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Original Research Article

Prognostic significance of immunohistochemical markers in Primary CNS lymphomas

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ABSTRACT

Introduction: Primary CNS lymphoma (PCNSL) is a rare form of extra nodal Non-Hodgkin Lymphoma that is typically confined to the brain, spinal cord, lepto meninges and eyes. We studied the clinico pathological features of PCNSLs, immuno histochemical (IHC) markers expressed and the association of morphological features & IHC markers with clinical outcome.

Materials and Methods: 30 cases of primary CNS lymphomas were studied. 25 cases were diffuse large B cell lymphomas, which were sub classified using Hans algorithm into GCB and non-GCB. The IHC markers done were CD20, CD3, BCL2, BCL6, MUM-1, CD10 and c-myc. Mean proliferation index Ki was 80%. Follow up and survival data was collected and the association of each IHC marker and subtype with prognosis was assessed.

Conclusion: PCNSL forms around 2% of all lymphomas as well as primary CNS tumours. Non GCB type is more common (72%). Mean overall survival was 9.7 months. Ki-67 index of 80% or more is the only independent variable of prognostic significance. None of the other IHC markers or sub typing had any influence on the outcome.

1. Introduction

Primary CNS lymphoma (PCNSL) is a rare form of extra nodal Non-Hodgkin Lymphoma that is typically confined to the brain, spinal cord, leptomeninges and eyes. Lymphomas of the dura, intravascular large B cell lymphoma, lymphoma with evidence of systemic disease or secondary lymphomas and all immunodeficiency associated lymphomas are excluded.[1]

The majority of PCNSLs in immunocompetent patients are diffuse large B-cell lymphomas (DLBCL), which are histologically indistinguishable from DLBCLs of peripheral nodal origin (NL). However, the clinical course and prognosis of PCNSLs are quite different from those of NLs, and the biological difference between these two entities has not been clearly defined.[2]

The cellular origin of PCNSLs is uncertain, however, results of a few studies suggest a germinal center or post-germinal centre origin for the majority of PCNSLs. This can be identified by immunohistochemical (IHC) assay on paraffin sections using CD10, BCL6 and MUM1 which show variable expression. Even though PCNSLs represent stage-I extra nodal disease by definition, these markers can also be of the prognostic significance.[3]

The prognosis of patients with PCNSLs has improved during the last decade with the introduction of high dose methotrexate. However after treatment, prognosis is good

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in only half of the patients and late neurotoxicity is also possible.[4,5] Hence prognostic markers, both morphological & IHC need to be defined. This study attempts at finding out these possible factors.

Hence we attempted to study the clinico pathological features of PCNSLs, immunohistochemical markers expressed in them and to find out the association of morphological features & IHC markers with clinical outcome.

2. Materials and Methods

This is a retrospective analytical study of PCNSLs diagnosed at Department of Pathology over a period of 7 years (Jan 2012- Dec 2018). A total of 30 cases of PCNSL were identified. All patients satisfying the WHO definition of PCNSLs were included. Those with evidence of systemic disease, secondary lymphomas and immunodeficiency associated lymphomas were excluded.

Medical records of these patients were reviewed and their demographic (age, sex); clinical (presenting symptoms, location, & follow up); histopathological features (type, subtype & IHC markers) were tabulated and analyzed for the results.

Four microns (haematoxylin and eosin stained) sections of all the cases were reviewed. IHC markers done were CD20 (Clone L26, Monoclonal mouse anti-human CD20cy, BGX), CD3 (Monoclonal mouse anti-human CD3, BGX), Ki 67 (Clone MIB-1, Monoclonal mouse anti-human Ki-67 antigen, BGX). CD10 (GCB marker; liquid mouse monoclonal antibody, Clone 56C6, BGX), BCL-6 (GCB marker; liquid mouse monoclonal antibody, Clone PG B6p, Dako); MUM-1 (non-GCB marker; monoclonal mouse anti-human MUM1 protein, Dako). BCL2 (mouse monoclonal antibody; Clone BCL2/100, BGX), c-Myc (Rabbit monoclonal antibody; clone Ep121, Path insitu).

Immuno staining was performed by recommended protocol of HRP/DAB IHC detection kit. This protocol includes de paraffinisation and hydration of tissue, unmasking of antigens by pre-treatment solution, peroxide block, protein block, incubation of slides with primary antibody, addition of polymer HRP antibody, addition of DAB substrate solution, counterstaining with haematoxylin, dehydration of tissue and mounting.

For CD10, BCL-6 and MUM-1, positive expression was defined as positive staining in more than or equal to 30% of tumour cells. For CD10, only membrane and cytoplasmic staining were considered positive, and in the case of BCL2, BCL-6 and MUM-1, only diffuse or granular nuclear staining was considered positive;[1] CD10, BCL-6, and MUM-1 expression results were used to sub classify DLBCL cases into GCB or non-GCB subgroups as described below using Hans algorithm.[6] c-myc was reported positive when 40% of cells showed nuclear positivity. Ki 67 index was calculated in each case by counting the positively stained nuclei in 10 HPF in areas showing maximum count and taking an average of the count.

All standard protocols were followed for the diagnosis and treatment with informed consent obtained from all these patients (after Institutional Review Board approval).

2.1. Statistical analysis

Overall survival (OS) was defined as the time from study entry (time of biopsy) to death. OS was estimated by the Kaplan–Meier method. Prognostic significance of immunohistochemical markers with survival of patients was analyzed by the Fisher Exact test. Group comparison(patients with less than or equal to 12 months follow up expired and patients with more than 12 months follow up alive)was carried out using the log-rank test., of significance was 0.05. Commercially available software was used (SPSS for Windows, release 20.0). Mean values were compared using student's t test.

3. Results

A total of 30 cases of PCNSL were identified during the 7 years study period. The mean age was 59 years (range: 36–77 years) and male: female (M: F) ratio 17/13= 1.3:1. The mean duration of symptoms was 10 weeks (range: 2-30weeks). Among all 30 patients, brain was the most common location of the tumour (17; 56.6%), followed by spinal cord (10; 33.3%) and orbit (3: 10%). About 82.3% were supra tentorial, 1 was in the cerebellum and 2 were in periventricular location. Cognitive impairments and headache were most common symptoms in cases of brain involvement. The common symptoms in cases of spinal cord and eye involvement were back ache and blurring of vision respectively.

Histological review of slides identified 25 cases (83.3%) of DLBCL, 2 cases (6.6%) of follicular lymphoma, and 1 case each of marginal zone lymphoma, mantle cell lymphoma and anaplastic large cell lymphoma ALK positive. This constituted 2.1% of all lymphomas (total 1420) and 2.01% of brain tumours (total 1490).

We analysed the clinical, pathological and IHC features of all the 25 DLBCL patients while the minimum sample size obtained from a previous study was 21.[7]

Morphologically DLBCL was composed of cohesive clusters of atypical lymphoid cells arranged in sheets and in an angiocentric pattern with brisk mitotic activity and frequent apoptotic figures with areas of necrosis. The cells were large with round to oval nuclei containing one to two distinct nucleoli and moderate amounts of eosinophilic to vacuolated cytoplasm (Figure 1). All cases of DLBCL were positive for CD20. Few reactive CD3 positive T-cells were seen in the background. The cases were further sub classified using Hans algorithm into GCB (7; 28%)

and non-GCB (18; 72%) groups with additional markers: MUM-1(Figure 2), BCL6 and CD10, (Figure 3). BCL6 was positive in 50% of cases. c-myc was positive in 24% cases and BCL-2 in 84% cases. The proliferative index, Ki 67 ranged from 40-90 %, with a mean of 80% (Figure 4).

The follow-up information including treatment details and prognosis of all DLBCL patients was retrieved using medical records and tabulated for statistical analysis. To study the impact of age on the prognosis, all DLBCL cases were divided into two groups: Age<60 (n=11) & Age ≥60 years (n=14). No significant difference was noted in their median overall survival (8 months & 6 months respectively, p=0.81). The follow up period ranged from 6 to 84 months. Median overall survival of DLBCL cases was 9.7 months. Out of 25, 5 patients lost to follow up, 10 expired and 10 were doing well till the last visit. Out of 10 expired patients; 1 was GCB subtype and 9 were non GCB subtype. No statistical difference in the overall survival was noted among GCB (7months) and non GCB subtypes (6months).

14 patients received chemotherapy (CT), 5 received combined chemo-radiotherapy (CTRT) and 6 patients received only radiation therapy (RT) due to advanced age or co morbidities. No significant survival benefit was noted among these treatment groups.

Prognostic significance of different IHC markers was evaluated using chi square test. Only high Ki67 index (above or equal to 80%) was found to be statistically significant in predicting poor survival (p=0.002).

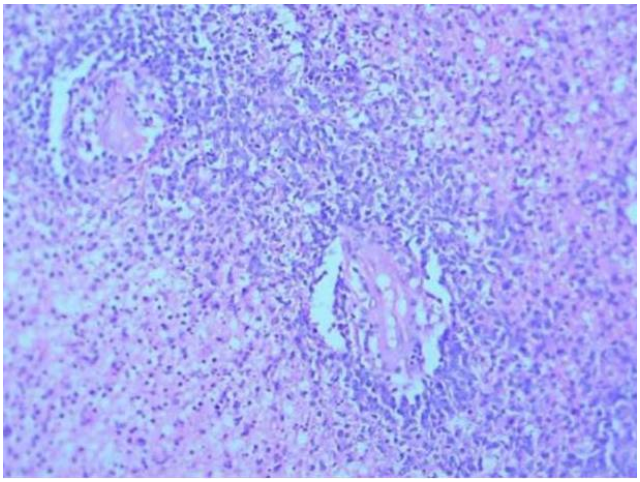

Fig. 1: Perivascular lymphoid infiltrate-H&E X20

4. Discussion

This study investigated the clinical features, immunoprofile, follow up and prognosis of PCNSL in a tertiary care centre in South India. The clinical profile of patients in our study is almost similar to as reported by previous studies from India in comparison to the Western studies. All the patients

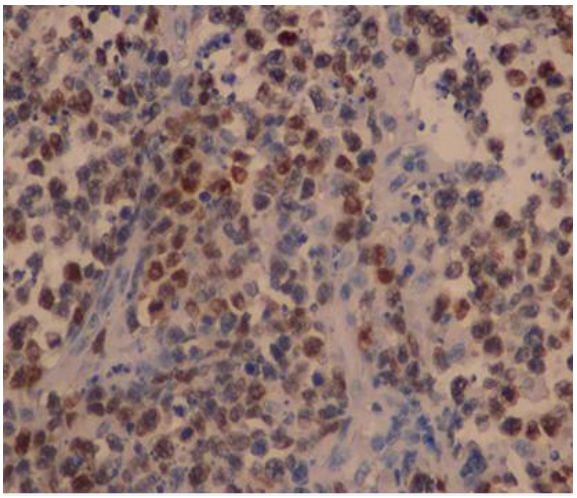

Fig. 2: MUM-1 IHCX40

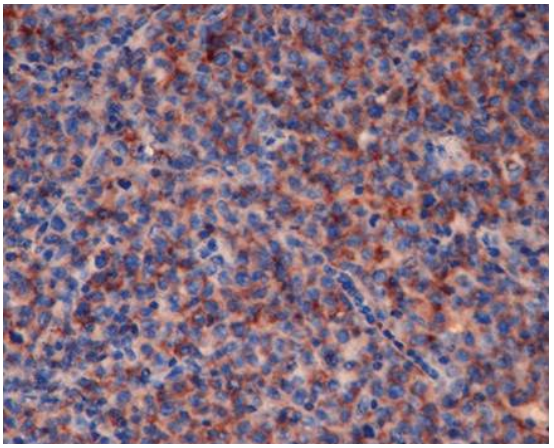

Fig. 3: CD10 IHCX40

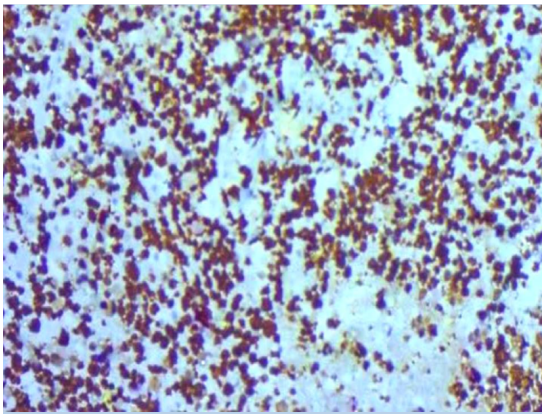

Fig. 4: IHC Ki 67 Neoplastic cells showing positivity

Table 1: Studies showing the distribution of cases in GCB andnon GCB subtypes of primary CNS lymphomas.

Study group (year)	Country	Algorithm used	Total no of cases	n%	
				GCB	Non GCB
Bimal et al2015	India	Hans	51	18(35.3)	33(64.7)
Stephan et al2017	Switzerland	Hans	119	29(26.6)	80(79)
Aki et al2013	Turkey	Hans	39	6(15.4)	33(84.6)
Mahadevan et al 2015	India	Hans and Chang	24	-	22(91.6)
Bhagavathi et al. 2008	USA	Hans	21	2 (9.5)	19 (90.5)
Cheng et al.2008	China	Chang	47	4 (8.5)	43 (91.5)
Hattab et al.2010	USA	Hans	31	5 (16.0)	26 (84.0)
Levy et al.2008	USA	Hans	38	5 (13.0)	33 (87.0)
Lin et al.2006	Taiwan	Hans	51	11(21.6)	40(78.4)
Raoux et al.2010	France	Hans	39	13 (25.7)	26 (74.3)
Kinoshita et al. 2010	Japan	Hans	29	8 (27.6)	21 (72.4)
Broet et al.2006 France	France	Hans and Chang	82	3 (3.7)	79 (96.3)
Momota et al.2010	Japan	Hans and Chang	23	1(4.4)	22(95.6)
Present Study	India	Hans	25	7(28)	18(72)

in the present study were immunocompetent. Similar to reports in the literature, cognitive impairment and headache were most common symptoms in our series. The majority (82.3%) of lesions were supratentorial in location similar to the other studies.[8] As described in the literature[9] we also found DLBCL (83.3%) to be the most common histological type involving the cerebral parenchyma. Other types of lymphomas are rare in the central nervous system.[10]

Several studies have shown that age as important independent variable in predicting the prognosis of DLBCL.[11] However in our study, we didn't find any prognostic significance of age as also noted in another study.[12]

There are several algorithms mentioned in the literature for sub typing PCNSLs[6,13] of which Hans algorithm appears the most frequently used algorithm. Algorithm described by Chang's additionally use CD138.[13] However, expression of CD138 is rare in PCNSL and hence not employed by several studies.[3,12] We too deferred using CD138 for sub typing.

The studies comparing the subtypes of DLBCL of central nervous system in the Western and Asian population had shown that non-GCB type was the most common subtype of DLBCL in both populations, however in varying proportions [table-1]. In the western cohorts, the incidence of the non-GCB type ranged from 84 to 96% of cases and the GCB subtype accounted for 4-25% of cases.[14] In the present study, though the non GCB subtype was more common (72%), the proportion of GCB subtype was significantly more (28%) as compared to the western population. Similarly, other studies from Asian subcontinent also had more than 20% cases of GCB subtype.

It is observed that, the GCB subtype has a better prognosis as compared to the non-GCB subtype.[15] The slightly more proportion of GCB subtype in the Asian population, may imply that PCNSL in this population has a better survival and would benefit from more aggressive therapy. However, in the present study, we did not find any significant difference in the survival between both the subtypes on analysis despite the aggressive therapy.

The Ki proliferative index represents the active growth fraction of the tumour and is a valuable immunohistochemical marker to distinguish indolent from aggressive lymphomas, particularly in small needle biopsies where exact typing may not be possible. We observed that high Ki expression is a predictor of poor prognosis in patients with PCNSL with cut off of 80%. We further analysed it by looking at the difference in the mean disease free survival (DFS). The mean DFS of patients with proliferation index less than 80% was 12.7 months whereas it was 6.6 months for those with Ki index above 80%. This also showed a significant difference (Students t test, p value 0.045)

In nodal DLBCL, a Ki 67 index of more than 70% has been shown to be associated with bad prognosis. Similarly the 3year survival was significantly lower in these patients with high proliferation.[16]

BCL-6 gene encoding a nuclear located Krüppel type zinc finger protein is expressed predominantly in normal GCB cells and may have an important role in regulating their differentiation. Its deregulated expression may contribute to lymphoma genesis and it is found to be rearranged in 30% of DLBCL. The CALGB 50202 study of the prospective G-PCNSL-SG1 trial found BCL-6 as an unfavourable prognostic biomarker in PCNSL.[17] We did not find any significant association between BCL-6 expression and overall survival in our study.

BCL-2, a proto-oncogene localized in mitochondria enhances cell survival by blocking apoptosis. Krogh-Jensen et al reported BCL-2 as non-significant indicator of prognosis of DLBCL, similar to our study.[18]

We did not find any association between c-myc expression and prognosis, which is in accordance with other studies done by Rubenstein and Kamraan Gill et al.[19]

CD10 is expressed in pre-B cells and germinal centre B cells and MUM-1 in post-GC cells.[20] In the present study, the expression of CD10 and MUM1 were not found to be correlating with prognosis, which is in accordance with previous analyses.[21] The percentage of CD10-positive tumours might have been low in these studies, as non GCB cases were more.

In our study, we also found a few rare types of lymphoma presenting as primary tumours of CNS. They included 2 cases of follicular lymphoma (spinal cord), 1 case each of mantle cell lymphoma (orbit), marginal zone lymphoma (orbit) and anaplastic large cell lymphoma ALK positive (brain). Among them, only the patient with mantle cell lymphoma expired after 9 months of diagnosis while others are doing well till the last follow up. Good prognosis of low-grade B-cell lymphomas of CNS is also reported by Taekyu Lim et al.[22]

Some studies have shown significant survival benefit of combined CTRT over CT or RT alone.[23] However in our study, no survival difference was noted in different treatment groups.

5. Conclusion

PCNSL is a rare and aggressive tumour, constituting around 2% of all lymphomas as well as primary CNS tumours. Although non GCB type is more common, GCB subtype contributed to slightly more proportion (28%) in our population when compared with western incidence. Median overall survival was 9 months. The proliferation marker, Ki-67 index of 80% or more was the only independent variable of prognostic significance. None of the other IHC markers or sub typing had any influence on the outcome.

6. Conflict of Interest

The authors declare that there are no conflicts of interest in this paper.

7. Source of Funding

None.

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