**MYC/BCL2/BCL6 triple-hit lymphoma that presented with a suprasellar tumor and meningeal dissemination: A case report**

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We herein describe a case of a 54-year-old woman who presented with inappropriate secretion of antidiuretic hormone, diplopia, weakness in the left leg, and bladder and rectum disturbances. Imaging studies of the brain revealed a suprasellar tumor that extended into the posterior portion of the sella turcica. Cerebrospinal fluid contained lymphoma cells that expressed CD10, CD19, CD38, and CD138, but not CD5, CD20, or surface/cytoplasmic immunoglobulins. Fluorescence in situ hybridization using a series of probes showed that lymphoma cells concurrently carried “triple-hit” rearrangements involving MYC, BCL2, and BCL6, and their partners not only in the immunoglobulin heavy chain gene, but also in the non-immunoglobulin gene loci. The patient failed to respond to chemoradiotherapy and died of disease progression shortly thereafter. A postmortem examination revealed lymphoma infiltration into the hypothalamus, frontal lobe, choroid plexus, dura mater, and spinal nerves, and lymphoma tissues exhibited high-grade B-cell lymphoma histopathology. This case represents a rare presentation of triple-hit lymphoma, which was initially confined to the central nervous system (CNS). The development of new and effective agents for double/triple-hit lymphoma associated with CNS disease is a significant unmet medical need.

Keywords: triple-hit lymphoma, FISH, suprasellar tumor, meningeal dissemination

**INTRODUCTION**

B-cell lymphomas with recurrent cytogenetic rearrangements affecting the chromosomal loci of MYC and either BCL2 and/or BCL6 are referred to as double- or triple-hit lymphomas (DHL/THL).¹³ These lymphomas often present with poor prognostic factors, including an advanced stage of the disease, elevated serum lactate dehydrogenase (LDH) level, and bone marrow involvement, and their clinical course is aggressive and treatment outcome remains poor.¹³ The central nervous system (CNS) is a frequent site of involvement in patients with DHL/THL.² In two studies on MYC/BCL2 DHL in Japan, CNS involvement was observed in 21% and 9% of the patients studied, respectively.¹³ Another study showed that the cumulative incidence of CNS involvement was 13% at 3 years.⁶
We herein describe a case of a patient with B-cell lymphoma who initially presented with a suprasellar tumor and meningeal dissemination. Fluorescence in situ hybridization (FISH) applied to lymphoma cells in the cerebrospinal fluid (CSF) revealed concurrent rearrangements of the MYC, BCL2, and BCL6 genes. The characteristic presentation, clinical outcome, and autopsy findings are described.

**CASE REPORT**

*Case presentation*

A 54-year-old woman presented with nausea and paresthesia of the right arm, and was admitted to our hospital due to marked hyponatremia. Her previous medical history was unremarkable. Her blood pressure was 116/67 mmHg, pulse rate was 56 beats per minute, and temperature was 36.0°C. There were no palpable lymph nodes in the neck, axillary regions, or groin. The neck was supple. Heart and breath sounds were normal. The liver and spleen were not palpable. Soon after admission, she developed weakness in the left leg, diplopia, and bladder and rectum disturbances. Her right pupil was 4 mm and left was 2 mm in diameter, and both were reactive. She shortly thereafter developed right oculomotor nerve palsy, right facial nerve palsy, and paraplegia of the lower extremities. Her hemoglobin level was 14.4 g/dL, white cell count was 5,630/µL, and platelet count was 310 × 10³/µL. Lactate dehydrogenase (LDH) was 196 IU/L, creatinine was 0.5 mg/dL, uric acid was 4.3 mg/dL, and soluble interleukin 2 receptor was 216 U/mL. The serum anti-human immunodeficiency virus antibody was negative.

Electrolyte levels in the serum and urine and their osmolality showed hyponatremia with concomitant plasma hypo-osmolality and contradictory high osmolality of the urine; i.e. serum sodium, 119 mmol/L (reference level, 139 to 147); serum potassium, 3.7 mmol/L (3.5 to 4.8); serum chloride, 85 mmol/L (101 to 111); serum osmolality, 245 mOsm/kg (275 to 290); urinary sodium, 96 mmol/L; urinary potassium, 34 mmol/L; urinary chloride, 89 mmol/L; and urinary osmolality, 643 mOsm/kg. Antidiuretic hormone (ADH) was detectable at a level of 1.9 pg/mL (reference level, 0 to 4.2), fulfilling the diagnostic criteria for the syndrome of inappropriate secretion of ADH (SIADH). Adrenocorticotropic hormone (ACTH) was 27.6 pg/mL (7.2 to 63.3), thyroid stimulating hormone was 0.375 µU/mL (0.490 to 4.060), prolactin was 57.83 ng/mL (4.91 to 29.32), cortisol was 9.2 µg/dL (10.4 to 26.4), and free thyroxine was 1.06 ng/dL (0.82 to 1.67). A rapid ACTH test showed a normal cortisol response.

Computed tomography (CT) and magnetic resonance image (MRI) of the brain revealed a suprasellar tumor that extended into the posterior portion of the sella turcica. The tumor was hypo-intense on T1- and T2-weighted images, and homogeneously enhanced with gadolinium contrast medium (Figure 1A). Diffusion-weighted imaging and an apparent diffusion coefficient map showed marked diffusion restriction, suggesting the hyper-cellular density of the tumor. Bilateral posterior limbs of the internal capsule and the optic fascicules showed hyper-intensity on T2-weighted images. [¹⁸F]-fluorodeoxyglucose positron emission tomography combined with CT revealed the accumulation of the tracer within the suprasellar tumor (Figure 1B). No other hyper-metabolic lesions were detected.

An examination of the CSF revealed proteins of 746 mg/dL, glucose of <10 mg/dL, and mononuclear cells of 579 cells/µL, predominantly composed of lymphoma cells of a defective B-cell immunophenotype; flow cytometry showed that these cells expressed CD10, CD19, CD38, and CD138 on their surface, but not CD5, CD20, or surface/cytoplasmic immunoglobulins (Figure 2). The DNA index was 1.06 relative to normal diploid cells. A microscopic examination of a cytospin smear slide revealed that the cells had irregular nuclear contours with immature chromatin and conspicuous nucleoli, and a modest amount of a basophilic cytoplasm (Figure 2). Differentiation to plasma cells was not apparent under microscopy. Bone marrow aspiration and biopsy showed...
Figure 1. Imaging studies of the suprasellar tumor. (A) Sagittal sections of MRI of the brain at the level of the pituitary gland. Left, T1-weighted image; middle, T2-weighted image; and right, T1-weighted image after the administration of gadolinium. The suprasellar tumor was homogeneously enhanced by contrast medium and the enhancement was concentrated at the rim of the tumor. (B) Axial (left) and coronal (right) sections of FDG-PET/CT images, showing the slightly stronger uptake of the tracer in the tumor than in the brain cortex (arrows).

Figure 2. Flow cytometry and cytomorphology of lymphoma cells in the cerebrospinal fluid (CSF) at presentation. (A) Multicolor flow cytometry of lymphoma cells. Cells expressed CD19, CD38, CD45RA, and CD138, but not CD20 or surface immunoglobulins. (B) Cytospin preparation of lymphoma cells (Wright stain; original magnification, ×20 [top] and ×100 [bottom] objective lens). Lymphoma cells showed a high nucleus-cytoplasm ratio, irregular nuclear contours, prominent nucleoli, and a basophilic cytoplasm.
no evidence of the invasion of lymphoma cells.

**Cytogenetic and FISH studies**

G-banding of metaphase spreads prepared from CSF specimens revealed a complex karyotype with two 14q+ marker chromosomes, indicative of a double immunoglobulin heavy chain gene (IGH) translocation. However, since there were many other unidentified marker chromosomes, karyotyping was not completed.

In order to determine IGH translocations, we first applied the IGH break-apart (BA) probe to the cytospin smear slide, showing that both IGH loci had split to generate three 5’ IGH (green) and two 3’ IGH (red) signals in the nuclei (Figure 3A). We next performed a FISH analysis using the BCL2 (red) and IGH (green) dual-fusion (DF) probe, showing three BCL2-IGH fusion (yellow) signals (Figure 3A). FISH using the MYC BA probe revealed one germline MYC (yellow) and two pairs of BA signals, i.e. two 5′ MYC (red) and two 3′ MYC (green), and the BCL6 BA probe showed two germline BCL6 (yellow) and one pair of BA signals, i.e. one 5′ BCL6 (red) and one 3′ BCL6 (green) (Figure 3A). We finally applied the MYC (red) and IGH (green) DF probe to metaphase spreads, showing that MYC-IGH fusion (yellow) signals were localized at der(8)t(8;14)(q24;q32), der(14)t(8;14)(q24;q32), der(11)t(11;?)(q25;?), and an unknown marker chromosome, while normal chromosome 8 and another marker chromosome were labeled with the MYC and IGH signals, respectively (Figure 3B). These results indicated that lymphoma cells carried numerical and structural cytogenetic abnormalities involving 8q24/MYC, 18q21/BCL2, and 3q27/BCL6, and their partners were not only 14q32/IGH, but also non-immunoglobulin gene loci, leading to rearrangements in MYC, BCL2, and BCL6. A tentative karyotype based on both G-banding and FISH was: 46~49,X,der(X)add(X)(p22)add(X)(q28),−2,add(2)(q37),−3,−4,del(6)(p23),add(6)(q27),t(8;14;11;?)(q24;q32;q25;?),−9,add(14)(q32),−16,−18,+5~11mar[cp14].

**Treatment course**

The patient was first treated with intrathecal methotrexate (MTX), cytarabine (Ara-C), and prednisolone, followed by high-dose MTX, high-dose Ara-C, and DeVIC (dexamethasone, etoposide, ifosfamide, and carboplatin) combination chemotherapy. According to the Bonn protocol, an Ommaya reservoir was placed, and fractionated doses of MTX and AraC were repeatedly injected into the cerebral ventricle. However, she failed to respond to these treatments, and developed hydrocephalus due to enlargement of the suprasellar tumor, requiring drainage of the CSF through the reservoir and radiation therapy to relieve her neurological symptoms. During the treatment course, she underwent hormone replacement therapy for hypopituitarism. Infiltration to the spinal nerves became apparent in imaging studies (Figure 4A). She died of disease progression 91 days from initial presentation.

**Postmortem examination**

A postmortem examination revealed lymphoma infiltration into the hypothalamus, frontal lobe, choroid plexus, dura mater, and spinal nerves (Figure 4B, Figure 5A). The involvement of the extra-cranial organs was demonstrated in the gallbladder and ascending colon. Histological sections of the tumor in the posterior cranial fossa were composed of diffuse proliferation of monomorphic lymphoma cells; there was no follicular pattern of proliferation within the tumor (Figure 5B). The lymphoma cells were medium-sized, and had scant cytoplasm and round nuclei. They did not show plasmablastic or immunoblastic features. A large number of mitotic figures were observed, indicating the rapid growth rate of the tumor. Immunohistochemistry revealed that lymphoma cells were positive for CD79a, CD10, BCL2, BCL6, and MUM1, and negative for CD20, CD10, and CD5. CD138 was negative by immunohistochemistry. The Ki-67 proliferation index was more than 90% (Figure 5C). These results matched those of the category of B-cell lymphoma, unclassifiable, with features intermediate
Figure 3. FISH. (A) FISH of interphase nuclei prepared from the CSF. Cytospin smear slides were hybridized with the Vysis LSI *IGH* dual-color BA rearrangement probe (top, left), Vysis LSI *IGH*/BCL2 dual-color, DF translocation probe (top, right), Vysis LSI *MYC* dual-color, BA rearrangement probe (bottom, left), and Vysis LSI BCL6 (ABR) dual-color BA rearrangement probe (bottom, right). The hybridization signals of each probe are indicated by the arrowheads of each color. (B) Hybridization of a metaphase spread with the Vysis LSI *IGH*/MYC/CEP 8 tri-color DF probe. G-banding (top) and a FISH picture (bottom) are shown. The MYC (red), IGH (green), and MYC-IGH fusion (yellow) signals are indicated by the arrowheads of each color. CEP 8 (blue) signals are not visible in this picture.

Figure 4. Involvement of spinal nerves. (A) Coronal sections of T2-weighted STIR MRI at the level of the lumbar vertebra. Top, spinal nerves were swollen and showed hyper-intense signals (arrows). Bottom, The tumor formed along the cauda equina within the subarachnoid space (arrows). (B) Postmortem appearance of the cauda equina of the spinal cord. The tumor showed a “bouton-like” appearance (arrows).
Figure 5. Postmortem examination of the dura matter tumor of the posterior cranial fossa. (A) Gross appearance of the tumor. (B) Histological sections of the tumor (hematoxylin & eosin staining: left, ×20 and right, ×40 objective lens). (C) Immunohistochemistry (×20 objective lens). a, CD20; b, CD79a; c, CD3; d, CD10; e, BCL6; f, MUM1; g, BCL2; h, CD5; and i, Ki67.
between diffuse large B cell lymphoma (DLBCL) and Burkitt lymphoma (BL) proposed by the 2008 WHO classification.\textsuperscript{8}

**DISCUSSION**

We herein described a case of a female patient who initially presented with SIADH, the cause of which was determined to be the suprasellar development of lymphoma of the B-cell type. The lymphoma showed meningeal dissemination at presentation, and neurological symptoms related to sensory and motor disturbances were likely accounted for by lymphomatous invasion of the cranial and spinal nerves. Most importantly, a series of FISH studies demonstrated that lymphoma cells carried the $\text{MYC} / \text{BCL2} / \text{BCL6}$ TH gene rearrangement, which was most likely responsible for the aggressive clinical behavior and refractoriness to chemoradiotherapy. A postmortem examination finally confirmed the diagnosis of B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL.

Although large series of DHL/THL have described high frequency of CNS involvement at presentation, the site of involvement within the CNS has not been fully described.\textsuperscript{4,6} Nevertheless, the majority of cases with CNS disease are recognized by CSF examination and, in general, positivity for CSF-cytology is regarded as CNS involvement.\textsuperscript{6} The present case indicates that DHL/THL can present with not only CSF-positivity, but also formation of tumors within the brain parenchyma detected by neuroimaging studies.

Since the initial lymphoma lesion of the present case was restricted to the brain, the disease may be included in the primary CNS lymphoma (PCNSL) category. The most common sites of PCNSL are periventricular regions, followed by frontal, parietal, temporal, and occipital lobes.\textsuperscript{9} Sporadic case reports of PCNSL that developed in the intra- and/or parasellar region, the incidence of which is less than 1% of sellar masses,\textsuperscript{10} have been reported.\textsuperscript{11-14} In a review of 19 cases of primary “pituitary” lymphoma by Koiso et al.,\textsuperscript{15} the median age was 49 and male-to-female ratio was 13:6, and 16 cases had the B-cell and 3 had the T or NK/T-cell phenotype. These patients presented with symptoms related to cranial nerves and/or constitutional symptoms, and endocrine abnormalities included anterior hypopituitarism in 14 and diabetes insipidus in 7. Thus, the presentation with SIADH and widespread meningeal dissemination observed in the current case appears to be unusual for PCNSL.

On the other hand, the chromosomal translocation and/or rearrangement of the oncogene in PCNSL has been investigated by FISH studies applied to paraffin-embedded biopsy specimens. In a large series of 75 cases of PCNSL, $\text{BCL6}$ was rearranged in 13 (17\%) and $\text{MYC}$ in 2 (3\%).\textsuperscript{16} In another series of 50 CNSL cases, $\text{BCL6}$ and $\text{MYC}$ rearrangements were detected in 26\% (12 of 47) and 8\% (4 of 49), respectively, while no $\text{BCL2}$-rearrangement case was identified.\textsuperscript{17} The lack of the $\text{BCL2}$ rearrangement is consistent with PCNSL with DLBCL histopathology showing the non-GCB type of expression profile, in which the $\text{BCL2}$ translocation is generally absent.\textsuperscript{18}

Regarding DH, one case of $\text{MYC}/\text{BCL6}$ DH was included in Cady’s series.\textsuperscript{16} Although $\text{CCND1}/\text{MYC}$ DH PCNSL was reported, this case appeared to represent a rare transformation of mantle cell lymphoma with $\text{CCND1}-\text{IGH}$ that secondarily acquired $\text{MYC}-\text{IGH}$.\textsuperscript{19} These literature reviews suggest that the concurrent occurrence of $\text{MYC}$, $\text{BCL2}$, and $\text{BCL6}$ rearrangements, observed in the current case, is unusual in PCNSL. Thus, although extra-neural involvement was not detected at the initial presentation, it is likely that this case represents a rare presentation of systemic lymphoma with TH rearrangements, which was initially confined to the cerebrospinal axis. The involvement of visceral organs was identified postmortem.

The treatment course of the present case was dismal. In a multicenter phase II trial for aggressive B-cell lymphoma associated with CNS involvement at diagnosis or relapse with high-dose MTX/AraC followed by high-
dose sequential chemoimmunotherapy and autologous stem-cell transplantation, 24 (63%) out of 38 cases achieved a complete response (CR), and the 2-year event-free survival rate was 50%, and 5-year overall survival rate was 41%. On the other hand, for DHL/THL, due to the \( MYC \)-driven aggressive nature and propensity for CNS involvement, intensified regimens successful in BL, such as hyper-fractionated cyclophosphamide, doxorubicin, vincristine, dexamethasone, methotrexate, cytarabine with rituximab (R-HyperCVAD/MA), dose-adjusted rituximab, etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin cyclophosphamide (DA-EPOCH-R), and vincristine, doxorubicin, methotrexate, ifosfamide, etoposide, cytarabine with rituximab (R-CODOXM/IVAC), have been applied for induction. Unfortunately, these intensified treatment protocols were not applicable to the current patient due to her poor performance status at presentation. Rituximab may have been ineffective because of the lack of CD20 expression in lymphoma cells. As it is unlikely that the combination of currently available cytotoxic drugs will lead to marked improvements in the treatment of DHL/THL associated with CNS disease, the development of new and effective agents for these difficult cases is a significant unmet medical need.

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鞍上部腫瘤と髄膜播種で発症した triple-hit lymphoma の 1 例

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症例: 54 歳女性. SIADH, 複視, 左下肢筋力低下, 膀胱直腸障害のため入院した。
検査結果: 頭部 MRI 検査で鞍上部からトルコ鞍にかけて広がる腫瘤を認めた。髄液検査でリンバ細胞を認め, CD10, CD19, CD38, CD138 陽性, CD20, CD5 陰性, 細胞表面・細胞質免疫グロブリン陰性であった。FISH 解析では, IGH および IGH 以外の遺伝子をパートナーとする MYC, BCL2, BCL6 の再構成が判明し, triple-hit lymphoma と考えられた。
経過: 化学療法, 放射線療法に反応なく, 診断から約 3 か月後に原病増悪のため死亡した。病理解剖では, 視床下部, 前頭葉, 脈絡叢, 硬膜, 脊髄神経への浸潤が確認され, 高悪性度 B 細胞リンパ腫の組織型であった。
考察: 本症例は triple-hit lymphoma が CNS に生じるという稀な発症様式を呈したものと考えられた。本症例を始めとする double-hit / triple-hit lymphoma に対する有効な治療法の開発が望まれる。

キーワード: triple-hit lymphoma, FISH, 鞍上部腫瘤, 髄膜播種