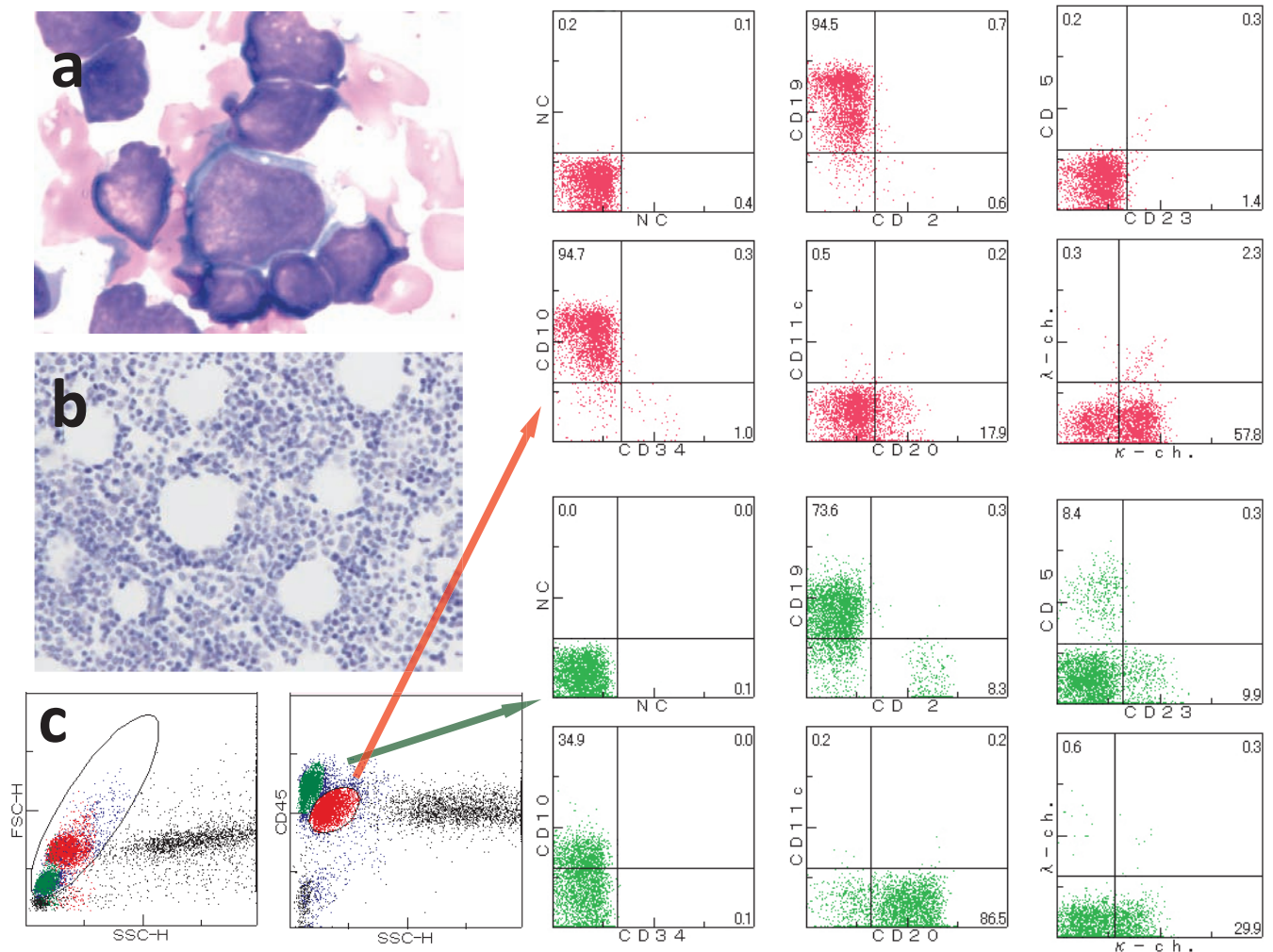
Fig. 2. Bone marrow features on admission. (*2a*) The smear shows the diffuse infiltration of follicular lymphoma (FL) cells. Large cells with cytoplasmic vacuoles were scattered among FL cells (original magnification, × 1,000). (*2b*) Immunohistochemically, the proliferating cells were negative for terminal deoxynucleotidyl transferase (original magnification, × 400). (*2c*) Flow cytometric analysis showed positivity for CD10, CD19, CD20, and Igκ.

early November. Peripheral blood analysis revealed a white blood cell count of 2.9×10^9/L, which comprised 44% neutrophils, 26% lymphocytes, 14% monocytes, 7% basophils, and 9% blasts. Her hemoglobin level was 78 g/L, her platelet count was 89×10^9/L, and her LDH level was 5,565 IU/L. Although CT showed no evidence of recurrent lymphadenopathy and there was no sign or symptom suggestive of extranodal or central nervous system involvement, her almost normocellular bone marrow (223×10^9/L) revealed the proliferation of the abnormal lymphoid blasts. Among the bone marrow cells, 79.2% were large immature lymphocytes with cytoplasmic vacuoles, which were positive for CD10, CD19, and TdT and negative for cytoplasmic immunoglobulin expression as well as surface Igκ (Fig. 3). Chromosomal analysis of the bone marrow aspirate showed t(14;18) and t(8;22) in the same cells as follows : 48, XX, +X, dup (1)

(q21q32), t(8;22) (q24;q11.2), der (9) (p?), +del (12) (q24.1), t(14;18) (q32;q21) in 4 out of the 19 mitotic cells analyzed. We then performed fluorescent *in situ* hybridization (FISH) analysis of the bone marrow aspirate, which demonstrated *BCL2* translocation in 66% of interphase cells and *MYC* translocation in 82% of interphase cells (Fig. 4a & 4b). On the other hand, FISH analysis of the inguinal lymph node that was re-biopsied after the fourth course of therapy showed only *BCL2* translocation in 97% of interphase cells without *MYC* translocation (Fig. 4c & 4d).

The patient received the hyper-CVAD regimen, the high-dose MTX/Ara-C regimen, and an L-asparaginase-based regimen, but the disease was resistant to these chemotherapies. The patient died of uncontrollable disease progression in February 2009.
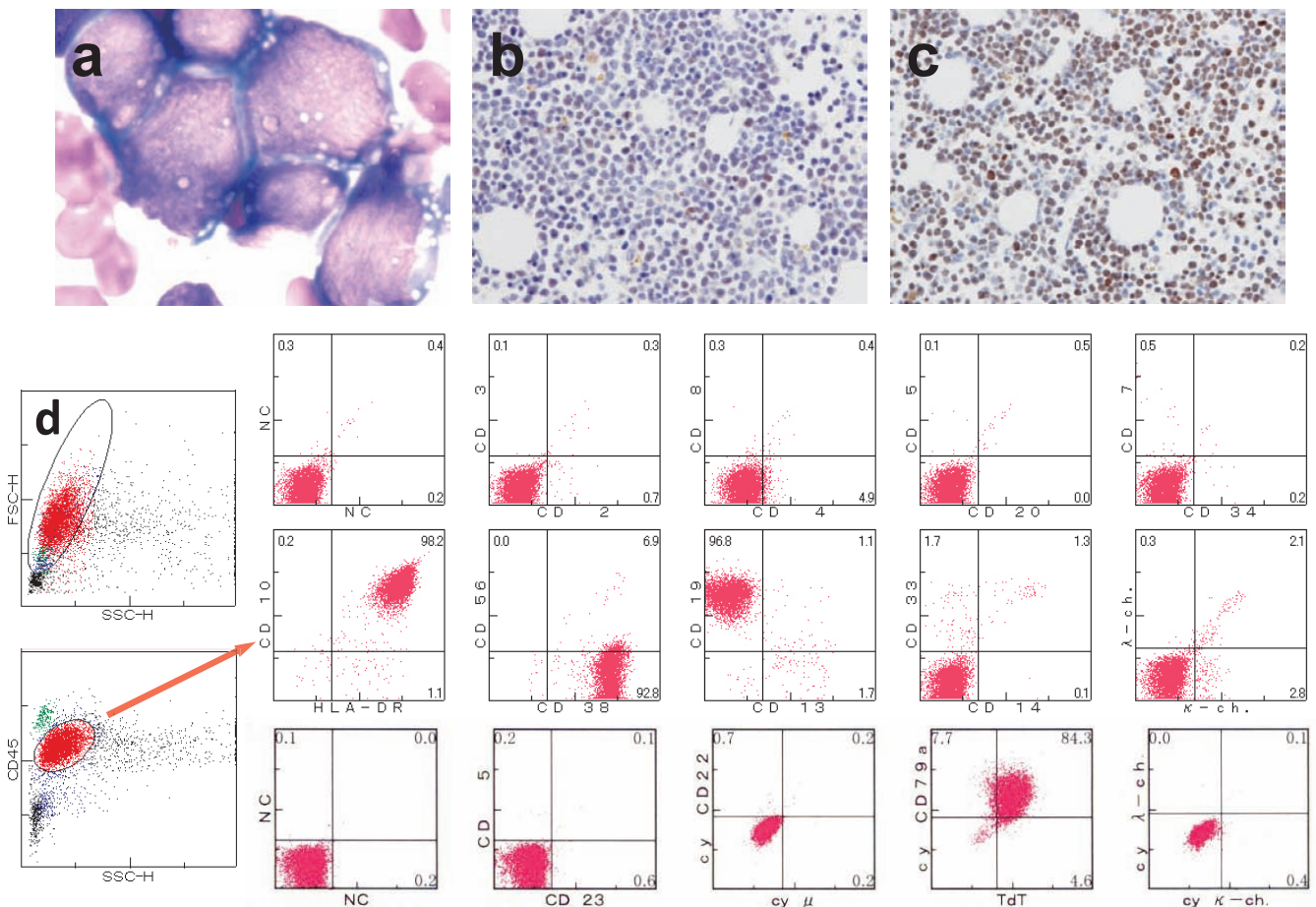
Fig. 3. Bone marrow features at the blastic transformation. (*3a*) The smear shows the diffuse infiltration of large neoplastic cells with cytoplasmic vacuoles resembling Burkitt's lymphoma/leukemia (original magnification × 1,000). (*3b*) Immunohistochemically, the proliferating cells were terminal deoxynucleotidyl transferase-positive. (*3c*) The MIB-1 (anti-Ki-67) index was estimated to be 70% (*3b-3c* ; original magnification, × 400). (*3d*) Flow cytometric analysis showed positivity for CD10, CD19, CD38, CD79a, HLA-DR, and terminal deoxynucleotidyl transferase.

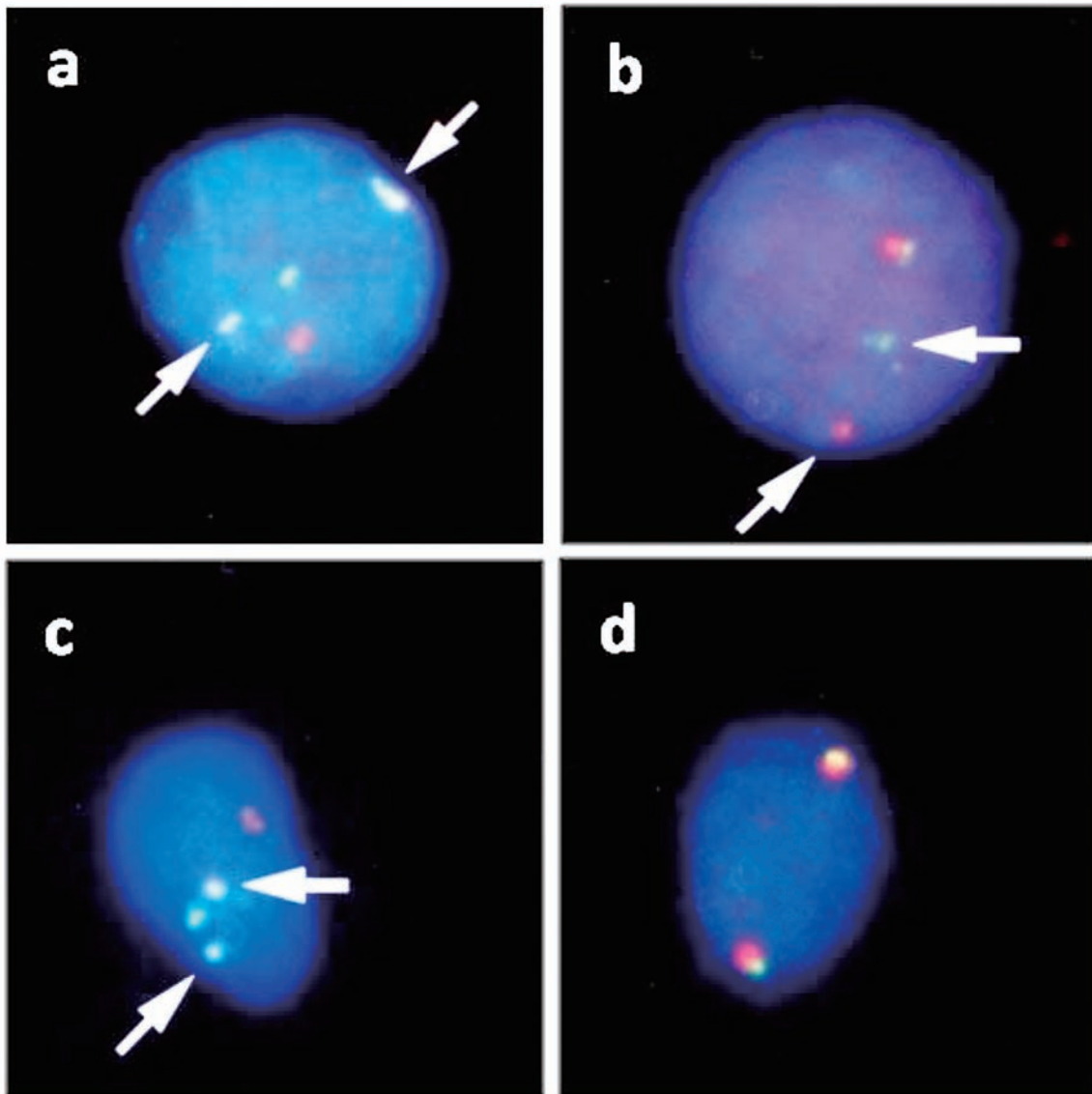**Fig. 4.** Interphase fluorescent *in situ* hybridization (FISH) analysis of the bone marrow aspirate (*4a* & *4b*) and of the inguinal lymph node (*4c* & *4d*). (*4a*) Fusion signals indicating t(14;18) (q32;q21) were seen in 66% of interphase cells. The green and red signals represent the *IGH* (14q32) and *BCL2* probes (18q21), respectively. The fusion signals (*yellow*) are indicated by *arrows*. (*4b*) Split signals for the *MYC* gene were seen in 82% of interphase cells (*arrows*). The green and red signals represent *3'MYC* and *5'MYC*, respectively. (*4c*) Fusion signals indicating t(14;18) (q32;q21) were seen in 97% of interphase cells (*arrows*). (*4d*) Split signals for the *MYC* gene were seen in 0% of interphase cells.

DISCUSSION

We described a patient with FL associated with clonally related B lymphoblastic lymphoma. Because of the deterioration in the general condition, we started chemotherapy using the CHOP regimen immediately after a tentative diagnosis of FL, grade 1, stage IV, had been made. However, chromosomal analysis of the first bone marrow aspiration in our hospital subsequently disclosed both t(14;18) and t(8;22) in the same cells. Although this dual translocation, along with a population of large cells that was observed in the hypercellular bone marrow, indicated a sign of transformation, because the patient's initial response to the therapy was excellent, we continued the initially planned R-CHOP regimen until the sixth course, paying full attention to the t(8;22) abnormality in her bone marrow. Despite our apprehension, re-biopsy of the inguinal lymph node after the fourth course showed no pathological changes. Therefore, we carried out two more cycles

of the chemotherapy. At the overt blast transformation, chromosomal analysis of the bone marrow aspirate showed both t(14;18) and t(8;22) abnormalities in the same cells, and this was confirmed by FISH. Interestingly, analyses of the inguinal lymph node consistently showed the *BCL2* translocation alone and the typical morphology of pure FL, grade 1.

In a recently reported retrospective study, concurrent *BCL2* and *MYC* translocations were identified in about 200 cases, and a variety of evidence suggested that *BCL2* translocation precedes *MYC* events in lymphomagenesis of DHL.[10] Analysis of 54 cases with DHL disclosed that *MYC* rearrangements were found at the time of the initial lymphoma diagnosis in about 60%, while, in about 40%, *MYC* rearrangement occurred at the time of histological transformation from pre-existing FL (19/54), DLBCL (3/54), or chronic lymphocytic leukemia (1/54). The morphology of DHLs of this type frequently presents as intermediate DLBCL/BL (36/54) or DLBCL (17/54), and only one sample presented as FL, grade 2.[11] Another recent study from Japan analyzed 27 cases of DHL involving both *BCL2* and *MYC* translocations. At the time of the diagnosis, extranodal involvement was found in 25 cases (93%) and central nervous system involvement was noted in 15 cases (56%). These patients had an extremely poor prognosis ; median survival was 6 months, and the 1-year survival rate was 22%.[12] The response to chemotherapy of this type of lymphoma is limited, and most cases relapse. No standard therapy has been established.[10]

In our case, at the initial evaluation, the bone marrow examination revealed that few BL-like larger cells were admixed with clonally related FL cells : phenotypically, CD10^{low+}, CD19$^+$, CD20$^+$, Igϰ$^+$ FL cells vs. CD10$^+$, CD19$^+$, CD20^{low+}, Igϰ$^+$ BL-like cells (Fig. 2). Cytogenetically, both *BCL2* and *MYC* translocations were detected, which corresponds to DHL. Soon after the completion of R-CHOP therapy, bone marrow examination revealed the proliferation of blasts. They were medium to large in size with cytoplasmic vacuoles and displayed a high proliferation rate. Phenotypically, these cells displayed CD10, CD19, CD38, TdT losing CD20, Igϰ, cytoplasmic immunoglobulin expression, although the disappearance of CD20 epitope may have been due to a down-modulating effect of the CD20 antigen by rituximab therapy.[13] Metamorphosis into more immature cells might have progressed. According to previous reports,[4-5,7-9] a diagnosis of B lymphoblastic leukemia, instead of intermediate DLBCL/BL, may be preferred, while the classification of such cases is still controversial.[3] Furthermore, the pathological diagnoses made after two consecutive biopsies of the coexisting inguinal lymph nodes were both FL, grade 1, without any signs of lymphoblastic transformation. Cytogenetically, *BCL2* translocation alone was detected in the biopsied specimen. Therefore, there were two related clones coexisting in the patient : one displaying the *BCL2* translocation alone and the other demonstrating both

BCL2 and *MYC* translocations. The former was found in the lymph nodes, while the latter was detected in the bone marrow. It may be natural to assume that histological transformation from FL with t(14;18) to B lymphoblastic leukemia involving the acquisition of an additional *MYC* rearrangement occurred. The detection of the few possible precursors of the blasts at the initial assessment and of the overt blasts that emerged after the therapy for FL was consistently restricted in the patient's bone marrow.

Since 1988, nine similar cases in which FL transformed to B lymphoblastic leukemia/lymphoma have been reported in the literature (Table 1).[4-9] In these nine cases, age at initial diagnosis ranged from 33 to 68 years old, with a median of 59 years old. The interval from the initial diagnosis to the lymphoblastic transformation ranged from early in the disease course to 12 years. Exceptionally, in case 2, B lymphoblastic lymphoma was detected at the initial diagnosis, presenting as composite lymphoma.[5] Transformed B lymphoblastic leukemia/lymphoma revealed *BCL2* and *MYC* gene rearrangements in all cases except for case 8. In 5 cases, the transformed cells expressed TdT. After blast transformation, these cases underwent multiagent chemotherapy or radiation therapy, but the survival after the final diagnosis was extremely short, ranging from 0.75 to 9 months, with a median survival of 4 months. Our case exhibited a similar clinical course. However, a unique characteristic of our case is that the lymphoblastic component was detected at the initial diagnosis of FL, as in case 2, but it had a different organ distribution from the FL.

The transformation was believed to have occurred sequentially from FL, as reported in the case described by Kroft *et al.* in which the lymphoblastic lymphoma shared the same immunoglobulin heavy chain gene rearrangement as the FL.[8] However, recent studies have indicated that FL and its transformants can also arise by divergent evolution from common progenitor cells in VDJ rearrangement.[14,15] This suggests that transformation of the cells with *BCL2/IGH* may originate in less differentiated cells. Therefore, another explanation for our case is that progenitor cells (FL precursor cells) containing the t(14;18) translocation acquired an additional *MYC* translocation in the bone marrow, thus transforming to TdT-positive B lymphoblastic leukemia. We therefore might have observed the ongoing transformation from FL (or its precursor) cells to B lymphoblastic leukemia cells in the bone marrow, which may afford a preferable milieu for transformation.

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Table 1. Reported cases of B-acute lymphocytic leukemia (ALL)/lymphoblastic lymphoma (LBL) transformed from preceding follicular lymphoma (FL)

Case	Age/ Sex	Initial			Interval (mo)	Transformed				Survival from final diagnosis (mo)	Reference
		Diagnosis	Involved sites	Gene rearrangement		Diagnosis	Involved sites	TdT	Gene rearrangement		
1	50/F	FL	hard plate	NA	65	DLBCL, B-ALL	BM, PB	+	t(14;18) t(8;14)	unknown	[4]
2	44/M	FL+ B-LBL	LN	NA	0	FL+B-LBL	LN, BM, PB	+	t(14;18) t(8;14)	9	[5]
3	62/M	FL	LN	NA	21	B-ALL	PB, PE	NA	t(14;18) t(8;14)	4	[6]
4	64/M	FL	LN	NA	93	FL+B-LBL	LN, BM, PB	−	t(14;18) t(8;14)	1	[6]
5	68/M	FL	LN	NA	4	B-ALL	BM, PB	−	t(14;18) t(8;22)	4	[7]
6	59/F	FL	LN	NA	25	acute undifferen-tiated leukemia	BM, PB	weak	t(14;18) t(8;22)	3	[7]
7	55/F	FL	LN	NA	10	Burkitt leu-kemia	BM, PB, LN, liver, spleen	NA	t(14;18) t(8;22)	0.75	[7]
8	33/F	FL	LN	negative for *BCL2/MYC* rearrangement	139	B-LBL	LN	+	negative for *BCL2/MYC* rearrangement	9	[8]
9	59/ F	FL+ DLBCL	LN	t(14;18)+t(8;14) in DLBCL areas	6	B-LBL	LN	+	t(14;18) t(8;14)	7	[9]
10	65/F	FL	LN, BM	t(14;18)	0	B-ALL	BM, PB	+	t(14;18) t(8;22)	3	Present case

LN ; lymph node, BM ; bone marrow, PB ; peripheral blood, PE ; pulmonary effusion, NA ; not available, DLBCL ; diffuse large B-cell lymphoma, BM ; bone marrow

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