

# Squash cytology of primary central nervous system lymphoma in an immunocompetent patient

Aneel Myageri, Vandana U.G., Sateesh Melkundi<sup>1</sup>, Preetam B. Patil<sup>2</sup>, Padmaja Kulkarni

Departments of Pathology, <sup>1</sup>Neurosurgery, and <sup>2</sup>Radiology, SDM College of Medical Sciences and Hospital, Sattur, Dharwad, Karnataka, India

## ABSTRACT

The diagnosis of primary central nervous system lymphoma (PCNSL) can be suggested by magnetic resonance imaging (MRI). Cerebral PCNSL in immunocompetent patients generally shows marked homogeneous contrast enhancement in MRI. However, confirmation of it requires demonstration of lymphoma cells in stereotactic biopsy of the lesion or in cerebrospinal fluid. In the present case, with radiologic diagnosis of glioma, complete resection of the lesion by micro-neurosurgical technique was performed. The tumor tissue was examined by squash smear, frozen section and paraffin sections. Cytologic appreciation of tumor cells as lymphoid origin in squash smears negated the other possibilities. The immunocytochemistry on squash smear and immunohistochemistry on paraffin sections confirmed the diagnosis of diffuse large B cell lymphoma of non-germinal center B cell phenotype.

**Key words:** Immunocytochemistry, primary central nervous system lymphoma, squash smear

## Introductions

Primary central nervous system lymphoma (PCNSL), a rare malignancy of the craniospinal axis, is fairly common in immunocompromised patients. It is also seen in immunocompetent individuals. The symptoms of PCNSL may be indistinguishable from other CNS tumors. The post-contrast characteristic enhancement in magnetic resonance imaging (MRI) of the lesion, which usually is in supratentorial periventricular location, would indicate the possibility of PCNSL. Confirmation of PCNSL however relies upon biopsy features of the lesion and ruling out previous or concurrent non-Hodgkin lymphoma or leukemia elsewhere.

We report an immunocompetent patient with PCNSL, which was diagnosed by squash cytology and later confirmed by immunohistochemistry on squash smear and paraffin sections.

## Case Report

This was a case report of a 38-year-old male patient who was admitted to neurosurgery with a history of headache and neck pain since 2 months. No history of antecedent head trauma, febrile illness, photophobia, seizures or previous diagnosis of any malignancy was present. He didn't have lymphadenopathy or hepatosplenomegaly. Neurologic examination was not revelatory as no abnormality was found. His peripheral smear examination was normal. He was negative for human immunodeficiency virus (HIV).

MRI of the brain revealed a well-defined lobulated focal mass measuring 4.5 cm × 3.6 cm × 3.5 cm with extensive adjacent white matter edema in right parieto-occipital lobe causing mass effect on right lateral and third ventricle and basal ganglia region with contralateral midline shift of 9 mm. On T1-weighted images mass was isointense to gray matter [Figure 1a]. On T2-weighted and fluid-attenuated inversion recovery images, it appeared iso to mildly hyperintense [Figure 1b]. There was mild restricted diffusion. The lesion showed intense homogeneous enhancement on post contrast study [Figure 1c and d]. Small area of central necrosis was seen within the mass. MR spectroscopy revealed an elevated choline and lipid lactate. MRI findings were concluded as glioma.

Patient underwent craniotomy for micro-neurosurgical complete resection by aspiration of the suspected glioma. Squash preparations were made from tissue received for frozen section and stained with hematoxylin and eosin.

Access this article online	
Quick Response Code:	Website: www.asianjns.org
	DOI: 10.4103/1793-5482.125673

### Address for correspondence:

Dr. Aneel Myageri, 613, Manya Towers, First Floor, SDM Medical Staff Quarters, Sattur, Dharwad - 580 009, Karnataka, India.  
E-mail: leenamyaageri@gmail.com

Monomorphic lymphoid tumor cells were seen without any fibrillary background [Figure 2a]. The cells had large nuclei with prominent nucleoli and scant to moderate cytoplasm. Further, lymphoid cells having a blast like appearance with cytoplasmic vacuoles were recognized better on Leishman stain [Figure 2b]. Tingible body macrophages and lymphoglandular bodies were seen. The frozen sections showed the tumor cells in sheets, but the cytologic details were not clear.

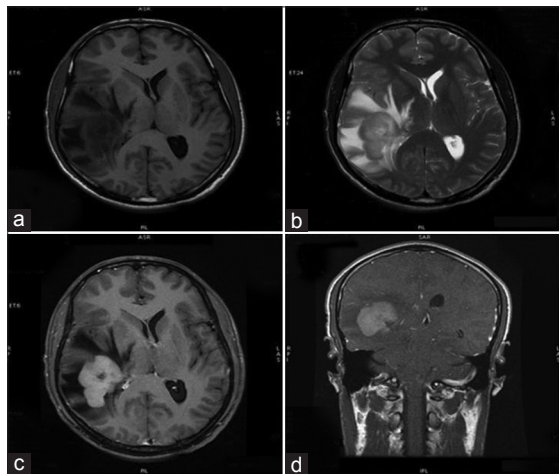
With intraoperative diagnosis of lymphoma infiltration on squash smears, on table bone marrow aspiration was done from the sternum to rule out hemopoietic malignancy in the marrow. The bone marrow smears showed normal marrow cells.

A squash unstained smear was further studied with immunocytochemistry for CD45 (with a satisfactory positive

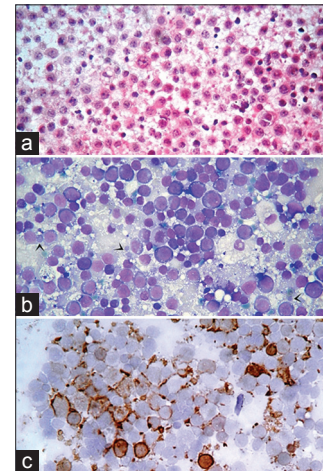
control). The smears showed cytoplasmic CD45 positivity in intact tumor cells [Figure 2c].

The paraffin sections revealed the typical lymphoma cells displaying perivascular pattern and invading the brain [Figure 3a and c]. The starry sky appearance was also seen [Figure 3b]. The diagnosis of PCNSL was conferred. The immunohistochemical studies showed CD20 positivity [Figure 3d] in lymphoma cells while they were negative for CD3, CD10, Bcl2, Bcl6 [Figure 4a-d] and CD99. CD3 positive reactive T cells were seen amidst the lymphoma cells [Figure 4a]. The negativity of lymphoma cells for CD10, Bcl2, Bcl6 and positivity to CD20 indicate the non-germinal center B-cell phenotype.

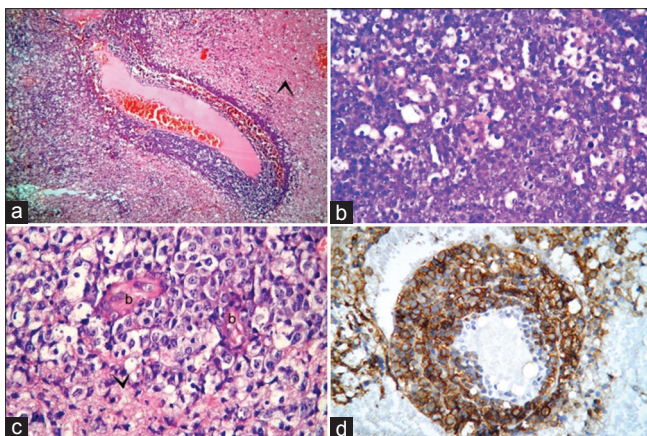
The final diagnosis of diffuse large B cell primary CNS lymphoma (DLBCL) was given. Post-operative contrast CT showed complete removal of the lesion and the patient



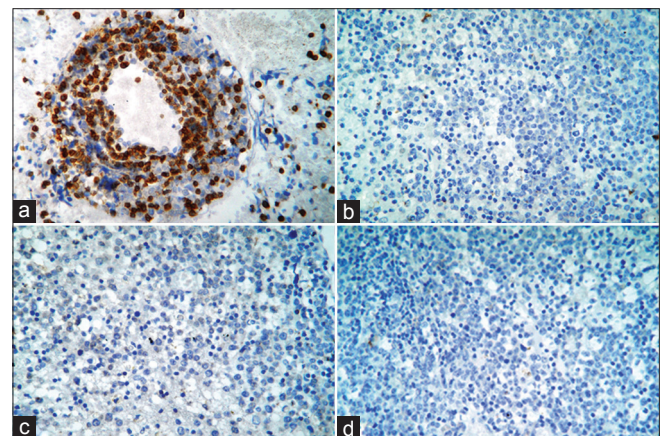
**Figure 1:** (a) Axial T1-W and (b) T2-W magnetic resonance imaging images showing solid mass in the right parieto-occipital region with surrounding edema. (c) Post contrast axial and (d) coronal images showing intense homogenous enhancement of mass



**Figure 2:** (a) Discohesive monomorphic lymphoma cells without fibrillary background (squash smear, H and E, ×40). (b) Monomorphic lymphoma cells in sheets with cytoplasmic vacuole and lymphoglandular bodies (arrow heads) (squash smear, Leishman, ×40). (c) Positivity for CD 45 in the cytoplasm of lymphoma cells (squash smear, ×40)



**Figure 3:** Paraffin sections showing lymphoma cells (a) in perivascular pattern and invading brain tissue (arrow head). (b) With a starry sky appearance. (c) Around the blood vessel (b) invading into adjacent brain tissue (arrow head). (d) Positive for CD20



**Figure 4:** Immunohistochemistry studies showing lymphoma cells. (a) Negative for CD3 while reactive T lymphocytes are CD3 positive. (b) Negative for CD10. (c) Negative for Bcl2. (d) Negative for Bcl6



received chemo and radiotherapy. MRI after 2½ years follow-up showed no evidence of recurrence.

## Discussion

PCNSL is rare extranodal lymphoma seen in both immunocompetent and immunocompromised patients especially those with HIV infection. The age in immunocompetent patients ranges from 2<sup>nd</sup> to 9<sup>th</sup> decade with most patients above 60 years.<sup>[1,2]</sup> Sarkar C and coauthors reported that in India, incidence of PCNSL is stabilized since two decades and also patients of PCNSL are decade younger compared with the western patients.<sup>[3]</sup> The incidence of PCNSL is declining in the HIV affected patients probably due to effective antiviral treatment<sup>[1]</sup> or as in India possibly due to early death by opportunistic infections.<sup>[3]</sup> Patients show variable symptoms but the symptoms of raised intracranial pressure and personality changes are more frequent.<sup>[2]</sup> Occurrence of seizures is infrequent because of deep location of the lesion in the brain.<sup>[1]</sup> In our study the patient was 38-year-old and had symptoms and signs suggestive of raised intracranial tension. Most PCNSLs are supratentorial periventricular cerebral lesions. On T2-weighted MRI images the lesions appear hypointense to isointense relative to gray matter.<sup>[4]</sup> This is because of high cellularity in lymphoma.<sup>[5]</sup> Herrlinger *et al.* opine that such clinical symptoms and radiologic findings are suggestive, but not conclusive of PCNSL.<sup>[2]</sup> The lesion can simulate radiologically inflammatory/infectious diseases, primary and metastatic malignancies.<sup>[1]</sup> According to Elder JB and Chen TC stress that enhancement along Virchow-Robin spaces is considered a highly specific feature of PCNSL.<sup>[6]</sup> However biopsy is required to confirm the diagnosis.<sup>[7,8]</sup> Resection of the tumor is not an accepted surgical procedure for PCNSL, because it may increase the risk of spread and the lesions are infiltrative.<sup>[6,9]</sup> Sierra del Rio *et al.* suggest that PCNSL is extremely sensitive to radio and chemotherapies and biopsy only is enough for pathologic diagnosis.<sup>[1]</sup> In our case, the patient underwent complete resection by micro-neurosurgical technique without any neurological deficits. The reason for not performing the stereotactic biopsy was that the MRI studies were suggestive of the lesion could be a glioma; the lesion was solitary and was amenable for resection; the patient was immune-competent and lymphoma was not the first diagnosis. Alécio-Mattei *et al.* recommend surgery as the gold standard therapy for early excision combined with post-operative radiotherapy.<sup>[8]</sup>

Typical discohesive lymphoma cells with lymphoglandular bodies without fibrillary background on squash smears would help to identify the lesion as hematopoietic origin.<sup>[10]</sup> Ruling out of hematopoietic malignancy in bone marrow, lymphnodes and other organs is necessary to diagnose PCNSL. Post contrast typical enhancement of lesion on MRI in supratentorial periventricular area correlated with the cyto-histologic diagnosis of primary brain lymphoma. Starry sky appearance, monomorphic large tumor cells in sheets

with scant cytoplasm and multiple nucleoli and perivascular pattern with no malignant lymphoid cells within the vascular lumen on paraffin sections supported the diagnosis of primary brain lymphoma. Starry sky appearance in paraffin sections in DLBCL has been documented by Makhdoomi *et al.*<sup>[11]</sup> In a recent article by Yamanaka it is concluded that most PCNSLs belong to DLBCL.<sup>[12]</sup> The tumor cells were positive for CD45 on squash smear. Paraffin sections showed tumor cells positive for CD45, CD20 and negative for CD3, CD10, Bcl2, Bcl6 and CD99, which confirmed the diagnosis of DLBCL of non-germinal center cell origin. Ferry JA summarizes that germinal center B cell phenotype (CD10+, bcl6+ or CD 10-, bcl6+, MUM1-) form a minority in most PCNSLs.<sup>[13]</sup> Bcl2 over-expression in lymphoma cells indicates germinal center cell origin. Bcl2 over expression should be studied by immunohistochemistry as t(14; 18) (q32; q21) may not be associated with over expression of the bcl2 protein.<sup>[14]</sup> High degree of suspicion is required to diagnose PCNSL when MRI shows characteristic features and necessitates the use of stereotactic biopsy. Use of CD45 immunocytochemistry marker on squash smear would help in further selection of markers to specify lymphoma.

## Acknowledgment

The authors would like to thank Dr. Laxmi Rao, Professor and HOD, Department of Pathology, Kasturba Medical College, Manipal, Karnataka, India for providing immunohistochemistry slides of the case.

## References

1. Sierra del Rio M, Rousseau A, Soussain C, Ricard D, Hoang-Xuan K. Primary CNS lymphoma in immunocompetent patients. *Oncologist* 2009;14:526-39.
2. Herrlinger U, Schabet M, Bitzer M, Petersen D, Krauseneck P. Primary central nervous system lymphoma: From clinical presentation to diagnosis. *J Neurooncol* 1999;43:219-26.
3. Sarkar C, Sharma MC, Deb P, Singh R, Santosh V, Shankar SK. Primary central nervous system lymphoma: A hospital based study of incidence and clinicopathological features from India (1980-2003). *J Neurooncol* 2005;71:199-204.
4. Koeller KK, Smirniotopoulos JG, Jones RV. Primary central nervous system lymphoma: Radiologic-pathologic correlation. *Radiographics* 1997;17:1497-526.
5. Haque S, Law M, Abrey LE, Young RJ. Imaging of lymphoma of the central nervous system, spine, and orbit. *Radiol Clin North Am* 2008;46:339-61.
6. Elder JB, Chen TC. Surgical interventions for primary central nervous system lymphoma. *Neurosurg Focus* 2006;21:E13.
7. Mi W, Mittal S. Primary lymphoma of the central nervous system in an immunocompetent patient. *Hosp Physician* 2001;34-8.
8. Alécio-Mattei T, Alécio-Mattei J, Aguiar PH, Ramina R. Primary central nervous system lymphomas in immunocompetent patients. *Neurocirugia (Astur)* 2006;17:46-53.
9. Eichler AF, Batchelor TT. Primary central nervous system lymphoma: Presentation, diagnosis and staging. *Neurosurg Focus* 2006;21:E15.
10. Joseph JT. Hematological tumors. In: *Diagnostic Neuropathology Smears*. Philadelphia: Lippincott Williams and Wilkins; 2006. p. 145-64.
11. Makhdoomi R, Nayil K, Rayees A, Kirmani A, Ramzan A, Khalil MB, *et al.* Primary CNS lymphoma in immunocompetent: A review of literature and our experience from Kashmir. *Turk Neurosurg* 2011;21:39-47.

12. Yamanaka R. Primary central nervous system lymphoma-Recent advance on clinical research. In: Lichtor T, editor. *Clinical Management and Evolving Novel Therapeutic Strategies for Patients with Brain Tumours*: InTech; 2013. Available from: <http://www.intechopen.com/books/clinical-management-and-evolving-novel-therapeutic-strategies-for-patients-with-brain-tumours/primary-central-nervous-system-lymphoma-recent-advance-on-clinical-research> [Last accessed on 2013 May 10].
13. Ferry JA. Lymphomas of the nervous system and the meninges. In: Ferry JA, editor. *Extranodal Lymphomas*. Philadelphia: Elsevier Saunders; 2011. p. 7-33.
14. Buccoliero AM, Castiglione F, Caldarella A, Rossi Degl'Innocenti D, Taddei A, Ammannati F, *et al.* BCL-2 in primary central nervous system lymphomas. *Immunohistochemistry and molecular biology. Pathologica* 2004;96:425-9.

**How to cite this article:** Myageri A, G VU, Melkundi S, Patil PB, Kulkarni P. Squash cytology of primary central nervous system lymphoma in an immunocompetent patient. *Asian J Neurosurg* 2013;8:195-8.

**Source of Support:** Nil, **Conflict of Interest:** None declared.