PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA: CLINICOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL PROFILE

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ABSTRACT

BACKGROUND

Primary central nervous system lymphoma (PCNSL) is a rare form of extranodal non-Hodgkin lymphoma (NHL) confined to the brain, spinal cord and/or eye, occurring in immunocompetent individuals. Histologically, they are diffuse large B-cell lymphomas. Over the last few decades there has been a gradual increase in their incidence.

AIM

To study the clinical, histopathological and immunohistochemical profile of primary central nervous system lymphoma.

SETTING AND DESIGN

Retrospective audit of seven cases of PCNSL diagnosed over a period of five years in a tertiary referral hospital of North India.

MATERIAL AND METHODS

The clinical, radiological and laboratory findings were retrieved from the hospital records. Histopathology slides were reviewed, studied in detail and a panel of immunohistochemical markers comprising of CD3, CD5, CD20, CD10, BCL6, BCL2, MUM1, CD30, EBV (LMP1), Ki-67 and p53 was done on all cases.

RESULTS

The male to female ratio was 3:4 with a median age of 60 years. The most common form of presentation was neurological deficits and altered sensorium. Imaging showed contrast enhancing, single or multiple, deep seated lesions within the cerebral hemispheres. Histologically, all were high-grade diffuse large B-cell lymphomas showing typical angiocentricity and a median Ki-67 proliferative index of 80%. Based on immunohistochemistry (Hans classifier) three cases had germinal centre B-cell (GCB) and four had non-germinal centre B-cell (non-GCB) phenotype. p53 was expressed in all cases with strong expression in four of them. Four patients died before treatment could be initiated, one received palliative chemo-radiotherapy and two did not follow up after diagnosis.

CONCLUSIONS

Primary CNS lymphomas are high-grade diffuse large B-cell lymphomas which show high Ki-67 proliferative indices and frequent overexpression of p53. Irrespective of histological subtype, GCB or non-GCB, outcome is uniformly poor. Early and prompt diagnosis is essential to improve the dismal prognosis of these patients. Developing new targeted therapies is the need of the hour.

KEYWORDS

CNS lymphoma, DLBCL, immunocompetent, immunohistochemistry, p53.

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INTRODUCTION: Lymphomas can involve the central nervous system (CNS) as a primary event or as secondary spread from systemic lymphoma.

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They can be encountered in both immunocompetent and immunocompromised individuals, incidence being much higher in the latter.[1] According to the current WHO definition, the term Primary CNS Lymphoma (PCNSL) is restricted to diffuse large B-cell lymphoma (DLBCL) confined to the brain and/or eye in immunocompetent patients.[2] A variety of other lymphomas, which may also manifest primarily or exclusively in the CNS, such as lymphomas of the dura, immunodeficiency-associated lymphomas and intravascular large B-cell lymphoma (IVLBCL) are excluded

from this definition. Congenital or acquired immunodeficiency is the only established risk factor for development of CNS lymphoma.[3] We present the clinicopathological features and immunohistochemical (IHC) profile of seven cases of PCNSL in immunocompetent patients.

MATERIAL AND METHODS: All cases of primary CNS lymphoma diagnosed over a period of five years (2011-2015), in our tertiary referral hospital, were included in the study. The pathology requisition forms and case files were scrutinized and the clinical, radiological and laboratory findings were noted down. Histopathology slides were reviewed in detail and a panel of IHC markers comprising of CD3 (PS1, Biogenex), CD5 (4C7, Novocastra), CD10 (56C6, Biogenex), CD20 (L-26, Novocastra), BCL6 (LN22, Novocastra), BCL2 (124, Dako), MUM1 (MUM1p, Dako), CD30 (1G12, Novocastra), EBV-LMP1 (CS1-4, Novocastra, dilution 1:100), Ki-67 (MM1, Novocastra), and p53 (DO-7, Dako) was done on all cases. All the primary antibodies were pre-diluted except EBV-LMP1. The detection kit used was 'NovoLink polymer' from Leica Biosystems, Newcastle Upon Tyne, United Kingdom. For IHC staining, standard protocols were followed as per the manufacturer's instructions.

Categorization of DLBCL into germinal centre B-cell (GCB) and non-germinal centre B-cell (non-GCB) phenotypes was done based on the Hans IHC algorithm.[4] The cut off values used for CD10, BCL6 and MUM1 positivity was 30%. For the other IHC markers more than 10% staining in tumour cells was considered positive. Ki-67 and p53 staining were semi-quantitatively assessed as percentage of tumour cells positive, recorded in multiples of ten. In addition, intensity of p53 staining was also recorded as weak (+), moderate (++) or strong (+++).

RESULTS: Three hundred and forty cases of lymphoma were diagnosed on tissue biopsies over a period of 5 years, out of which 284 (83.5%) were NHL. Seven cases of primary CNS lymphoma were identified which comprised 2% of all lymphomas and 2.5% of all NHL. The relevant clinical features, radiological findings and serum lactate dehydrogenase (LDH) levels are summarised in table 1.

Case	Age/sex	Clinical presentation	MRI findings	LDH (U/L)
1	66/M	Seizures Left hemiparesis	Single, enhancing, deep seated, nodular lesion, right frontal lobe. Subacute infarct left MCA. Small chronic subdural hematomas.	380
2	60/M	Headache Vomiting Previously diagnosed as a case of seizure disorder on phenytoin x 20 years.	Multiple enhancing lesions in right frontal lobe, periventricular location, corpus callosum and right basal ganglia.	801
3	52/F	Left hemiparesis Left facial nerve palsy Previously diagnosed as a case of seizure disorder on non-allopathic medication x 8 years	A large heterogeneously enhancing complex haemorrhagic lesion, right deep temporo-occipital region with satellite nodules	ND
4	62/M	Altered sensorium	Single enhancing lesion in the left frontal lobe adjacent to frontal horn of left lateral ventricle	ND
5	46/F	Right facial nerve palsy Right brachial paresis Aphasia	Multiple enhancing deep seated lesions in bilateral cerebral hemispheres (frontal and temporal areas), midbrain lesion +	500
6	70/F	Left hemiparesis Giddiness Altered sensorium	Single large well-defined lobulated intensely enhancing lesion involving caudate nucleus, anterior lentiform nucleus, right ganglio-capsular region and thalamus.	337
7	60/F	Acute memory loss Drowsiness Altered sensorium	Single large enhancing lesion centred in the genu of corpus callosum, extending into bilateral basi-frontal regions, right caudate nucleus and ganglio-capsular region	683

Table 1: Clinical features, radiological findings and LDH levels in seven PCNSL patients

LDH: lactate dehydrogenase, **MCA:** middle cerebral artery, **MRI:** magnetic resonance imaging, **ND:** not done.

The age at presentation ranged from 46 to 70 years with a median of 60 years. The male to female ratio was 3:4. At presentation, focal neurological deficits were present in 4/7, altered sensorium in 4/7, signs of raised intracranial tension (ICT), seizures, acute memory loss and aphasia in one patient each. Two patients gave previous history of seizure disorder and were on anticonvulsant therapy. All the patients tested negative for HIV. LDH levels were available in five cases, and were raised in all with a mean of 540 U/L. Systemic workup did not reveal any lymphadenopathy, hepatosplenomegaly or bone marrow involvement. Two patients underwent CSF examination which showed no lymphomatous infiltration.

Contrast Magnetic Resonance Imaging (MRI) in all the patients showed enhancing deep-seated lesions in the cerebrum with variable involvement of the basal ganglia, periventricular regions, corpus callosum, thalamus and midbrain (Figure 1). Large solitary lesions were encountered in four patients while the rest had multiple lesions. All patients underwent open surgical biopsies with partial or complete removal of tumour.

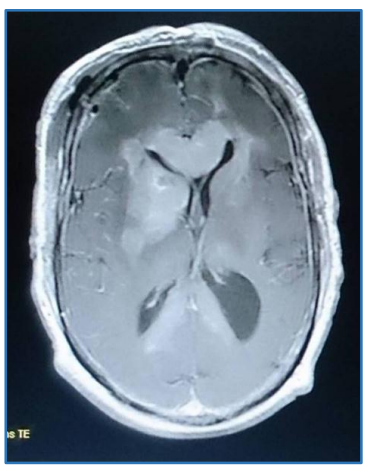

Fig. 1: MRI showing a contrast enhancing lesion centred in genu of the corpus callosum with extension into bilateral frontal lobes

Histopathology showed cohesive sheets of centroblasts with typical angiocentricity (Figure 2a and b). Single cell infiltration into the surrounding brain parenchyma was also common (Figure 2c). Mitotic activity was high, ranging between 3-13/HPF, with numerous apoptotic nuclear fragments (Figure 2d). Starry sky-pattern and necrosis were identified in two cases. Meningeal involvement was seen in one case.

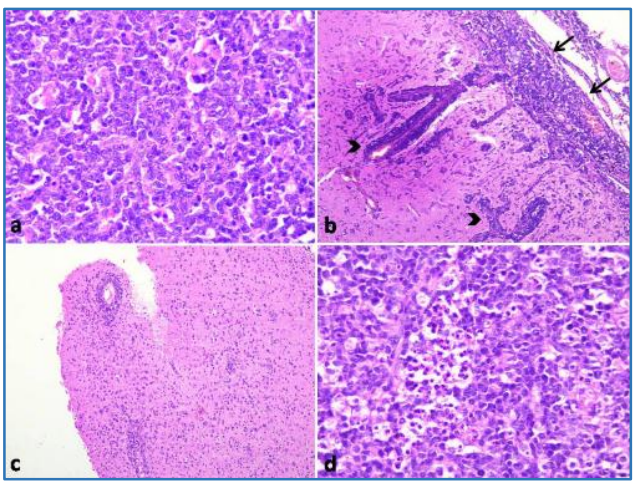

Fig. 2: Histological features of PCNSL at various magnifications. a) Centroblastic morphology. b) Angiocentric growth pattern (arrowheads) with meningeal infiltration (arrows). c) Single cell infiltration into the adjoining brain parenchyma. d) Prominent apoptosis. H&E

Case	CD20	CD10	Bcl6	MUM1	BCL2	Cyclin D1	EBV	CD30	CD5	CD3	Ki67%	p53%[a]
1	+	+	+	+	+	-	-	-	-	-	60	20 ++
2	+	-	-	+	-	-	-	-	-	-	60	20 ++
3	+	+	+	+	+	-	-	-	-	-	90	40 ++
4	+	+	+	+	-	-	-	-	-	-	80	70 +++
5	+	-	+	+	+	-	-	-	-	-	80	90 +++
6	+	-	+	+	+	-	-	-	-	-	70	30 +++
7	+	-	+	+	-	-	-	-	-	-	90	90 +++

Table 2: Immunohistochemical profile in seven PCNSL patients

+: positive, -: negative, [a] intensity of p53: ++ moderate, +++ strong.

IHC profile of the seven cases is depicted in table 2. All the cases were CD20+ (Figure 3a) and diagnosed as diffuse large B-cell lymphoma. A variable number of small reactive T-cells were seen admixed with neoplastic B cells, their concentration being more toward the periphery of tumour. Perivascular T-cell cuffing was identified in three cases in the peripheral areas (Figure 3b). Positive staining for CD10 was seen in three cases, BCL6 in five, MUM1 in all and BCL2 in four cases (Figure 3c and d). Based on Hans algorithm, three cases (case no. 1, 3 & 4) had GCB and the rest had non-GCB phenotype.[4] In all cases, the neoplastic lymphoid cells were negative for cyclinD1, EBV (LMP-1), CD3, CD5 and CD30. Ki-67 proliferative index ranged between 60-90% with a median of 80% (Figure 3e). Positivity for p53 was present in all the cases ranging between 20-90% (Figure 3f). Intensity of nuclear staining was strong in four while it was moderate in the remaining three.

Out of the seven patients, one received palliative treatment with 6-mercaptopurine and methotrexate (MTX) regimen and whole brain radiotherapy, 45Gy/25F/5 weeks. At his last follow up at 3 months, this patient had significant residual disease. Four patients died within one month of diagnosis, before any chemotherapy or radiotherapy could be initiated. Two patients were lost to follow up after diagnosis.

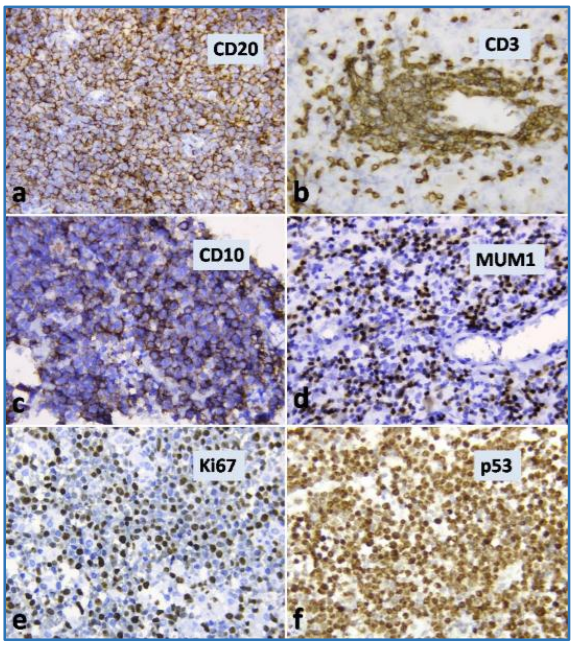

Fig. 3: Immunohistochemistry findings in PCNSL a) Diffuse CD20 positivity b) Perivascular cuff of reactive CD3+ T-cells c) CD10 positivity d) MUM1 positivity e) High Ki-67 proliferative index f) Strong p53 expression +++. Diaminobenzidine chromogen, 400x

DISCUSSION: Epidemiology: Owing to the rarity of PCNSL in the immunocompetent, the disease has been very challenging to study. Various studies have shown that primary CNS lymphoma comprises 1-7% of primary CNS neoplasms and less than 1% of all NHL.[2,5-8] Studies in immunocompetent western population have shown a gradual increase in their incidence, which can partly be attributed to improved investigative modalities. Studies in Indian population have, however, shown no increase in their incidence.[7,8] In our institutional 5-year study, PCNSL comprised 2.5% of all NHL.

Clinical Features: The peak age for PCNSL in the immunocompetent population is fifth or sixth decade of life with a male to female ratio of 3:2.[1] The age of occurrence is much younger in patients with any form of immunodeficiency.[1] The median age in our study was 60 years with a male to female ratio of 3:4.

Clinical presentation largely depends upon the site of involvement and mass effect. Patients can present with focal neurologic deficits (50-80%), neuropsychiatric symptoms (20-30%), increased intracranial pressure (10-30%), seizures (5-20%) or eye symptoms (5-20%).[1,9] Seizures are less common than with other types of brain tumours, probably because PCNSL involves predominantly the subcortical white matter rather than epileptogenic grey matter.[10] Patients rarely present with B symptoms that are commonly associated with systemic forms of NHL. In our series, neurological deficits were the most common presenting symptoms, followed by altered sensorium. Only one case presented with seizures and none had B symptoms. Occult systemic lymphoma may be detected in up to 8% of patients initially presenting with CNS lymphoma.[11] In our study, systemic workup revealed no evidence of lymphoma elsewhere.

Two of our patients were on long term therapy with anticonvulsants. Long term use of phenytoin may be associated with development of lymphoma, although this has not been convincingly proven.[12]

Imaging: Contrast enhanced cranial MRI is the imaging modality of choice which displays isohyperintense T2 signals with restricted diffusion. They enhance avidly and homogeneously after contrast administration with perilesional oedema.[13,14] About 60-85% of cases involve the supratentorial compartment, including frontal (20-15%), temporal (8-15%), parietal (7-18%) and occipital (3-4%) lobes.[1,9,13] They are usually located in the deep portions of the cerebral white matter and can involve the basal ganglia, periventricular regions and corpus callosum. Multifocal lesions are more common in immunocompromised (60-85%) as compared to immunocompetent population (25-50%).[1,13] The differential diagnosis on imaging includes lymphoma, glioma and metastasis. In our series, all cases were located in the deep white matter with three of them being multifocal. Multifocal lesions raised a concern for metastasis. One case had a lesion centred in the genu of corpus callosum with extension into bilateral frontal lobes resembling a glioma.

Histopathology and Immunohistochemistry: Stereotactic brain biopsy is recommended to establish a diagnosis of CNS lymphoma. More than 95% of lymphomas primarily involving the CNS are DLBCL. The rest include Epstein-Barr virus-associated lymphoproliferative disorders, low-grade B-cell lymphomas, Burkitt lymphoma, IVLBCL, T-cell lymphomas, anaplastic large cell lymphoma, and Hodgkin lymphoma.[1,10,14] The malignant large lymphoid cells in DLBCL can have centroblastic or immunoblastic morphology and show typical angiocentricity, which is an important clue to diagnosis. The latter feature was striking in all our cases and could be identified even at low power examination. They are high grade tumours and show prominent mitotic activity with or without necrosis. Besides cohesive sheets, single cell infiltration into the adjoining brain parenchyma can also be seen.[15] Effect of steroids on histopathology includes increase in apoptosis with infiltration by macrophages, decreased CD20 cells and increased number of CD3 T-cells.[15] Since all our patients received steroids prior to surgery, apoptosis was a prominent histological feature in all. It is recommended to avoid glucocorticoid therapy prior to surgery as steroids may cause rapid tumour lysis rendering a pathologic diagnosis difficult.[10]

On IHC, PCNSL are positive for B-cell markers like CD20, PAX5 and CD79a. Other markers which can be positive include CD10 (10-20%), BCL6 (60-80%), BCL2 (22-100%) and MUM1 (90-100%).[15,16,17] Being high-grade tumours, Ki-67 proliferative index is generally high (50-90%). IHC findings in our cases are in accordance to those described in

literature. CNS lymphoma in the immunocompetent, unlike HIV positive patients, is not associated with Epstein-Barr virus (EBV). All our cases were negative for EBV (LMP1) by IHC.

Primary CNS lymphoma is thought to arise from late-germinal centre or post-germinal centre lymphoid cells. Gene expression profiling shows PCNSL to be distributed among the spectrum of activated B-cell like and germinal centre type DLBCL.[18] Using IHC surrogates for molecular subgrouping, like the Hans algorithm, three cases in our study had GCB phenotype while the rest were non-GCB type. Although of clinical interest, prognostic significance of subgrouping of PCNSL, unlike nodal DLBCL, is not clear.[14]

Overexpression of p53 protein can be seen in 20-100% of PCNSL.[15,18,19] All our cases had some degree of p53 expression supporting the fact that alteration of p53 is important in the pathogenesis of PCNSL. Overexpression cannot be readily regarded as a reflection of an underlying genetic abnormality and may be a result of epigenetic events.

In one study, the presence of reactive perivascular T-cell infiltrates were associated with better overall survival, in patients treated with high-dose MTX.[20] Three patients in our series showed perivascular CD3+ T-cell cuffing, of which one patient expired before therapy could be initiated. The other two did not follow up after diagnosis.

Treatment and Prognosis: In patients younger than 60 years the intent of treatment is curative. Polychemotherapy based on high-dose MTX or alternatively high-dose chemotherapy with autologous stem cell rescue can be offered. For patients over 60 years of age no curative regimen with acceptable toxicity has yet been established. A MTX-based chemotherapy, in combination with temozolamide is recommended.[9]

PCNSL is a highly aggressive lymphoma with a poor prognosis. Various prognostic parameters have been studied in PCNSL. In an international large multicentre study in immunocompetent patients, age, performance status, LDH serum level, CSF protein concentration, and involvement of deep structures of the brain were independent predictors of survival.[21]

In the largest polychemotherapy trial including MTX as the most efficient cytostatic drug, the Bonn protocol achieved a median overall survival of 50 months, with the best treatment results in patients younger than 61 years (5 year survival: 75%).[22]

The search for tissue biomarkers of prognosis for patients with PCNSL is an active area of investigation. p53 and c-Myc expression were associated with poor outcome in one study.[23] However, overexpression of BCL6 has shown conflicting results with regard to prognosis in different studies.[23,17] Increased expression of STAT6, a mediator of IL-4 signalling, is associated with a shorter survival in patients treated with MTX.[24]

CONCLUSION: Primary CNS lymphomas are high-grade diffuse large B-cell lymphomas with poor outcome. Both the GCB and non-GCB subtypes have high Ki-67 proliferative index with frequent overexpression of p53. Since immunocompetent patients can tolerate systemic therapy better than immunocompromised patients, it is essential to achieve a better understanding of the genetic landscape of this disease so that targeted therapies can be developed with the aim of cure.

REFERENCES:

1. Deckert M, Paulus W. Malignant lymphomas. In: Louis DN, Ohgaki H, Wiestler OD, et al. eds. WHO classification of tumours of central nervous system. Lyon, France: IARC 2007;4th ed:p188-192.
2. Kluin PC, Deckert M, Ferry JA. Primary diffuse large B-cell lymphoma of the CNS. In: Swerdlow S, Campo E, Harris NL, et al. eds. WHO classification of tumours of haematopoietic and lymphoid tissue. Lyon, France: IARC 2008; 4th ed:p240-241.
3. Bhagavathi S, Wilson JD. Primary central nervous system lymphoma. Arch Pathol Lab Med 2008;132:1830-1834.
4. Hans CP, Weisenburger DD, Greiner TC, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 2004;103(1):275-282.
5. Miller DC, Hochberg FH, Harris NL, et al. Pathology with clinical correlations of primary central nervous system non-Hodgkin's lymphoma: the Massachusetts general hospital experience 1958-1989. Cancer 1994;74(4):1383-1397.
6. Ostrom QT, Gittleman H, Fulop J, et al. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the united states in 2008-2012. Neuro Oncol 2015;17(4):iv1-iv62.
7. Sarkar C, Sharma MC, Deb P, et al. Primary central nervous system lymphoma – a hospital based study of incidence and clinicopathological features from India (1980-2003). J Neurooncol 2005;71(2):199-204.
8. Powari M, Radotra B, Das A, et al. A study of primary central nervous system lymphoma in northern India. Surg Neurol 2002;57(2):113-116.
9. Schlegel U. Primary CNS lymphoma. Ther Adv Neurol Disord 2009;2(2):93-104.
10. Gerstner ER, Batchelor TT. Primary central system lymphoma. Arch Neurol 2010;67(3):291-307.
11. Abrey LE, Batchelor TT, Ferreri AJ, et al. International primary CNS lymphoma collaborative group. Report of an international workshop to standardize baseline evaluation and response criteria for primary CNS lymphoma. J Clin Oncol 2005;23(22):5034-5043.
12. Singh G, Driever PH, Sander JW. Cancer risk in people with epilepsy: the role of antiepileptic drugs. Brain 2005;128(pt 1):7-17.

13. Eichler AF, Batchelor TT. Primary central nervous system lymphoma: presentation, diagnosis and staging. Neurosurg Focus 2006;21(5):E15.
14. Giannini C, Dogan A, Salomao DR. CNS lymphoma: a practical diagnostic approach. J Neuropathol Exp Neurol 2014;73(6):478-494.
15. Commins DL. Pathology of primary central nervous system lymphoma. Neurosurg Focus 2006;21(5):E2.
16. Boonstra R, Koning A, Mastik M, et al. Analysis of chromosomal copy number changes and oncoprotein expression in primary central nervous system lymphomas: frequent loss of chromosome arm 6q. Virchows Arch 2003;44(3):164-169.
17. Braaten KM, Betensky RA, de Leval L, et al. BCL-6 expression predicts improved survival in patients with primary central nervous system lymphoma. Clin Cancer Res 2002;9:1063-1069.
18. Montesinos-Rongen M, Brunn A, Bentink S, et al. Gene expression profiling suggests primary central nervous system lymphomas to be derived from a late germinal center B cell. Leukemia 2008;22(2):400-405.
19. Bergmann M, Blasius S, Bankfalvi A, et al. Primary non-Hodgkin lymphomas of the CNS -proliferation, oncoproteins and Epstein-Barr-virus. Gen Diagn Pathol 1996;141:235-242.
20. Ponzoni M, Berger F, Chassagne-Clement C, et al. Reactive perivascular T-cell infiltrate predicts survival in primary central nervous system B-cell lymphomas. Br J Haematol 2007;138(3):316-323.
21. Ferreri AJM, Blay JY, Reni M, et al. Prognostic scoring system for primary CNS lymphomas: the international extranodal lymphoma study group experience. J Clin Oncol 2003:21(2);266-272.
22. Pels H, Schmidt-Wolf IG, Glasmacher A, et al. Primary central nervous system lymphoma: results of a pilot study and phase II study of systemic and intraventricular chemotherapy with deferred radiotherapy. J Clin Oncol 2003;21(24):4489-4495.
23. Chang CC, Kampalath B, Schultz C, et al. Expression of p53, c-Myc, or Bcl-6 suggests a poor prognosis in primary central nervous system diffuse large B-cell lymphoma among immunocompetent individuals. Arch Pathol Lab Med 2003;127(2):208-212.
24. Rubenstein JL, Fridlyand J, Shen A, et al. Gene expression and angiotropism in primary CNS lymphoma. Blood 2006;107(9):3716-3723.