Introduction

Primary CNS lymphoma (PCNSL) is most frequently an aggressive diffuse large B cell lymphoma (DLBCL) confined to the CNS and is associated with poor prognosis. It is an interesting B cell lymphoma as very few B lymphocytes, if any, are found in the CNS under normal circumstances [1]. More than 95% of PCNSL cases have immunophenotypic features consistent with activated B cell subtype of DLBCL (ABC-DLBCL) [2].

In 2008, a new entity of systemic non-Hodgkin lymphoma, called B cell lymphoma with features intermediate between diffuse large B cell lymphoma and Burkitt lymphoma (DLBCL/BL) was recognized by World Health Organization (WHO) [3]. It is characterized by mixed features of DLBCL, often of germinal center B cell phenotype (GCB-DLBCL), and Burkitt lymphoma. Interestingly, systemic DLBCL/BLs are aggressive lymphomas associated with high incidence of the CNS involvement and poor prognosis [3]. It is not currently known if a DLBCL/BL can manifest in the CNS alone without systemic involvement.

Herein, we reported a case of PCNSL with genetic and pathologic features consistent with DLBCL/BL. We suggest that primary CNS DLBCL/BL lymphoma (PCNS-DLBCL/BL) does exist and is a very aggressive subtype of PCNSL.

Materials and methods

The study was approved by the Mayo Clinic institutional review board. Clinical information was obtained by chart review. Three observers (H.W.T., L.J. and D.M.M.) evaluated the immunohistochemical (IHC) results. Fluorescence in situ hybridization (FISH) findings were analyzed and interpreted by R.P.K.

Patient summary

The patient was a 69 year old male who developed rapidly progressive bilateral lower extremity weakness and pain, and urinary urgency. Magnetic resonance imaging (MRI) of the brain and thoracic/lumbar spine demonstrated a
2×3.2×2.2 cm mass in the right temporal lobe, a 1.9×1.6×1 cm mass in the right occipital lobe, and abnormal linear enhancement of the cauda equina (Figure 1). The right temporal lobe mass was biopsied with findings consistent with diffuse large B-cell lymphoma. Cerebrospinal fluid (CSF) cytology was positive for large lymphoma cells. A staging evaluation including computed axial tomography (CAT scans) of chest, abdomen, and pelvis, bone marrow biopsy, and ophthalmological evaluation were negative. HIV was negative. Due to rapid clinical deterioration, a course of radiation to the whole brain and thoracic spine to sacrum (T12-S3) was started as an initial treatment. Ten days into radiation therapy, he had sudden onset of bilateral upper extremity weakness. MRI of the cervical spine demonstrated an intramedullary lesion in the right lateral column of cervical spinal cord from C5 - C7 associated with mass effect (Figure 1). By this time, results on FISH and IHC came back consistent with DLBCL/BL. He was switched to a systemic therapy with high-dose Methotrexate and Ara-C in combination with Rituximab. He responded well with significant recovery of strength in all four extremities. Unfortunately, he developed infectious complications related to the systemic therapy. He was recovering well when he had a sudden death. No autopsy was performed.

Histology and immunohistochemistry

The morphologic and immunohistochemical features were studied on formalin-fixed and paraffin-embedded tissue section. Single antibody staining was performed for CD20 (predilute; Dako, Carpinteria, CA, USA), CD3 (predilute; Lab Vision, Fremont, CA), CD10 (1:10; Leica, Buffalo Grove, IL), BCL-6 (predilute; Dako), PAX-5 (predilute; Lab Vision), CD138 (1:50; Dako), CD79a (1:50; Dako), MUM-1 (predilute; Dako), BCL-2 (predilute; Dako), and osteopontin (1:10; R&D Systems, Minneapolis, MN). Immunostaining was performed with an automated immunostainer (Dako Cytomation Immunostainer Plus) according to the company's protocols with minor modifications. Antibody was detected with EnVision FLEX + labeled polymer (Dako). Sections were counterstained with Gill 1 hematoxylin (Richard-Allan/Thermo Fisher Scientific, Inc, Fremont, California), and cover-slipped using Cytoseal 60 mounting medium. Positive and negative controls were performed.

Fluorescence in situ hybridization

Formalin-fixed paraffin-embedded 5-µm sections were prepared and mounted onto positively charged glass slides. Slides were placed in a 90 °C oven for 15 min; slides were then deparaffinized with xylene (2 times, 15 min each) at room temperature (RT), dehydrated in 100% ethanol for 5 min at RT and placed in 80 °C 10 mM citric acid (PH 6.0) for 45 min. Following this procedure, the slides were immersed in 2 x standard saline citrate for 5 min at 37 °C followed by digestion in 0.2% pepsin working solution at 37 °C for 48 min. Immediately after digestion, the slides were dehydrated using an ethanol series (70, 85, and 100%) for 2 min each at RT. Probes included D8Z2/MYC/IGH, MYC, IGH/BCL2; and BCL6 (Abbott Molecular, Des Plaines, Ill.), and c-MYC/IGL (Mayo homebrew). MYC and BCL6 are break-apart probes.
Primary CNS B cell lymphoma with unique features

(BAP), others are fusion probes. Working solution of each probe except c-MYC/IgL was made by mixing 1 ul of concentrated probe with 9 ul of LSI/WCP hybridization buffer (Abbott). The working solution of c-MYC/IgL was prepared by mixing 3 parts MYC probe with 7 parts LSI/WCP buffer then add 2 x volume of 3 parts IGL probe with 7 parts LSI/WCP buffer. The combined working solution was applied to the target areas, coverslipped, sealed with rubber cement, co-denatured with a HYBrite TM at 80 °C for 5 min, and hybridized overnight in a 37 °C humidified oven. Following hybridization, slides were soaked in RT 2 x SSC/0.1% NP-40 to remove coverslips, placed in the same solution at 74 °C for 2 min and then placed in same solution of RT for 2 min. The slides were stained with 4'-6-diamidino-2-phenylindole (DAPI; Vysis) and coverslipped. The formalin-fixed paraffin-embedded samples were analyzed using standard fluorescence microscopy methods [4].

Results

Morphologic and immunohistochemical findings

Right temporal lobe mass biopsy showed diffusely infiltrating large neoplastic lymphocytes with brisk mitoses (Figure 2). They were positive for CD20, CD79a, and PAX-5; and negative for CD3, indicating the B-cell lineage. They showed strong expression of BCL-2, were focally positive for CD10, strongly expressed osteopontin, and were negative for BCL-6, CD138, and MUM-1. The proliferative rate by MIB-1 was nearly 100%.

Florescence in situ hybridization findings

FISH studies were performed on paraffin sections of the right temporal lobe brain specimen. We used a break-apart strategy with probes that recognize the 5' and 3' regions of BCL6 and the 5' and 3' regions of MYC. We also used a dual-color, double-fusion FISH strategy with probes for MYC/IGH, IGH/BCL2 and MYC/IGL.

MYC/IGH fusion signals were identified in 100% of the tested nuclei (Figure 3), indicating the presence of t(8;14)(q24;q32). Three copies of BCL2 were also detected in 100% of nuclei (Figure 3), indicative of gain of BCL2. BCL2 did not show any disruption as demonstrated by a break-apart probe. Further analysis using immunoglobulin light (IGL) chain probes did not show any fusion between BCL2 and IGL. There were

Figure 2. The brain biopsy demonstrates a diffuse infiltrate of lymphoma into the white matter (A, H&E x 10, normal white matter at left side of the image; B, H&E x 40). Immunohistochemical studies show that neoplastic lymphocytes are positive for CD20 (C, x 10), CD10 (D, x 10), BCL-2 (E, x 20), and osteopontin (F, x 10, normal white matter at top of the image).
Primary CNS B cell lymphoma with unique features

Discussion

Our report identifies the first patient with a DLBCL/BL lymphoma presenting as a primary central nervous system lymphoma without any systemic involvement. The findings in our patient indicate that PCNSDLBCL/BL is characterized by an aggressive clinical course and multifocal involvement in the CNS. Pathologically, it demonstrates typical DLBCL morphology and germinal center B cell phenotype with CD10 expression and negative MUM1 expression. It expresses osteopontin strongly with nuclear and cytoplasmic staining pattern similar to other PCNSL cases [5-7]. Proliferation index by Ki-67 is almost 100%. Molecularily, our case of PCNSDLBCL/BL over-expresses MYC due to t(8;14) and BCL2 due to a gain. BCL2 overexpression was confirmed by IHC and by FISH with several probes. Lack of t(14;18) indicates that this case cannot be labeled as a double-hit lymphoma [8]. The pathologic and genetic features clearly show that PCNSDLBCL/BL has a mixture of features of GCB-DLBCL and Burkitt lymphoma. The aggressive nature of PCNSDLBCL/BL is likely explained by a combination of MYC overexpression driving excessive lymphoproliferation and anti-apoptotic activity of BCL2 overexpression.

PCNSDLBCL/BL appears to be a very rare variant of PCNSL. More than 95% of PCNSL cases have ABC phenotype with the remainder having GCB phenotype [2]. Primary CNS Burkitt lymphoma is very rare. As such, the incidence of PCNSDLBCL/BL must be extremely low.

We have previously shown that osteopontin (OPN) is the most upregulated gene in PCNSL compared to non-CNS DLBCL [7]. Our microarray findings were backed up by Rubenstein JL et al [9]. We have shown by double immunostaining for CD20 and OPN that B lymphoma cells in PCNSL overexpressed OPN [5, 6]. Yuan J et al have also shown that OPN is strongly expressed by B lymphoma cells in PCNSL compared to non-CNS lymphoma [10]. IHC in our patient confirmed that PCNSDLBCL/BL also strongly expresses OPN. Therefore, the strong expression of OPN is a unique feature of the CNS signature of PCNSL.

In conclusion, we recommend that a comprehensive FISH analysis for MYC, BCL2, and BCL6

Figure 3. The lymphoma cells demonstrate MYC/IGH fusion and three copies of BCL2 in 100% of nuclei by FISH. The arrows in A indicate the nuclei with fusion signals (yellow) of MYC (orange) and IGH (green) probes. The arrows in B indicate nuclei with three IGH (green) and three BCL2 (orange) signals; no fusion was identified.
should be performed in all PCNSL cases with aggressive clinical course, multifocal involvement of the CNS, and high proliferation index to make the correct diagnosis of PCNSDLBCL/BL. Based on our experience, patients with PCNSDLBCL/BL should be treated aggressively with a systemic therapy as early as possible. Eligible patients should be considered for high-dose chemotherapy followed by autologous stem cell transplant following intensive induction therapy consisting of high CNS penetrating agents such as high-dose Methotrexate and Ara-C combined with Rituximab. Additional research is necessary to better understand PCNSDLBCL/BL and to determine an effective therapeutic approach.

Acknowledgements

This work is supported by Mayo Scholarly Opportunity Award and James C. and Sarah K. Kennedy Mayo Clinic Research Career Development Award (HWT).

Address correspondence to: Dr. Han W Tun, Department of Hematology and Oncology, Mayo Clinic Florida, 4500 San Pablo Road, Jacksonville, Florida 32224 USA Tel: 904-953-6460; Fax: 904-953-2315; E-mail: Tun.Han@mayo.edu

References


