Annals of Diagnostic Pathology 48 (2020) 151610

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

journal homepage: www.elsevier.com/locate/anndiagpath

Original Contribution

Analysis of primary central nervous system large B-cell lymphoma in the era of high-grade B-cell lymphoma: Detection of two cases with *MYC* and *BCL6* rearrangements in a cohort of 12 cases

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ARTICLE INFO

Keywords:
Double-hit lymphoma
High-grade B-cell lymphoma
c-myc
Primary central nervous system lymphoma
BCL6

ABSTRACT

High-grade diffuse large B-cell lymphoma (HG-DLBCL) refers to DLBCL with *MYC* and *BCL2* and/or *BCL6* rearrangements (double-hit or triple-hit DLBCL) that exhibits poor prognosis. Double-expressor DLBCL (c-myc +/bcl-2+) has intermediate prognosis when compared to HG-DLBCL. Primary central nervous system lymphoma (PCNSL) has distinct pathophysiology (frequent non-germinal center-like subtype and double-expressor) and has worse prognosis than systemic DLBCL. By fluorescence *in situ* hybridization (FISH), 25–30% of PCNSLs harbor *BCL6* abnormalities with rare alterations in *MYC*, *BCL2*, double-hit or triple-hit events. We describe the clinicopathologic features and status of *MYC*, *BCL2* and *BCL6* in 12 PCNSLs (7 women, 5 men; median age 63 years; range: 28–79). Six cases showed focal starry-sky pattern. Immunohistochemically, all (100%) were of non-germinal center-like subtype, and 8/10 (80%) cases were double-expressors. Ki-67 ranged from 70 to 100%. FISH was positive in 9/12 (75%) cases: 4 (33%) harbored a *BCL6* rearrangement, 3 (25%) had a gain of *BCL2*, 2 (17%) cases each had a gain of *BCL6* and gain of *IGH*, and gain of *MYC* and deletion of *MYC* were observed in 1 case each (8%). Two (16%) cases were *MYC/BCL6* double-hit PCNSLs. No *MYC/BCL2* or triple-hit cases were identified. Eleven (92%) patients received chemotherapy and one also received whole brain radiation. The median time of follow-up was 4.4 months (range, 0.3–40.3). Seven (58%) patients are alive, 4 (33%) have died, and 1 (8%) had no follow-up. Five alive patients are in remission, including one *MYC/BCL6* double-hit PCNSL. Our results add two new cases of rare double-hit PCNSL to the literature.

1. Introduction

Primary central nervous system large B-cell lymphoma (PCNSL) is defined as lymphoma confined to the brain, spinal cord, leptomeninges, and the eye without evidence of systemic disease at presentation [1]. It accounts for ~1% of all non-Hodgkin lymphomas and < 5% of all CNS malignant neoplasms [1]. Morphologically, PCNSL is identical to DLBCL outside the brain and exhibits a characteristic perivascular distribution with variable degrees of brain parenchymal involvement. PCNSL has distinct pathophysiology and worse prognostic features when compared to systemic DLBCL [2].

Current standard of care for the diagnosis of systemic DLBCL includes determination of the "cell of origin" by immunohistochemistry using a classifier that works as a surrogate of the molecular signature detected by gene expression profiling, separating DLBCL into germinal center origin (GCB) and non-germinal center/activated B-cell (non-GCB/ABC) origin. The most widely known classifier to date is the one implemented by Hans et al. in 2004 [3] which divides DLBCL into GCB (CD10+/bcl-6+/MUM1-) and non-GCB/ABC (CD10-, bcl-6−/+, MUM1+) subtypes. The importance of classifying DLBCL into these groups is prognostic, with better outcome for DLBCLs of GCB subtype compared to poor outcome in DLBCLs of non-GCB/ABC subtype [4]. In addition, detection of bcl-2 and c-myc by immunohistochemistry (double-expressor DLBCL) has been used as a potential prognostic indicator with inferior survival as compared to non-double expressor DLBCL [5-8]. More importantly, several studies have demonstrated the clinical and biological relevance of the presence of *MYC*, *BCL2* and *BCL6* gene rearrangements in DLBCL by fluorescence *in situ* hybridization (FISH) [8,9]. The 2017 revised 4th edition World Health Organization (WHO) classification of hematopoietic and lymphoid tissues designates the category "high-grade BCL" for cases of DLBCL with rearrangements of *MYC* and *BCL2* and/or *BCL6*, also referred to as

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https://doi.org/10.1016/j.anndiagpath.2020.151610

double-hit (*MYC*, and *BCL2* or *BCL6*) or triple-hit (*MYC*, *BCL2* and *BCL6*) lymphomas [10]. These tumors have worse prognosis when compared to DLBCL without gene rearrangements or those with single gene rearrangement, including those with advanced stage, bone marrow involvement, extranodal disease, high levels of serum lactate dehydrogenase, aggressive clinical course, and poor response to therapy [10-15]. For reasons that are not entirely understood, overexpression of c-myc, bcl-2 and/or bcl-6 proteins does not translate into the detection of *MYC*, *BCL2* and/or *BCL6* rearrangements, making FISH the only method to date to best determine prognosis in DLBCL [10].

PCNSL is usually of non-GCB/ABC subtype with frequent overexpression of c-myc and bcl-2 proteins by immunohistochemistry [16-18]. Some studies have suggested that c-myc alone or c-myc and bcl-2 protein expression are associated with poor prognosis in PCNSL [19-22], whereas other studies have not shown an association of these markers with clinical outcome [23,24]. By FISH, PCNSL harbors frequent abnormalities of the *BCL6* gene (25–30% of cases, similar to systemic DLBCL) but rare alterations in *MYC* and *BCL2*, and only sporadic cases of double-hit or triple-hit lymphoma [1,13,18-20,22,23,25-32]. To the best of our knowledge, only 2 prior cases of PCNSL with *MYC* and *BCL6* rearrangements have been described in two large case series from 2008 and 2013 (1 case in each study) [18,26].

In this study, we present 12 cases of PCNSL diagnosed at our institution over a period of 7 years in which the clinical, imaging, histopathologic and immunohistochemical features, as well as the status of *MYC*, *BCL2*, and *BCL6* by FISH were available. We identified two new cases of PCNSL with *MYC* and *BCL6* rearrangements in our cohort that qualify for the designation of double-hit PCNSL according to the 2017 WHO classification [10]. Our results add two new cases of PCNSL with very rare *MYC* and *BCL6* gene rearrangements with available clinicopathologic features to the medical literature.

2. Material and methods

2.1. Patient selection

The archives of our Institution were searched from 2013 through January 2020 for cases diagnosed as PCNSL that have available clinical, radiologic, histopathologic, immunohistochemical and fluorescence *in situ* hybridization analysis information. At total of 12 patients were identified. Approval for this study was obtained from the Institutional Review Board.

2.2. Collection of clinical data

Clinical, relevant imaging, and laboratory data were retrieved from the electronic medical record. Laboratory data collected for each patient included: serum lactate dehydrogenase (LDH) levels and human immunodeficiency virus serologic status (HIV-1/2) at diagnosis. Beta-2 microglobulin serum levels are not routinely performed at our institution. We also collected available treatment information for the PCNSL, as well as information related to follow-up and outcome for each patient.

2.3. Histopathologic, immunohistochemical and flow cytometric evaluation

Hematoxylin and eosin-stained (H&E) slides, immunohistochemical stains and *in situ* hybridization studies were reviewed in all cases. Additional immunohistochemistry using an anti-c-MYC rabbit monoclonal antibody (Y69, prediluted; Ventana Medical Systems, Tuczon, AZ) was performed on paraffin-embedded tissue sections from those cases where a paraffin block or unstained slides were available and did not have a c-myc immunostain, following the manufacturer's instructions (VENTANA ultraView Universal DAB Detection Kit using the BenchMark XT instrument). For the markers CD10, bcl-6, and MUM1 a cut off of > 30% positive tumor cells for each marker was used to classify a case as positive. For the c-myc immunostain a cut off of > 40% positive tumor cells was used to classify a case as positive. For bcl-2 a cut off of > 50% positive tumor cells was used to classify a case as positive. Where available, flow cytometry immunophenotypic reports were also reviewed.

2.4. Fluorescence in situ hybridization (FISH) studies

Interphase FISH was performed on formalin-fixed paraffin-embedded tissue using break-apart probes (Abbott Molecular, Des Plains, IL) to assess the presence of gene rearrangements involving 3q27 (*BCL6*) and 8q24 (*MYC*). In addition, a dual-color, dual fusion probe set (Abbott Molecular) was used to assess the presence of the t(14;18)(q32;q21) *IGH/BCL2* translocation. All probes were used according to the manufacturer's instructions. H&E-stained sections were marked off corresponding to area of greatest tumor involvement. The marked area was transcribed onto unstained slides cut from the same tissue block. Four-micron thick sections were baked for 1 h at 60 °C followed by deparaffinization and rehydration. Pretreatment was performed at 80 °C for 20 min followed by protease treatment for 22 min at 37 °C. This was followed by dehydration and hybridization at 73 °C for 3 min and 37 °C overnight. Post-hybridization wash was performed at 75 °C for 3 min and then the slides were stained with 4′,6-diamidino-2-phenylindole (DAPI) to visualize the nuclei followed by placement of a glass coverslip. Each probe set was applied to a separate slide. Slides were analyzed under a 100× oil immersion objective and an Olympus BX-61 fluorescence microscope coupled with the BioView Duet™ computer-assisted imaging system (Billerica, MA). A minimum of 50 nuclei were scored for each case.

The normal staining pattern for a break-apart probe set (*BCL6* and *MYC*) is two fusion signals per nucleus. If the observed pattern was one red signal, one green signal and one fusion signal in > 11.6% of nuclei, the case was considered positive for the appropriate gene rearrangement. Variations on this pattern are also indicative of duplications and/or deletions of the target gene locus. For example, for the *BCL6* probe set, the presence of three fusion signals in > 16.2% of nuclei would be consistent with the presence of either a duplication of the 3q27 locus or a trisomy 3. The normal staining pattern for a dual color, dual fusion probe set (*IGH/BCL2*) is two red (*BCL2*) and two green (*IGH*) signals per nucleus. A signal pattern of one red signal, one green signal and two fusions or a one red, one green and one fusion pattern in > 5.8% of nuclei is indicative of the presence of the t(14;18)(q32;q21) translocation. Variations in these patterns are also indicative of duplications and/or deletions. For example, a signal pattern of one red and two green signals in > 16.2% of nuclei would indicate a deletion 18q21 or a monosomy 18.

3. Results

3.1. Clinical data

The study group included 7 women and 5 men with a median age of 63 years (range, 28–79). Clinically, the patients presented with a wide spectrum of neurologic symptoms depending on the region involved by lymphoma. The most common symptoms included headache, altered mental status, and weakness of one or more extremities. LDH levels were performed in 9 of the 12 patients (75%). The medium LDH serum level at diagnosis was 199 IU/L (range, 116–255; reference range 100–248 IU/L). Serologic studies for HIV were available in 7 patients (58%) with only 1 positive case (case 5). None of the cases in this study had a prior or concurrent history of lymphoma outside the CNS. The details for each patient are summarized in Table 1.

3.2. Imaging data

Information on computed tomography (CT) scans and/or magnetic

Table 1
Clinico-radiologic, pathologic, cytogenetic and prognostic features in cases of primary CNS lymphoma.

Case No	Age at dx	Sex	LDH (100–248 IU/L)	HIV (serology)	Neurologic symptoms before dx	Imaging findings (MRI and/or CT scan)	Biopsy site and type of specimen	Pathology dx (date)
1	60	M	144	Neg	Vertical diplopia, blurriness and imbalance for 2 weeks	No pre-operation MRI available	Left cerebellum Biopsy	HG-BCL with *MYC* and *BCL6* rearrangement
2	73	F	199	ND	Sudden motor aphasia, difficulty to initiate movement to walk, right mouth drooping, alteration of tasting sensation	Four enhancing lesions in left cerebral hemisphere involving inferior frontal gyrus, external and internal capsule (largest 2.8 × 1 cm). All lesions with surrounding edema and mild RD. Mild mass effect, with effacement of left lateral ventricle	Parietal lobe Craniotomy with resection	HG-BCL with *MYC* and *BCL6* rearrangement
3	42	F	ND	ND	Rapid loss of left eye vision, left temporal headache followed by right eye vision loss	Enhancing mass along cisternal component of left optic nerve within left aspect of optic chiasm and left optic tract. Circumferential enhancement along canalicular and intraorbital portion of left optic nerve. No leptomeningeal dissemination	Left optic nerve Biopsy	DLBCL
4	79	M	246	ND	Unsteady gait, forgetfulness, dizziness	CT: Hyperdense enhancing mass (5 × 2.6 cm) posteromedial to effaced atrium and occipital horn of right lateral ventricle MRI: Right peri-atrial and corpus callosum splenium mass with extensive vasogenic edema	Right hemisphere Biopsy	DLBCL
5	28	M	184	Pos (known HIV infection)	Confusion, fatigue, progressive lower extremities weakness. Weight loss for 6 months	Multiple masses in bilateral periventricular basal ganglia, left thalamus, left caudate, left cerebellum, and left frontal lobes. All T2 and T1 hypointense with peripheral enhancement. Midline shift to right side, and mass effect on upper brainstem. Effacement of 4th and 3rd ventricles. No leptomeningeal or dural enhancement	Left frontal lobe ST bx	EBV-positive DLBCL
6	58	F	134	Neg	Cognitive decline in few weeks followed by severe headaches	Left frontal periventricular region/basal ganglia mass (4 × 3.7 cm) with homogeneous contrast enhancement and RD. Right midline shift of 1.5 cm with surrounding white matter edema and obstructive hydrocephalus of both lateral ventricles	Left frontal lobe Biopsy	DLBCL
7	62	F	116	Neg	AMS, headaches,	Heterogeneously enhancing lesions in right periventricular frontal lobe	Right frontal lobe	DLBCL

(*continued on next page*)

Table 1 (*continued*)

Case No	Age at dx	Sex	LDH (100–248 IU/L)	HIV (serology)	Neurologic symptoms before dx	Imaging findings (MRI and/or CT scan)	Biopsy site and type of specimen	Pathology dx (date)
					repeated falls for 7 days, generalized weakness	extending to anterior right aspect of corpus callosum (4.5 × 4 cm) with prominent RD. Surrounding edema at right frontal and temporal lobes and right basal ganglia. Midline shift to left (1.5 cm) at the level of the ventricular atrium. Mild mass effect on upper brainstem. Effacement of right lateral ventricle with mild dilatation of left temporal horn. FLAIR hyperintensities extending to right thalamus and right midbrain	Biopsy	
8	64	F	ND	ND	Rapid onset of AMS and seizures	Enlarging supratentorial and infratentorial T2 hypointense homogeneously enhancing lesions throughout. Enlarging T1 hypointense lesion (3.2 cm) in periventricular region of the right temporal horn extending to right lateral ventricle. Additional periventricular homogeneously enhancing mass within atrium of left lateral ventricle (1.5 cm). Enlarging enhancing lesions effacing the 4th ventricle with extension into dentate nucleus and foramen of Luschka. Subependymal enhancement extending to left temporal horn and to left frontal horn. Few foci of RD within enhancing lesion in the right temporal horn. Marked T2/FLAIR hyperintense signal. Midline shift to left side (7 mm) at the level of foramen of Monro	Site NS ST bx	DLBCL
9	54	M	225	Neg	Progressive weakness of right lower extremities for 1 month, fall, and 2 days of slurred speech	Extensive abnormal signal in bilateral frontoparietal white matter with extension into bilateral basal ganglia. More extensive on left than right hemisphere. Nodular areas of enhancement (largest 2.5 cm) at bilateral posterior periventricular white matter	Left frontal lobe Biopsy	DLBCL
10	74	M	ND	ND	Lower extremities weakness with increased falls, difficulty	Right frontal lobe lesion (7.5 × 4 cm) with involvement of right cingulate and corpus callosum with prominent enhancement and RD. Smaller lesion in right lateral putamen/posterior limb of internal capsule	Right frontal lobe ST bx	DLBCL

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Table 1 (*continued*)

Case No	Age at dx	Sex	LDH (100–248 IU/L)	HIV (serology)	Neurologic symptoms before dx	Imaging findings (MRI and/or CT scan)	Biopsy site and type of specimen	Pathology dx (date)
					with speech and memory for 2–3 weeks	(1 × 1.2 cm) also with prominent enhancement and RD.		
11	75	F	208	Neg	Worsening confusion and intermittent right side weakness for 2 weeks	Multiple enhancing irregular lesions in bilateral cerebral hemispheres with solid homogeneous enhancement involving bilateral frontal, parietal, and temporal lobes and sparing the posterior fossa and brainstem. The largest lesion (1.6 cm) is in the left posterior frontal/anterior parietal lobe and shows moderate RD. Additional two peripherally enhancing lesions in the left centrum semiovale with peripheral RD. All lesions show significant T2 and FLAIR vasogenic edema. No midline shift. Basal cisterns are patent	Bilateral ST bx	DLBCL
12	77	F	255	Neg	Gradual onset of confusion, depression, gait imbalance and falls, time NS	Homogeneously enhancing lobulated ependymal and subependymal masses along the walls of the lateral ventricles, 3rd and 4th ventricles. The largest component (1.5 cm) is along the wall of the right lateral ventricle. In the 3rd ventricle, the mass is along the floor of the ventricle with prominent RD. Mild ventriculomegaly with periventricular edema. No midline shift or transtentorial herniation. No abnormal leptomeningeal enhancement seen	Bilateral periventricular ST bx	DLBCL

Case No	Morphologic findings	IHC results	Flow cytometry	GCB *vs* ABC	FISH (HR lymphoma panel *MYC*, *BCL2*, *BCL6*)	Treatment for CNS lymphoma	Time of FU (months)	Alive (A) Dead (D)
1	Centroblastic with starry-sky pattern	Pos: CD20, CD45, CD79a, MUM1, bcl-6, Ki-67–100%, bcl-2, c-myc > 90% Neg: CD3, CD10, CD30, CD43, CD138, EMA, ALK1, EBER	Pos: CD19, CD20, kappa LC Neg: CD5, CD10, lambda LC, T-cell markers	ABC	3q27 (*BCL6*) rearrangement 8q24 (*MYC*) rearrangement del 18q21 (*BCL2*)	Ferrari regimen, 3 cycles Rituximab single agent 3 doses	36	A (FOD)

Table 1 (*continued*)

Case No	Morphologic findings	IHC results	Flow cytometry	GCB *vs* ABC	FISH (HR lymphoma panel *MYC*, *BCL2*, *BCL6*)	Treatment for CNS lymphoma	Time of FU (months)	Alive (A) Dead (D)
2	Centroblastic with focal pleomorphic cells, starry-sky pattern	Pos: CD20, CD43, CD45, CD79a, MUM1, bcl-6, Ki-67 70%, bcl-2 c-myc ND Neg: CD10, CD30, ALK1, CD138, EBER	ND	ABC	3q27 (*BCL6*) rearrange-ment 8q24 (*MYC*) rearrange-ment and 8q24 gain gain 18q21 (*BCL2*)	HD-MTX + rituxi-mab 1 cycle, stopped due to poor performance status	3.9	D (AMS and seizures)
3	Centroblastic with starry-sky pattern	Pos: CD20, CD45, CD79a, MUM1, Ki-67–100%, bcl-2, c-myc 60% Neg: CD3, CD5, CD10, CD23, CD30, CD43, CD138, bcl-6, ALK1, cyclin D1, EBER	ND	ABC	gain 18q21 (*BCL2*)	Ferrari regimen + rituximab Carmustine followed by auto-SCT	40.3	A (FOD)
4	Centroblastic No starry-sky pattern	Pos: CD5, CD20, CD45, bcl-6, MUM1, Ki-67 90%, bcl-2 c-myc ND Neg: CD3, CD10, CD23, CD30, ALK1, EBER	Pos: CD5, kappa LC	ABC	3q27 (*BCL6*) rearrange-ment	MATRix regimen, 2 cycles	2.5	D (NS)
5	Centroblastic with a polymorphic background, suggestive of HG-LyG[a]	Pos (large cells): CD20, CD30 focal, CD45, CD79a, PAX5, MUM1, EBER, Ki-67 70%, bcl-2 Neg (large cells): CD3, CD4, CD5, CD8, CD56, CD68, CD138, bcl-6, ALK1, c-myc	Scant B-cell and T-cell populations, no definitive abnormalities	ABC	Negative	MATRix regimen, 3 cycles, last without thiotepa	4.8	A (FOD)

Table 1 (*continued*)

Case No	Morphologic findings	IHC results	Flow cytometry	GCB *vs* ABC	FISH (HR lymphoma panel *MYC*, *BCL2*, *BCL6*)	Treatment for CNS lymphoma	Time of FU (months)	Alive (A) Dead (D)
6	Centroblastic with starry-sky pattern	Pos: CD20, CD45 weak, CD79a, bcl-6, MUM1, Ki-67–100%, bcl-2, c-myc > 90% Neg: CD3, CD5, CD10, CD43, CD138, ALK1, EBER	Pos: CD19, CD20, kappa LC Neg: CD5, CD10, lambda LC	ABC	gain 14q32 (*IGH*)	MATRix regimen had 2 relapses 1st relapse: MTR + WBRT and temozolo-mide, 10 cycles 2nd relapse: palliative care, no tx	10.4	D (at OS facility)
7	Centroblastic with starry-sky pattern	Pos: CD5 focal weak, CD20, CD43 focal weak, CD45, CD79a, MUM1, Ki-67 90%, bcl-2, c-myc 60% Neg: CD3, CD10, CD15, CD30, CD138, cyclin D1, bcl6, ALK1, EBER	ND	ABC	gain 3q27 (*BCL6*)	MATRix regimen, 4 cycles Auto-SCT	29.1	A (FOD)
8	Centroblastic No starry-sky pattern	Pos: CD20, bcl-6, MUM1, Ki-67 90–100% Neg: CD3, CD10, EBER, bcl-2, c-myc	ND	ABC	Negative	No treatment (patient decision)	0.3	D (NS)
9	Centroblastic with starry-sky pattern	Pos: CD20, CD45, MUM1, Ki-67 90–100%, bcl-2, c-myc 60–70% Neg: CD3, CD5, CD10, CD30, bcl-6, EBER	ND	ABC	3q27 (*BCL6*) rearrange-ment gain 14q32 (*IGH*)	MATRix regimen, 4 cycles Auto-SCT	21.2	A (on tx)
10			ND	ABC	Negative		0.9	

(*continued on next page*)

Table 1 (*continued*)

Case No	Morphologic findings	IHC results	Flow cytometry	GCB *vs* ABC	FISH (HR lymphoma panel *MYC*, *BCL2*, *BCL6*)	Treatment for CNS lymphoma	Time of FU (months)	Alive (A) Dead (D)
	Centroblastic No starry-sky pattern	Pos: CD20, CD45, bcl-6, MUM1, Ki-67 90–100%, bcl-2, c-myc 50% Neg: CD3, CD10, CD30, EBER				Rituximab weely X4 and WB-XRT X1 stopped on 3/2019		NK Lost to FU
11	Centroblastic No starry-sky pattern	Pos: CD20, CD45/LCA, bcl-6, MUM1, Ki-67–100%, bcl-2, c-myc 80% Neg: CD3, CD5, CD10, CD30, EBER	ND	ABC	del 8q24 (*MYC*) gain 3q27 (*BCL6*)	Rituximab + HD-MTX 1 cycle	1.8	NK Lost to FU
12	Centroblastic No starry-sky pattern	Pos: CD20, CD45/LCA, bcl-6, MUM1, Ki-67–100%, bcl-2, c-myc 40–50% Neg: CD3, CD5, CD10, CD30, EBER	ND	ABC	gain 18q21 (*BCL2*)	MATRix regimen, 1 cycle	0.9	NK Transferred to different facility and lost to FU

Abbreviations: CNS = central nervous system; IHC = immunohistochemistry; HR = high risk; FISH = fluorescence *in situ* hybridization; CT = computed tomography; dx = diagnosis; MRI = magnetic resonance imaging; RD = restricted diffusion; AMS = altered mental status; ABC = activated B-cell-like (non-germinal center) phenotype; GCB = germinal center B-cell-like phenotype; HG = high grade; LBCL = large B-cell lymphoma; DLBCL = diffuse large B-cell lymphoma; NHL = non-Hodgkin lymphoma; Pos = positive; Neg = negative; LyG = lymphomatoid granulomatosis; NK = not known; ND = not done; NS = not specified; NOS = not otherwise specified; EBV = Epstein-Barr virus; EBER = EBV encoded RNA *in situ* hybridization; FOD = free of disease; Tx = therapy; s/p = status post; MTX = methotrexate; Ferrari regimen = high dose MTX and high dose cytarabine; MATRix regimen = MTX, cytarabine, thiotepa, rituximab; R-CHOP = rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; auto-SCT = autologous stem cell transplant; BEAM = carmustine, etoposide, cytarabine, melphalan; LC = light chain immunoglobulin; WBRT = whole brain radiation therapy; ST bx = stereotactic biopsy; FLAIR = Fluid-attenuated inversion recovery.

[a] The patient did not have a previous diagnosis of LyG or any evidence of disease outside the CNS.

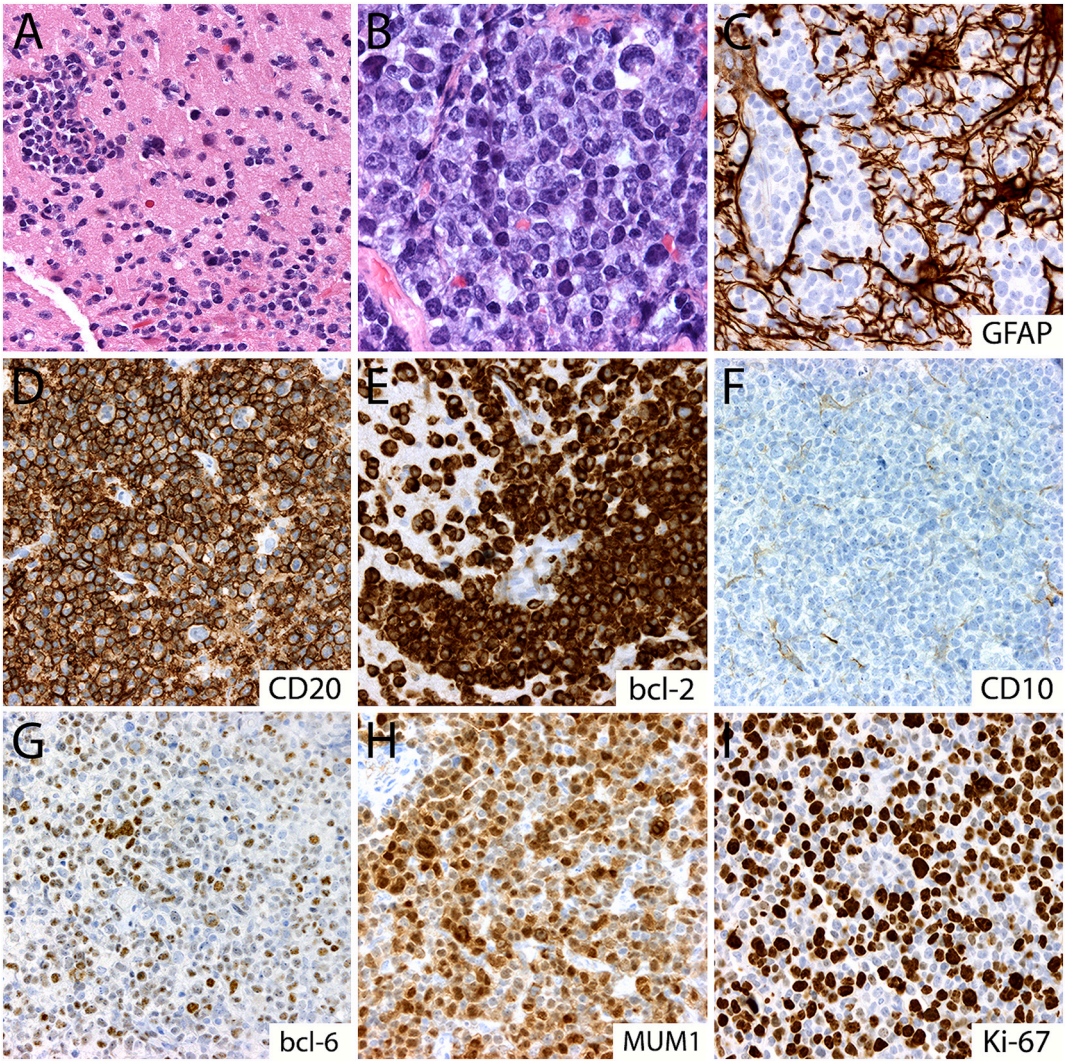

Fig. 1. Primary central nervous system lymphoma with *MYC* and *BCL6* gene rearrangements (double-hit PCNSL; case 2). A) Large cell lymphoma infiltrating brain parenchyma as single cells, small clusters of lymphoma cells, or arranging in a perivascular distribution. B) Other areas consist of diffuse sheets of lymphoma cells with centroblastic features and pleomorphism. Areas with a starry-sky pattern were also seen (not shown). C) Glial fibrillary acidic protein (GFAP) highlights entrapped reactive astrocytes. The lymphoma is positive for D) CD20, E) bcl-2, G) bcl-6, and H) MUM1, and negative for F) CD10, consistent with a large cell lymphoma of non-germinal center/activated B-cell-like subtype. I) The Ki-67 proliferation index is approximately 70%. (Original magnification: A, C–I: 200×; B: 400×).

resonance imaging (MRI) was available in 11 patients (92%). The majority of cases presented as supratentorial parenchymal lesions/masses with hyperintense signal on T2-weighted sequences, homogeneous contrast-enhancement, diffuse restriction, and with surrounding vasogenic edema. Detailed radiologic information for each case is listed in Table 1.

3.3. Histopathology

Eleven samples were stereotactic biopsies and 1 sample was obtained by craniotomy and resection (case 2). All cases were diagnosed as large B-cell lymphoma and were composed of sheets of large lymphoma cells with centroblastic features showing a variable degree of infiltration of the brain parenchyma with characteristic perivascular distribution (see Figs. 1A, B and 2A). Immunoblastic or anaplastic features were seen focally in most cases. Necrosis was variably present. Six cases (50%) showed a starry-sky pattern at least focally. In one case (8%) the background was predominantly composed of a polymorphic inflammatory infiltrate admixed with large lymphoma cells, but other areas of the tumor showed sheets of large lymphoma cells especially around blood vessels (case 5).

3.4. Immunohistochemistry

All cases (100%) showed expression of CD20 and when available, of additional B-cell markers, including PAX5 and/or CD79a. CD5 was positive in 2 of 8 tested cases (25%) and CD10 was negative in all 11 tested cases (100%). CD30 was positive (> 30% of lymphoma cells) in 1 of 10 tested cases (case 5). Immunohistochemistry for bcl-2, bcl-6 and MUM1 was tested in all 12 cases. Eleven cases (92%) were positive for bcl-2, 8 cases (67%) were positive for bcl-6, and all cases (100%) were positive for MUM1. c-myc was positive in 8 of 10 tested cases (80%). Using the Hans algorithm [3], all 12 cases (100%) were classified as large B-cell lymphoma of non-germinal center/ABC-like phenotype. Eight of 10 (80%) tested cases were positive for both bcl-2 and c-myc (double expressors). Ki-67 was performed in all cases (proliferation index range 70% to 100%) with 10 of 12 cases (83%) showing a proliferation index between 90 and 100%. Epstein-Barr virus encoded RNA *in situ* hybridization was positive in 1 of 12 cases (8%). Representative examples of the immunohistochemistry results in our cohort are shown

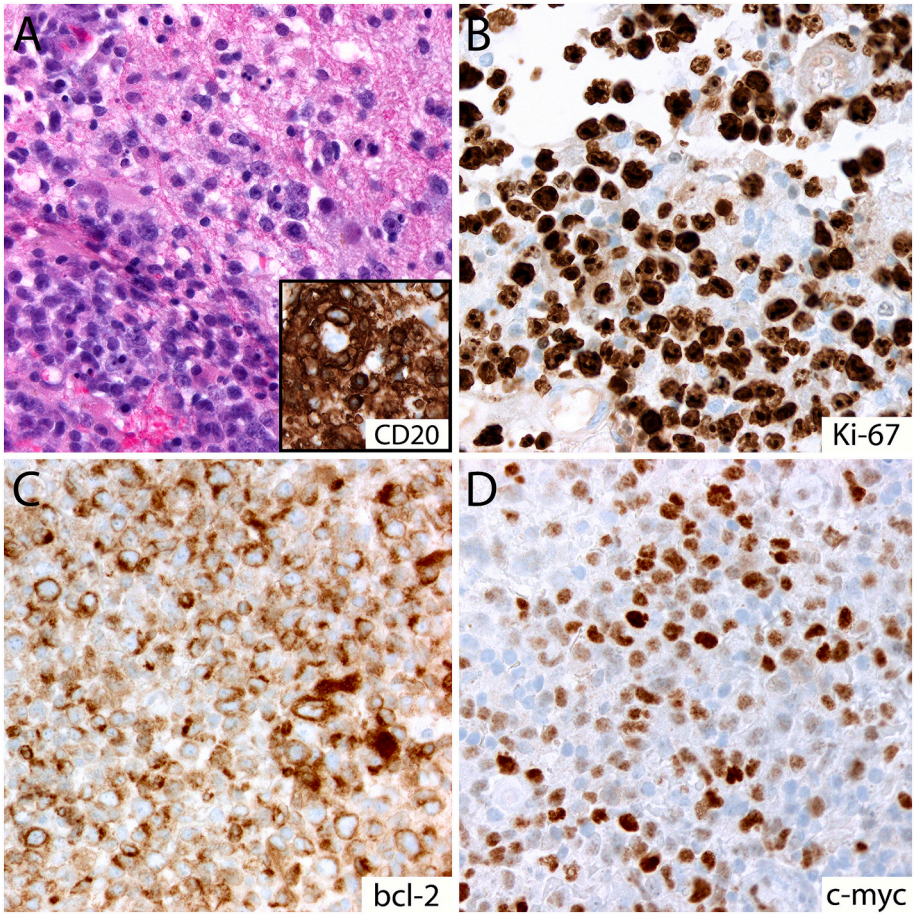

Fig. 2. Primary central nervous system lymphoma, negative for *MYC*, *BCL2*, and/or *BCL6* gene rearrangements (case 10). A) Large cell lymphoma with centroblastic morphology involving brain parenchyma (inset, CD20 immunostain). No starry-sky pattern was seen. B) The Ki-67 proliferation index is 90 to 100%. Despite negative gene rearrangements by fluorescence *in situ* hybridization, the lymphoma cells are positive for C) bcl-2 and D) c-myc (~50% of cells). (Original magnification: A–D: 200×).

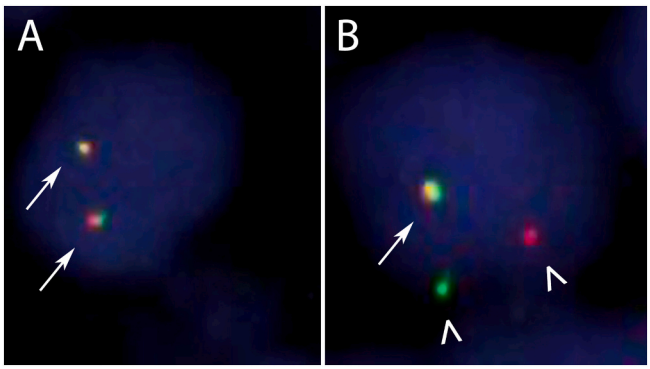

Fig. 3. Representative example of fluorescence *in situ* hybridization in primary central nervous system lymphoma (case 9). A) Normal result for *BCL6* break-apart probe demonstrating the presence of 2 fusion signals per nucleus (arrows). B) Positive break-apart probe result indicative of a t(3q27)/*BCL6* gene rearrangement demonstrating the 1 red and 1 green (arrowheads), and 1 fusion signal pattern (arrow); (Original magnification: A and B: 100×). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in Figs. 1 and 2.

3.5. Flow cytometry

Flow cytometry immunophenotypic studies were performed in 4 cases (33%). Three of these cases (cases 1, 4, and 6) showed a kappa monotypic B-cell population and 1 case (case 5) showed scant B-cell and T-cell populations with no definitive immunophenotypic abnormalities.

3.6. Fluorescence in situ hybridization (FISH)

The high-risk lymphoma FISH panel was performed in all 12 cases. Nine cases (75%) demonstrated FISH abnormalities whereas 3 cases (25%) were negative for genetic alterations. From the cases with abnormalities, 4 cases (33%) harbored a *BCL6* gene rearrangement, 3 cases (25%) showed gain of *BCL2*, 2 cases (17%) showed gain of *BCL6*, 2 cases (17%) showed gain of *IGH*, 1 case (8%) harbored a gain of *MYC*, and 1 case (8%) harbored a *MYC* deletion. Some of these genetic abnormalities were present in the same patient (see Table 1). Two of the cases (16%) with *BCL6* rearrangement showed a concurrent *MYC* rearrangement and met the current definition of double-hit lymphoma with *MYC* and *BCL6* gene rearrangements (cases 1 and 2) and could be referred to as double-hit PCNSLs using the current nomenclature from the 2017 WHO classification [10]. None of the cases showed single or concurrent rearrangements of *IGH* or *BCL2* genes, t(14;18)(q32;q21) *IGH/BCL2*, concomitant *MYC* and *BCL2* rearrangements, or concomitant *MYC*, *BCL2* and *BCL6* rearrangements (triple-hit lymphoma). A representative example of a normal signal for the *BCL6* probe and for a *BCL6* gene rearrangement are shown in Fig. 3.

3.7. Treatment and outcome

Eleven of 12 patients (92%) received chemotherapy and one of these patients also received whole brain radiation therapy. One patient (8%) denied treatment. The chemotherapy regimens included: methotrexate, cytarabine, thiotepa and rituximab (MATrix) in 6 patients (50%); high-dose methotrexate and high-dose cytarabine (Ferrari regimen) in 2 patients (17%); and methotrexate and rituximab in 2 patients (17%). Single agent rituximab and temozolomide were used in 1 patient each (8%). Three of 12 patients (25%) underwent autologous

stem cell transplant. The median time of follow-up for all patients was 4.4 months (range, 0.3–40.3 months). At the time of preparation of this study, 7 patients (58%) were alive, 4 have died (33%), and 1 patient was lost to follow-up (8%). From the 7 surviving patients, 5 (71%) were free of disease, including one of the patients with *MYC* and *BCL6* gene rearrangements (case 1) and in 2 patients follow up information is not available. Three of the 4 deceased patients died at an outside facility < 6 months after diagnosis and 1 patient died at 10.4 months of diagnosis (case 6). This patient (case 6) was the only one from all 12 individuals who had two PCNSL relapses.

4. Discussion

We present 12 cases of PCNSL with clinical, imaging, histopathology, immunohistochemistry, and FISH findings. Along with the classic morphologic description of PCNSL, half of the cases in this study demonstrated a starry-sky pattern in about 10% to 30% of the tumor, a feature that we have not seen mentioned or may have not been paid much attention to in PCNSLs. This finding was observed only in cases that harbored a FISH abnormality. Similar to what has been described in the literature in PCNSLs [1,16-20,22,23,29,30], all cases (100%) were of non-GCB/ACB-like subtype, and there was a high frequency of double expression of bcl-2 and c-myc by immunohistochemistry (80% of tested cases).

Our FISH results were consistent with the findings previously reported in the literature for PCNSLs [16,18,26-31]. The most frequent genetic abnormalities observed were in the *BCL6* gene; either as a gene rearrangement (t(3q27)) or as a gain of 3q27, which together were observed in 50% (6/12) of cases. Breakapart FISH probes, such as those used in this study, were designed for use where the gene locus of interest (*e.g.* 3q27, 8q24) may have multiple potential rearrangement partners. Unfortunately, while these probes identify the presence of a gene rearrangement involving the locus, they do not identify the specific partner. For the 3q27 (*BCL6*) locus, the most common rearrangement is the t(3;14)(q27;q32) translocation involving *BCL6* and the *IGH* heavy chain enhancer region (14q32). Additional variants that have been reported include the t(3;22)(q27;q11.2) translocation involving *BCL6* and *IGL* and the t(2;3)(p12;q27) translocation between *IGK* and *BCL6* [33]. The observed gains of 3q27 may represent a specific 3q27 duplication or a trisomy 3. Trisomy 3 is more common in T-cell lymphomas than in B-cell lymphomas, where it is relatively rare [34]. We also identified one case (case 11) with deletion of 8q24 (*MYC*) that has been reported in the literature only once by Tapia et al. [22] in 2015 in a series of 42 cases. The case in our series was also accompanied by a gain of 3q27 (*BCL6*) and showed overexpression of c-myc by immunohistochemistry in 80% of lymphoma cells. This finding further supports the lack of concordance between c-myc protein expression and genetic abnormalities detected at the *MYC* locus. No particular clinical, radiologic, histologic, or immunophenotypic differences were identified between this case and the rest of the cases.

Similarly, we identified two cases (16%) that harbored *MYC* and *BCL6* gene rearrangements by FISH (case 1 and 2). These two cases otherwise did not show any particular clinical, radiologic, histologic or immunophenotypic differences from the rest of the cases. Case 1 was a man diagnosed with PCNSL involving the left cerebellum at 60 years of age, received 3 cycles of Ferrari regimen and 3 cycles of rituximab as a single agent, and is free of disease at 36 months of last follow up. Case 2 was a woman diagnosed at 73 years of age who had multiple lesions in the left cerebral hemisphere and died 3.9 months after diagnosis despite treatment with one cycle of high-dose methotrexate and rituximab. We did not identify any case of *MYC/BCL2* double-hit lymphoma or any triple-hit lymphoma. To the best of our knowledge, only two cases of PCNSL with *MYC* and *BCL6* gene rearrangements have been previously reported in the literature. The first case was identified by Cady et al. in 2008 [26] in a series of 75 PCNLs cases (1% of their cases). The second case was identified by Brunn et al. in 2013 [18] in a series of 50 PCNSLs (2% of their cases). This information corroborates the rarity of *MYC/BCL6* double-hit PCNSLs similar to that of *MYC/BCL2* double-hit and triple-hit PCNSLs [31,32]. It is possible that *MYC/BCL6* double-hit PCNSLs have been under-reported as some large case series of PCNSLs with FISH analysis only focused on detecting *BCL6* gene alterations and *MYC* status was not evaluated [29].

The clinical implications of detecting *MYC* and *BCL6* rearrangements in a case of PCNSL are unclear as the number of cases available in the literature are too few to draw any conclusions (total of 4 cases including the two from this study). In systemic DLBCL, detection of *MYC* and *BCL6* rearrangements has prognostic significance with these cases showing a poor outcome [12,35-37]. For this reason, these cases have been included in the group of high-grade BCL in the 2017 WHO classification [10]. Although in this study we refer to those cases with *MYC* and *BCL6* rearrangements as “double-hit PCNSL”, it is also uncertain if they should really be considered “high-grade PCNSL”. For example, one of the patients with double-hit PCNSL in our study (case 1) is still alive and free of disease at 36 months of follow up. Therefore, although the natural tendency may be to apply the term double-hit or triple-hit to all LBCLs that demonstrate *MYC*, *BCL2*, and/or *BCL6* gene rearrangements, it might not be entirely accurate to designate specific subtypes of LBCLs (*e.g.* T-cell/histiocyte-rich LBCL, EBV+ LBCL, intravascular lymphoma, primary mediastinal LBCL, *etc*.) as high-grade BCLs as currently recommended by the WHO, but this remains as mere speculation. More cases of double-hit or triple-hit PCNSLs should be studied to further determine whether these genetic abnormalities have a significant impact on prognosis, as in systemic DLBCL. Furthermore, PCNSL has already a worse overall outcome when compared to systemic DLBCL, with the only additional negative prognostic factor being in an older age group (> 65 years), which is associated with reduced survival [1]. Reproducible and robust prognostic and predictive markers are still lacking in PCNSL and several studies have shown conflicting results between potential markers. Overexpression of bcl-6 protein and/or detection of a *BCL6* rearrangement have been associated with either favorable or poor prognosis in PCNSL [22,25,26,38,39]. Likewise, c-myc and/or bcl-2 protein expression and presence of *MYC* and/or *BCL2* rearrangements have also shown different outcomes [18-22,24,40]. Poor prognosis in PCNSL appears to be secondary to a decreased antitumor immune response, *e.g.* intratumoral T-cell and dendritic cell density, as compared to systemic DLBCL [2]. Adjuvant dendritic cell and T-cell immunotherapy may prove useful to boost treatment responses in this subtype of lymphoma [2].

A number of genomic abnormalities have also been identified in PCNSL [41]. In a comprehensive study [42], mutations in *MYD88*, specifically *MYD88* L265P mutation, and in *CD79B* were especially more frequent in the DLBCL of the immune-privileged sites such as CNS and testis, relative to nodal DLBCL, with up to 75% of PCNSL showing *MYD88* mutation. In addition, in that same study, DLBCLs of the immune-priviledged sites were found to be largely represented by cases of non-GCB/ABC phenotype and were essentially mutually exclusive with the high-risk lymphoma markers. These observations further support the notion that PCNSL, despite its histologic similarity to DLBCL, is a distinct subtype and these mutations can potentially be useful in the diagnosis and characterization of individual cases. More importantly, they can create opportunities for targeted therapies. A meta-analysis on the clinicopathologic significance of *MYD88* L265P mutation [43] found that there was no agreement on the effect of this mutation on survival, with variable reports in the literature. Whether inclusion of these mutations and other genomic alteration in the clinical work-up will be valuable remains to be seen and requires further studies and guidelines for uniform application.

Only one case in this study group (case 5) was positive for HIV and this was the only PCNSL positive for EBV. The morphology and immunophenotype of this case were diagnostic of an EBV+ LBCL and at least some areas of the tumor suggested that it could have progressed from grade 2 lymphomatoid granulomatosis. This patient was the

youngest in the study group (28 years old at diagnosis) and as of the latest follow-up, the patient had not developed lymphoma outside the CNS. c-myc was negative by immunohistochemistry and FISH analysis was negative for alterations in *MYC*, *BCL2* and *BCL6* genes.

In summary, we present a study of 12 cases of PCNSL with FISH results for all cases in the era of high-grade LBCL with detailed clinical, radiologic and additional laboratory data, and with the identification of additional double-expressor cases. We identified two new cases with very rare *MYC* and *BCL6* rearrangements that qualify for the WHO 2017 designation "double-hit PCNSL" or "PCNSL with MYC and BCL6 gene rearrangements". However, no differences were observed between these double-hit PCNSLs and the rest of the cases and one of these patients is alive and free of disease at last time of follow up. In addition, we identified a case of PCNSL with deletion of 8q24 (*MYC*) only reported once before in the literature.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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