

Case report

Composite Lymphoma as Co-occurrence of Advanced Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma Carrying Trisomy 12 and t(14;18) and Peripheral T-cell Lymphoma

Yumi Aoyama,¹⁾ Taiichi Kodaka,¹⁾ Yuriko Zushi,²⁾ Yuta Goto,¹⁾ Hiroko Tsunemine,¹⁾
Tomoo Itoh,³⁾ Takayuki Takahashi¹⁾

Composite lymphoma is defined as the co-occurrence of two types of lymphoma, comprising 1-4% of lymphomas, and the association of B-cell-type chronic lymphocytic leukemia (B-CLL)/small lymphocytic lymphoma and peripheral T-cell lymphoma (PTCL) is rare. Here, we report a case (77-year-old woman) of advanced B-CLL complicated by newly appearing PTCL. Two years after the onset of B-CLL, CLL cells acquired CD38 antigen expression and the disease entity became CLL/prolymphocytic leukemia. Trisomy 12 and t(14;18) karyotypes were observed. Five years after the onset of B-CLL, large abnormal cells with convoluted nuclei appeared in the peripheral blood and rapidly increased in number. These cells were positive for CD3, CD4, CD5, CD30 (partially), CD56, and $\alpha\beta$ -type T-cell receptor (TCR), in which PCR demonstrated monoclonal TCR- γ gene rearrangement. An additional diagnosis of PTCL, not otherwise specified was made. We treated her with an R-CHOP regimen, resulting in the marked reduction of B-CLL cells but progressive PTCL. Brentuximab vedotin had a transient effect, but the patient died of sepsis due to residual PTCL and pancytopenia. This case is highly informative for tumor biology of B-CLL in terms of emergence of both chromosomal abnormalities and PTCL with progression of this leukemia.

Key words: B-cell-type chronic lymphocytic leukemia, composite lymphoma, t(14;18) (q32;q21), trisomy 12, peripheral T-cell lymphoma

INTRODUCTION

Composite lymphoma is characterized by the simultaneous development of two types of lymphoma in a single patient, comprising 1-4% of malignant lymphomas with a variety of combinations.¹ The coexisting lymphomas, except for B-cell lymphoma with B-cell chronic lymphocytic leukemia (B-CLL)/small lymphocytic lymphoma (SLL), include peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS), anaplastic large cell lymphoma (ALCL), NK-cell leukemia, and Sezary syndrome. Among these T-cell lymphomas, 9 cases of PTCL-NOS have been reported, followed by 4 cases of ALCL.² These reports documented that the time from the onset of B-CLL to lymphoma development was 0.8 to 14 years, with a survival time of 2 days to 2 years after the diagnosis of composite lymphoma. Thus, composite lymphoma occurs even in the early stage of B-CLL.³ Epstein-Barr virus (EBV) was negative in all 17 reported cases

examined.²

In 10% of B-CLL cases, leukemic lymphocytes change from a typical small mature form to lymphocytes with prominent nucleoli.⁴ This change is known as transformation to prolymphocytes, and in the FAB classification, it is defined as CLL/prolymphocytic leukemia (PLL) when the percentage of prolymphocytes is 10% or more and less than 55%. Prolymphocytoid transformation occurs slowly over several years and is associated with resistance to conventional chemotherapy. Here, we report a rare case of CLL/PLL carrying trisomy 12 and t(14;18), which was complicated by newly appearing PTCL.

CASE REPORT

A 77-year-old woman was referred to our hospital because of leukocytosis diagnosed during a regular check-up in November 2011. Hematological examination revealed a

Received: September 26, 2017. Revised: December 15, 2017. Accepted: December 29, 2017. J-STAGE Advance Published: February 8, 2018
DOI:10.3960/jslrt.17033

¹⁾Departments of Hematology and ²⁾Cell Therapy, Shinko Hospital, and ³⁾Department of Diagnostic Pathology, Kobe University Graduate School of Medicine, Kobe, Japan

Corresponding author: Takayuki Takahashi, Department of Hematology, Shinko Hospital, 4-47, Wakihama-cho, 1-chome, Chuo-ku, Kobe 651-0072, Japan. E-mail: takahashi.takayuki@shinkohp.or.jp

Copyright © 2018 The Japanese Society for Lymphoreticular Tissue Research

WBC count of $27.7 \times 10^9/L$ with 78.3% lymphocytes. These lymphocytes were found to be positive for CD5, CD19, CD20, CD23, and smlgG- κ by flow cytometric analysis. Thus, a diagnosis of B-CLL at Rai clinical stage 0 was made. Chromosomal examination of bone marrow cells demonstrated a normal karyotype. In 2012, she underwent right upper pulmonary lobectomy because of adenocarcinoma and developed metastatic carcinoma in the lumbar spine 3 years later. In November 2013, she was treated with fludarabine for B-CLL because of bronchial stenosis due to hilar lymph node swelling and leukocytosis ($71.1 \times 10^9/L$). At this time, circulating lymphocytes were mature and small to medium in size, but 53.5% of these cells had prominent nucleoli, indicating the disease to be CLL/PLL. These CLL cells acquired CD38 expression in addition to the previous phenotype. Karyotype analysis of bone marrow cells revealed abnormalities of trisomy 12 and $t(14;18)(q32;q21)$. The Rai clinical stage at this time was intermediate risk. After fludarabine treatment, bronchial stenosis was improved with maintenance of partial remission. However, in February 2016, she complained of abdominal distension and facial edema. She also developed progressive thrombocytopenia and high serum LDH, and was admitted to our hospital again. Physically, she had systemic edema and lymphadenopathy at the neck and axilla. Laboratory examination (Table 1) demonstrated

Table 1. Laboratory findings on admission (March 2016)

Hematology		Chemistry	
WBC	$9.7 \times 10^9/L$	TP	5.4g/dL
Neu	35.6%	Alb	3.0g/dL
Eos	0%	AST	91IU/L
Bas	0%	ALT	20IU/L
Mon	3.2%	T-Bil	3.3mg/dL
Lym	27.6%	D-Bil	1.6mg/dL
Abnormal lym	33.6%	ALP	710IU/L
RBC	$482 \times 10^4/L$	LDH	1,440U/L
Hb	13.0g/dL	γ -GTP	168mg/dL
Ht	39.2%	BUN	9.7mg/dL
Plt	$1.5 \times 10^4 /L$	Cr	0.58mg/dL
		Na	142mEq/L
		K	4.1mEq/L
Coagulation		Cl	105mEq/L
PT	12.6S	Ca	7.7mg/dL
PT-INR	1.05	P	3.2mg/dL
APTT	28.4S	UA	7.1mg/dL
Fib	295mg/dL	CRP	0.46mg/dL
D-dimer	8.0 μ g/mL	sIL2-R	4,153U/mL

Abbreviations: atyp.lym: atypical lymphocytes, sIL-2: soluble interleukin-2 receptor. Normal ranges of sIL-2R and D-dimer are 145-519 U/mL and 0.0-1.0 μ g/mL, respectively.

a WBC count of $9.7 \times 10^9/L$ with a few large abnormal cells that had convoluted nuclei and cytoplasmic vacuoles (Figure 1A), which rapidly increased in number. These cells were positive for CD3, CD4, CD5, CD30 (partially), CD56, and $\alpha\beta$ -type T-cell receptor (TCR) by flow cytometry (FCM) performed at the Department of Cell Therapy of our hospital. Although the CLL cells were morphologically immature prolymphocytes (Figure 1B), the immunophenotype of these cells was identical with that of the previous cells. Monoclonal rearrangements for both immunoglobulin heavy chain (IgH) and TCR γ genes were found by PCR analysis of peripheral blood and bone marrow cells, suggesting the emergence of a T-cell tumor in advanced B-CLL.

Moderate lymphadenopathy in the left superior supraclavicular fossa, axilla, and the area of the para-abdominal aorta was observed on CT. However, lymph node biopsy could not be performed because of thrombocytopenia and systemic edema. Therefore, we made a cell block preparation at the time when abnormal T cells in the peripheral blood increased in number. The results of immunopathological examination of the cell block were mostly consistent with the phenotype of the abnormal T cells determined by FCM, but anaplastic lymphoma kinase (ALK), granzyme B (Figure 2A), CD30, Epstein-Barr virus (EBV)-encoded small RNA (EBER), and T-cell intracytoplasmic antigen (TIA)-1 (Figure 2B) were negative. CCR4 was weakly positive as examined by FCM, which was performed at SRL Inc. (Hachioji, Tokyo, Japan) (data not shown). An additional diagnosis of PTCL-NOS was made based on the immunopathological examination, although ALK-negative ALCL was not completely ruled out. Multiplex virus PCR analysis⁵ detected EBV in the peripheral blood, and the viral load was 5.7×10^4 copies/mL. Chromosomal analysis of marrow cells demonstrated a complex karyotype in addition to trisomy 12 and $t(14;18)(q32;q21)$. Changes in chromosomal abnormalities in this case are shown in Table 2.

We treated her with R-CHOP chemotherapy for both PTCL and transformed B-CLL, resulting in the marked reduction of

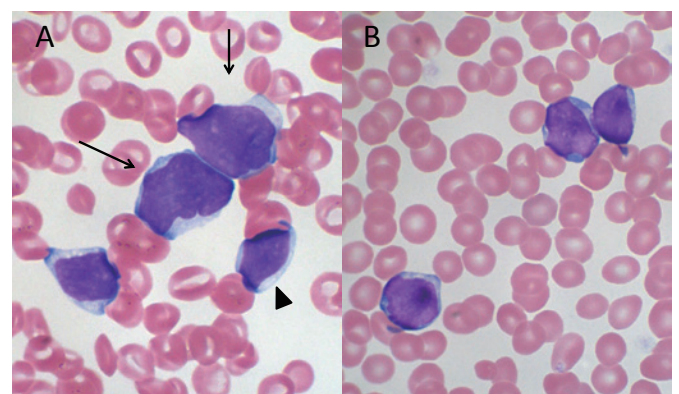


Fig. 1. Smear preparation of peripheral blood at the time of onset of composite lymphoma (March 2016).

A: Large abnormal lymphocytes with convoluted nuclei were observed (arrows) in addition to small immature lymphocytes (arrowhead), Wright-Giemsa staining ($\times 1,000$).

B: Small immature lymphocytes appeared to be B-CLL cells. They had nuclei with prominent nucleoli and fine chromatin network, Wright-Giemsa staining ($\times 1,000$).

Table 2. Cytogenetic analysis at respective clinical stages of the present patient

November 2011: 46,XX [20/20 cells analyzed]
December 2013: 47,XX,+12, t(14;18)(q32;q21) [3/18 cells analyzed]/46,XX [15/18 cells]
March 2016: 47, XX, +12, t(14;18)(q32;q21)[4/20 cells analyzed]/85, XX, -X, -X, add(1)(p13)×2, -2, -2, add(2)(p11.2)×2, del(3)(q21)×2, -4, -5, -5, -5, -6, -9, -9, add(9)(p13), -10, -12, -12, -13, -13, -14, t(14;18)×2, -15, -15, -15, -17, -17, +18, +18, -19, -22, +15mar [2/20 cells]/46, XX [14/20 cells]

November 2011: the time of diagnosis of B-CLL, December 2013: the period of CLL/PLL, March 2016: the period when abnormal T cells appeared in the peripheral blood.

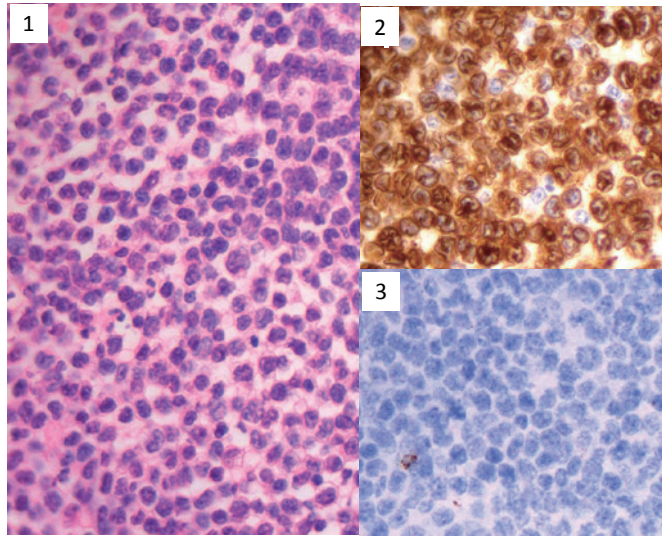


Fig. 2A

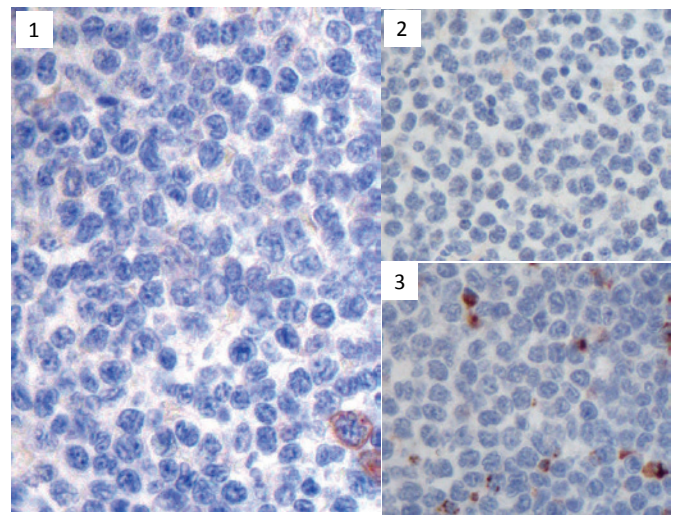


Fig. 2B

Fig. 2A and B. Cell block preparation with circulating mononuclear cells when abnormal large cells comprised more than 90% of white blood cells.

2A-1: Many large abnormal lymphocytes with convoluted nuclei were seen (H-E staining, ×400). **2A-2:** The majority of these cells were positive for granzyme B staining (×400). **2A-3:** Few cells were positive for CD30 (×400). **2B-1:** Only a few cells were positive for CD30 (×400). **2B-2:** These abnormal cells were negative for EBER-in situ hybridization (×400). **2B-3:** Only a few cells were positive for TIA-1 staining (×400).

B-CLL cells from $0.9 \times 10^9/L$ to $0.08 \times 10^9/L$ as evaluated by the WBC count and FCM, but there was rapid proliferation of PTCL cells (Figure 3). As the PTCL cells were weakly positive for CD30, we then treated her with brentuximab vedotin, causing a marked decrease in PTCL cells from $7.6 \times 10^9/L$ to $0.06 \times 10^9/L$. However, this effect was transient, and the second course of brentuximab vedotin was ineffective for the tumor cells and persistent severe pancytopenia. We subsequently treated her with mogamulizumab⁶ because the PTCL cells were weakly positive for CCR4, leading to a decreased number of PTCL cells below $0.5 \times 10^9/L$ without recovery of normal hematopoiesis (Figure 3). Low-dose chemotherapy with etoposide or gemcitabine hydrochloride was also ineffective, and she died of sepsis due to residual PTCL and pancytopenia 4 months after hospitalization.

DISCUSSION

In the present patient, PTCL manifested itself in a leukemic form at the advanced stage of B-CLL. Although we could not perform lymph node biopsy, the superficial

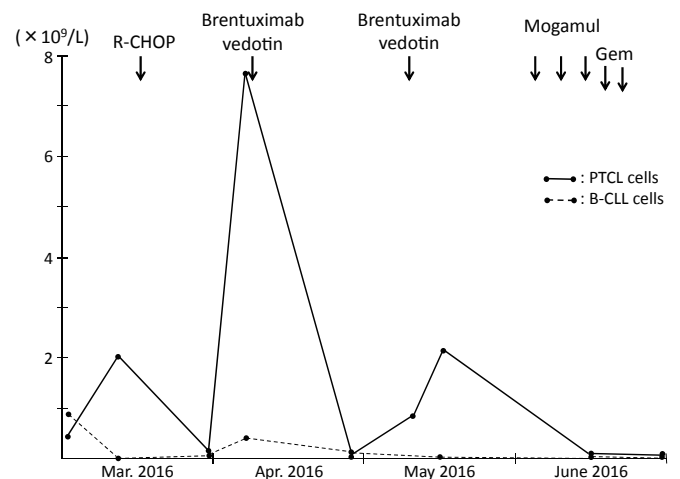


Fig. 3. Clinical course of the present patient. The number of B-CLL and PTCL cells was calculated from the ratio of respective tumor cells as evaluated by flow cytometry and the white blood cell count. After a single course of R-CHOP, B-CLL but not PTCL cells markedly decreased in number without regrowth. Although PTCL cells were suppressed by brentuximab vedotin, the effect was transient and a few PTCL cells remained in the peripheral blood. R-CHOP: rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone. Mogamul: mogamulizumab, Gem: gemcitabine (low-dose).

lymphadenopathy was unchanged in terms of the size and number during the progressive proliferation of the circulating abnormal T cells. Hepatosplenomegaly was also not noted; therefore, the origin of PTCL was unclear. Interestingly, the number of B-CLL cells was reduced to $0.9 \times 10^9/L$, suggesting a growth advantage of PTCL over advanced B-CLL.

Although the pathogenesis of composite lymphoma has remained unclear, the following 2 possibilities have been proposed:⁷ First, certain substances produced by lymphoma cells, such as inflammatory cytokines, may chronically stimulate normal lymphocytes and lead to their neoplastic transformation. Second, the immunosuppressive microenvironment due to the presence of lymphoma may allow the clonal evolution of normal lymphocytes with acquired genetic abnormality. In the present patient, PTCL may have emerged based on the latter because of treatment with fludarabine, which is known to be highly immunosuppressive. CLL/PLL itself may also provide an immunosuppressive environment because these advanced B-CLL cells occupy lymphoid organs and bone marrow for a long period, presumably resulting in impaired immunosurveillance by normal immunocompetent cells.

Interestingly, in the present patient, trisomy 12 and t(14;18)(q32;q21) appeared at the stage of CLL/PLL, suggesting multi-step tumor transformation possibly caused by genomic instability. Although trisomy 12 is common in B-CLL, t(14;18)(q32;q21) is rare. For example, Sen *et al.* identified only 2 cases with t(14;18)(q32;q21) and trisomy 12, but not sole t(14;18) abnormality among 2,215 B-CLL patients.⁸ The impact of t(14;18)(q32;q21) on the prognosis of B-CLL is controversial. Recently, DE Braekeleer *et al.* stated that the prognosis of B-CLL patients with immunoglobulin gene translocations was poor with the exception of those with t(14;18)(q32;q21),⁹ whereas Lu *et al.* found a correlation between CD38 expression, which is associated with a poor prognosis,¹⁰ and immunoglobulin heavy chain (IGH) gene rearrangement manifesting as t(14;19), t(8;14), or t(14;18).¹¹ Regarding the prognosis of B-CLL patients with t(14;18)(q32;q21) as a sole chromosomal abnormality, 2 of 4 such patients exhibited an unfavorable clinical course.^{12, 13} The present patient acquired t(14;18)(q32;q21) and trisomy 12 at the stage of CLL/PLL; therefore, collectively, t(14;18)(q32;q21) may be associated with a poor prognosis, although the accumulation of B-CLL cases with t(14;18)(q32;q21) is required. Regarding the relationship between the emergence of chromosomal abnormalities and disease progression, Cuneo *et al.* noted an increase in the trisomy 12-carrying cell number as the clinical stage advanced, including in CLL/PLL.¹⁴ Similar with in this study, increased incidence of chromosome 6q abnormalities with disease progression was reported.¹⁵ Although emergence of t(14;18) at the stage of CLL/PLL has not been reported, karyotypic instability with disease progression in B-CLL may be common.

The prognosis associated with composite lymphoma is poor with conventional chemotherapy.² In the present patient, R-CHOP continued to be effective against advanced B-CLL, whereas the anti-CD30 antibody exhibited transient

effects on PTCL. Previous studies demonstrated the efficacy of autologous or allogeneic hematopoietic stem cell transplantation (HSCT) for composite lymphoma during remission. Therefore chemoimmunotherapy with rituximab, brentuximab vedotin, or other monoclonal antibodies may help to achieve a level of remission sufficient for performing HSCT. In the future, further improvement of the prognosis of composite lymphoma is desired by the combination of new agents, such as molecular targeting agents, and autologous or allogeneic HSCT.

CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

REFERENCES

- 1 Thirumala S, Esposito M, Fuchs A. An unusual variant of composite lymphoma: a short case report and review of the literature. *Arch Pathol Lab Med.* 2000; 124: 1376-1378.
- 2 Boyer DF, Lindeman NI, Harris NL, Ferry JA. Peripheral T-cell lymphomas with cytotoxic phenotype in patients with chronic lymphocytic leukemia/small lymphocytic lymphoma. *Am J Surg Pathol.* 2014; 38: 279-288.
- 3 Martinez A, Pittaluga S, Villamor N, *et al.* Clonal T-cell populations and increased risk for cytotoxic T-cell lymphomas in B-CLL patients: clinicopathologic observations and molecular analysis. *Am J Surg Pathol.* 2004; 28: 849-858.
- 4 Melo JV, Catovsky D, Galton DA. The relationship between chronic lymphocytic leukaemia and prolymphocytic leukaemia. II. Patterns of evolution of 'prolymphocytoid' transformation. *Br J Haematol.* 1986; 64: 77-86.
- 5 Ito K, Shimizu N, Watanabe K, *et al.* Analysis of viral infection by multiplex polymerase chain reaction assays in patients with liver dysfunction. *Intern Med.* 2013; 52: 201-211.
- 6 Ogura M, Ishida T, Hatake K, *et al.* Multicenter phase II study of mogamulizumab (KW-0761), a defucosylated anti-cc chemokine receptor 4 antibody, in patients with relapsed peripheral T-cell lymphoma and cutaneous T-cell lymphoma. *J Clin Oncol.* 2014; 32: 1157-1163.
- 7 Küppers R, Dührsen U, Hansmann ML. Pathogenesis, diagnosis, and treatment of composite lymphomas. *Lancet Oncol.* 2014; 15: e435-e446.
- 8 Sen F, Lai R, Albitar M. Chronic lymphocytic leukemia with t(14;18) and trisomy 12. *Arch Pathol Lab Med.* 2002; 126 : 1543-1546.
- 9 DE Braekeleer M, Tous C, Guéganic N, LE Bris MJ, *et al.* Immunoglobulin gene translocations in chronic lymphocytic leukemia: A report of 35 patients and review of the literature. *Mol Clin Oncol.* 2016; 4: 682- 694.
- 10 Brachtl G1, Piñón Hofbauer J, Greil R, Hartmann TN. The pathogenic relevance of the prognostic markers CD38 and CD49d in chronic lymphocytic leukemia. *Ann Hematol.* 2014; 93: 361-374.
- 11 Lu G, Kong Y, Yue C. Genetic and immunophenotypic profile of IGH@ rearrangement detected by fluorescence in situ hybridization in 149 cases of B-cell chronic lymphocytic leuke-

11. mia. *Cancer Genet Cytogenet.* 2010; 196: 56-63.
12. Chen W, Miao Y, Wang R, *et al.* t(14;18)(q32;q21) in chronic lymphocytic leukemia patients: Report of two cases and a literature review. *Oncol Lett.* 2016; 12: 4351-4356.
13. Elyamany G, Fadalla K, Elghezal H, *et al.* Chronic Lymphocytic Leukemia with t(14;18)(q32;q21) As a Sole Cytogenetic Abnormality. *Clin Med Insights Pathol.* 2014; 7: 21-27.
14. Cuneo A, Wlodarska I, Sayed Aly M, *et al.* Non-radioactive in situ hybridization for the detection and monitoring of trisomy 12 in B-cell chronic lymphocytic leukaemia. *Br J Haematol.* 1992; 81: 192-196.
15. Finn WG, Kay NE, Kroft SH, *et al.* Secondary abnormalities of chromosome 6q in B-cell chronic lymphocytic leukemia: a sequential study of karyotypic instability in 51 patients. *Am J Hematol.* 1998; 59 : 223-229.