

Case Study

Richter Transformation in the Brain from Chronic Lymphocytic Leukemia

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Richter syndrome (RS) involves the development of an aggressive lymphoma in patients with chronic lymphocytic leukemia (CLL). Diffuse large B-cell lymphoma (DLBCL) is the most common type of RS. Extranodal RS occasionally occurs; however, isolated lesions in the central nervous system (CNS) of RS are rarely seen and the features have not been well described. We describe a Japanese patient who developed isolated involvements of the parenchyma of the CNS as a manifestation of RS two years after the initial diagnosis of CLL. DLBCL in the cerebrum was confirmed to be clonally related to the CLL cells by *immunoglobulin heavy chain (IGH)* gene analysis, utilizing the identical VH-D-J regions with additional mutations in the IGH variable region. [*J Clin Exp Hematop* 53(2) : 157-160, 2013]

Keywords: chronic lymphocytic leukemia, Richter syndrome, central nervous system, immunoglobulin gene

INTRODUCTION

Richter syndrome (RS) involves the development of an aggressive lymphoma in patients with chronic lymphocytic leukemia (CLL).¹⁻⁴ Diffuse large B-cell lymphoma (DLBCL) is the most common type of RS, which occurs in approximately 5-10% of CLL patients.⁵ In a cohort of 185 CLL patients, the median time to RS transformation was 23.0 months from the diagnosis and RS was not observed after 82.4 months.⁶ Nodal involvements account for the majority of cases of RS, but extranodal presentations of RS occasionally occur in the gastrointestinal tract, skin, liver, or other sites.^{7,8} Central nervous system (CNS) infiltrations of RS (CNS-RS) are rare and isolated parenchymal RS lesions in the CNS have been described in only a limited number of reports.⁹⁻¹³

We here present a Japanese patient who developed iso-

lated CNS-RS two years after the initial diagnosis of CLL. We also examined the clonal relationships of the CNS-RS lesion with the original CLL cells.

CASE REPORT

A 66-year-old female was referred to us because of leukocytosis in 2007. Lymphocytosis of 11,000/ μ L was recognized in the peripheral blood. The cells exhibited a mature lymphocytoid morphology with condensed round nucleus (Fig. 1), with immunophenotypes of CD5⁺CD10⁻CD20⁺

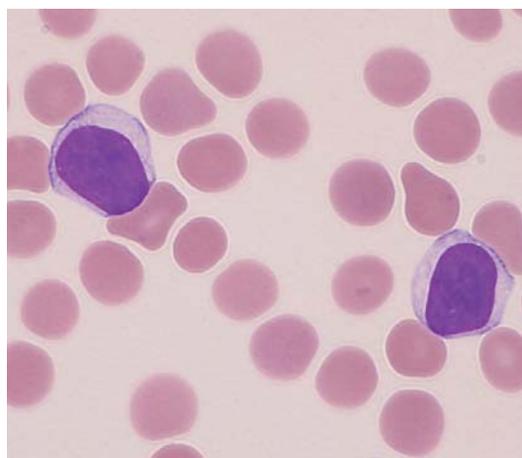


Fig. 1. Leukemic cells in the peripheral blood. Mature lymphocytoid cells with a condensed round nucleus were recognized. May-Giemsa stain.

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CD23⁺CD38⁻ and surface IgM and IgD positivity; λ light chain restriction was also recognized. There was no palpable lymphadenopathy or hepatosplenomegaly. The patient was diagnosed with CLL, Rai system stage 0, and was subsequently followed at another hospital. The administration of cyclophosphamide and prednisolone was initiated one year later because of progressive thrombocytopenia. In 2009, she began to show problems in her daily activities, complaining of fatigue and difficulty walking. She was then referred to our hospital again and admitted to the neurosurgery department. On physical examination, her consciousness was slightly impaired and left hemiplegia was recognized. No lymphadenopathy or hepatosplenomegaly was noted. There was an ill-defined tumor in the right hemisphere accompanying perifocal edema and midline shift by computed tomography (CT) (Fig. 2a). By magnetic resonance imaging, an irregularly shaped mass lesion of 40 mm was recognized in the right putamen and the caudate nucleus, which was enhanced with gadolinium medium (Fig. 2b). CT-guided biopsy of the tumor was performed and histological examination showed the findings of diffuse infiltration of atypical medium- to large-sized lymphoid cells into the perivascular space and cerebral parenchyma (Fig. 3). These cells were positive for CD20, Bcl-6 and MUM-1 and negative for CD3, CD5, CD10, CD23 and cyclin D1 by immunostaining. The MIB-1 index was 69%. A diagnosis of DLBCL, non-germinal center type by Hans classification,¹⁴ was considered from these findings. Splenomegaly and mediastinal and hilar lymph nodes enlarged up to 15 mm were seen by CT imaging. The patient's laboratory findings were as follows: lymphocytosis of 27,000/ μ L with a mature lymphocyte morphology of the same phenotype as at the initial presentation, including negativity of cyclin D1, mild thrombocytopenia, no anemia and a serum

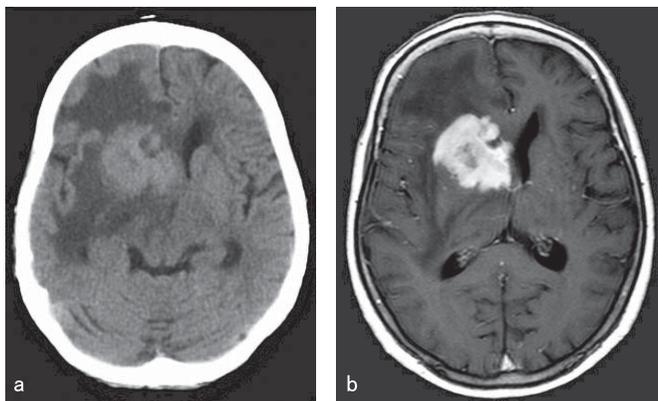


Fig. 2. Imaging studies for the intracranial lesion in a patient with chronic lymphocytic leukemia. (2a) Computed tomography. A poorly defined tumor with perifocal edema and midline shift was seen in the right hemisphere. (2b) Magnetic resonance imaging. There was a mass lesion of 40 mm with gadolinium enhancement in the right putamen to the caudate nucleus.

lactate dehydrogenase level of 265 IU/L (normal range: 114-220). RS in the cerebrum from CLL was diagnosed. The consciousness disturbance and midline shift were improved by intravenous dexamethasone and glycerol infusion. The patient was treated by radiation therapy at a total dose of 50 Gy for the cranial lesion and systemic rituximab administration in the hematology department, and was then discharged for further rehabilitation. However, the cognitive functions of the patient gradually deteriorated and, one year later, multiple cerebral lesions recurred.

Immunoglobulin heavy chain (IgH) gene analysis

Although the diagnosis of RS in the cerebrum was made, solitary RS in the brain parenchyma is quite rare and the relationship of the CNS lesion with peripheral CLL cells was not evident, so *IGH* gene analysis was performed following previously reported methods.¹⁵ Briefly, DNA was isolated from the mononuclear cells of the peripheral blood or formalin-fixed, paraffin-embedded tissues of the cerebral tumor. *IGH* genes were amplified by polymerase chain reaction (PCR) with the corresponding primers for the *IGH* variable regions including the heavy chain complementarity-determining region (HCDR) 3. PCR products were purified and directly sequenced. Sequence analysis was performed on the IgBlast database (<http://www.ncbi.nlm.nih.gov/igblast/index.html>), and the IGMT/Junction Analysis tool (http://www.imgt.org/IMG_T_jcta/jcta) was also utilized for the HCDR3 sequence.

Amplified PCR products from peripheral blood and the brain tumors were the same size, namely, 103 base pairs (Fig. 4). The *IGH* genes from these cells utilized IGHV4-34*01, IGHD3-9*01 and IGHJ4*01 and were identical, except for 3 base pairs.

The sequence of CLL cells in the peripheral blood was closer to the germline sequence of IGHV4-34*01 and IGHD3-

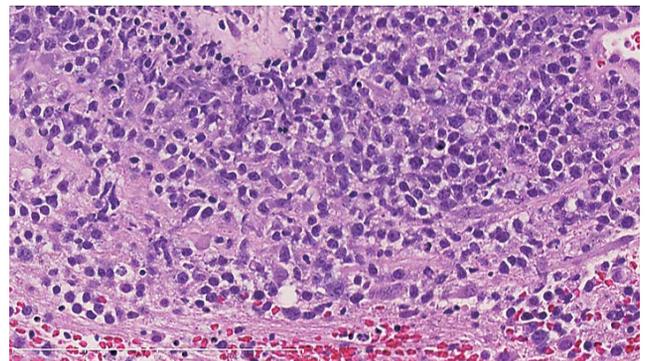


Fig. 3. Histological examination of the biopsy samples taken from the mass lesion in the right cerebrum. Atypical medium- to large-sized lymphoid cells with irregular nucleoli infiltrated into the parenchyma and perivascular spaces. H&E stain.

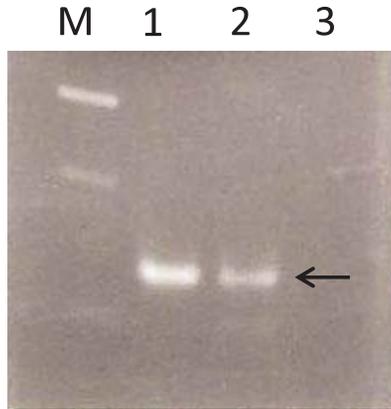


Fig. 4. Polymerase chain reaction (PCR) amplification of immunoglobulin heavy chain genes. *Lane 1*: the patient's sample DNA extracted from the formalin-fixed, paraffin-embedded tissues of the cerebral tumor, *Lane 2*: DNA isolated from the mononuclear cells of the peripheral blood (PB), *Lane 3*: no template control. M represents a molecular marker. The amplified PCR products from the brain tumors and PB were recognized at the same size of 103 base pairs (arrow).

9*01 and had mutated from these genes by one nucleotide each. Therefore, DLBCL of the brain was confirmed to be derived from the CLL clone. Although the length of the analyzed sequences was insufficient to define the mutation status conclusively, CLL in this patient was considered as mutated IGHV type and the HCDR 3 sequence carried non-stereotyped CDR3 with 20 amino acids.¹⁶

DISCUSSION

CNS-RS may occur during the course of CLL, as in the present case, or as *de novo* RS in some instances.³ Isolated leptomeningeal diseases were also reported.⁷ The incidence of CLL among Asians is as low as one-tenth that among Caucasians and several differences in their characteristics have been shown.¹⁷⁻¹⁹ The available data on RS, including CNS-RS, in Asian patients with CLL are currently insufficient.

A lack of CD5 and CD23 expression detected by immunohistochemical approaches, as in the present case, is common in RS² and similar to *de novo* DLBCL; however, molecular profiles such as genetic alterations and immunoglobulin gene mutations are distinct from *de novo* DLBCL.²⁰ It is also known that profiles of primary CNS lymphoma differ from nodal DLBCL;²¹ therefore, it would be interesting to compare those of CNS-RS and primary CNS lymphoma to obtain further insights into the lymphomatous infiltration in CNS.

With clonality analysis of RS and concurrent CLL cells, 78% of RS were shown to evolve from CLL, while in 22%, the RS cells were clonally unrelated.²² In the present study,

CNS-RS cells apparently originated from CLL cells with additional gains of mutation in *IGHV* genes. It is also possible that RS cells derive from CLL stem cells²³ with no relation to the development of CLL; further studies on these issues are necessary. TP53 deletion, NOTCH1 activation and MYC abnormalities have emerged as common genetic alterations of RS⁴ and CLL, with a specific *IGHV* gene, IGHV4-39, combined with a stereotyped receptor, BCR subset 8, being associated with a high risk of RS.²⁴ In this regard, RS, for which the prognosis is currently poor, could become more manageable in the future by obtaining such information on CLL.

Although therapy including rituximab-combined chemotherapies and/or allogeneic hematopoietic cell transplantation is indicated for RS,⁵ optimal approaches for CNS-RS are not known. Although treatments with dexamethasone, radiation and rituximab monotherapy were selected for the present case because of poor performance status and cognitive malfunctions, it is worth evaluating whether recently validated chemotherapeutic regimens for primary CNS lymphoma²⁵ are applicable to CNS-RS, together with novel agents for CLL.

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DISCLOSURE/CONFLICT OF INTEREST

The authors declare no competing financial interests.

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