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OCT4 and Nestin Expression in the Microenvironment of Primary Central Nervous System Lymphomas

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ABSTRACT

Background: Angiogenesis is essential for the survival of neoplasms. Our aim was to describe the clinical profile of primary central nervous system lymphoma (PCNSL) patients at our institution and explore the immunohistochemical expression of OCT4 and nestin in the tumor microenvironment especially in relation to angiogenesis.

Materials and Methods: All cases of PCNSL from 2016 to 2022 were retrospectively studied, and clinical and radiological characteristics of the patients were obtained. Descriptive statistics were used.

Results: 26 cases were studied; 24 cases (92.3%) were B-cell lymphomas: 23 diffuse large B-cell, and one Burkitt lymphoma. 7.7 % were of T lineage. 13 women and 13 men, had age ranges between 33-71 years (mean 58.16 years). Three patients (12 %) had immunosuppression. Nestin staining revealed hypertrophic astrocytes forming patches about blood vessels with positive cytoplasmic staining in endothelium and pericytes (5-10% of the intra-tumor arterioles). These findings were seen in both B and T lymphomas. OCT4 nuclear expression was only observed in five large B-cell lymphomas and seemed to have relationship with mitoses/HPF (high power field).

Conclusion: The novel finding of endothelial, pericytes and hypertrophic astrocytes staining with nestin, points to the involvement of stem cells promoting angiogenesis as a result of a dialogue between neoplastic cells and vascular stem cells. OCT4 expression seems to have a relationship with cell proliferation whose clinical significance should be investigated in prospective studies.

Keywords: PCNSL; T cell Lymphoma; Nestin; OCT4; Angiogenesis

INTRODUCTION

Primary Central Nervous System Lymphoma (PCNSL) is an extra-nodal variant of Non-Hodgkin Lymphoma (NHL) confined to the brain, leptomeninges, eye, and spinal cord. It represents 4-7% of all intracranial neoplasms and has become the second most common malignant brain tumor in the United States, behind gliomas[1]. The main risk factor is an immunodeficiency state, either acquired (HIV, immunosuppressive therapy) or congenital[2]. In Anglo-Saxon series, 95-98% of LPSNC are of B lineage. In the Unites States, 1500 new cases are reported annually and mainly affect individuals in the fifth or sixth decade of life[3].

The development of cancer goes through different stages, and in this process, the interaction between the neoplasia and the non-neoplastic parenchyma plays an important role. Participation of the immune system and especially of the macrophage in the modification of the microenvironment in favor of neoplasia has been studied[4].

In lymphoma tissue, high micro-vessel density is associated with greater biological aggressiveness[5]. Furthermore, the expression of endothelial growth factor in lymphoma cells and of CD105 (endoglin) in intra-tumoral small vessels, have been reported, suggesting endothelial proliferation and angiogenesis[6].

In the adult individual, angiogenesis, is the process of generating new blood vessels through sprouting or splitting of preexisting vessels. Vascular-resident stem cells are present in all 3 layers of the vessel wall[7]. Therefore, we wanted to investigate the possible participation of stem cells in the angiogenesis process in the tumor microenvironment. We decided to use two stem cell-associated markers, Oct4 and nestin.

OCT4 is a transcription factor involved in maintenance of pluripotency in primitive stem cells and germ cells. OCT4 nuclear expression is a specific marker for central nervous system (CNS) germinomas. In 145 cases of diffuse large B-cell lymphomas (DLBCL), its nuclear expression was reported in 18% of testicular lymphomas (2/11) and in 4.5% of non-testicular lymphomas (6/134), including 2/6 (33%) primary CNS DLBCLs [8]. OCT 4 nuclear positivity has also been reported in 2/18 testicular DLBCL (11.1%)[8].

On the other hand, nestin is an intermediate filament component of the cytoskeleton initially described in neural stem cells (NSCs), but whose expression has also been demonstrated in different types of progenitor cells[9]. It is also a biomarker of invasive and infiltrative phenotype, and is related to angiogenesis in several malignant tumors[10].

The aim of this work was to characterize the population of patients with PCNSL in a referral institution for neurological patients, and to investigate the expression of two stem cell markers, OCT-4 and nestin, in the tumor microenvironment of PCNSL, especially in relation to angiogenesis.

MATERIALS AND METHODS

The study was carried out in a national neurological reference hospital of the Ministry of Health. The files of the Department of Neuropathology from January 2016 to December 2022, and the corresponding clinical records, were reviewed. Cases of CNS lymphomas with histological slides and paraffin blocks were selected. The histological diagnosis was confirmed. An immunohistochemical panel was applied: CD45 (Common Leukocyte Antigen) (PDM009, Diagnostic BioSystems, The Hague, The Netherlands), CD3 (PDM 126, Diagnostic BioSystems, The Hague, The Netherlands), CD20 (AM537, BioGenex, Fremont, California., USA), OCT-4 (Anti-Human OCT4, EP143, AN724-10M, Biogenex), and Nestin (SC-21248, Santa Cruz Biotechnology Inc. Dallas, Texas, USA). The technique described by the providers was performed.

OCT 4. A positive staining was considered if 10% of the cells or more presented nuclear staining.

Nestin. All cytoplasmic staining was considered positive and the type and percentage of cells with positive staining were described, as well as its distribution in the tumor.

Ethical disclosure. Ethical approval was obtained from the Institutional Review Board & Ethics Committee (register number 66-22). Given that it was a retrospective study, in which personal data that allowed the identification of the patients was not handled, the Ethics Committee did not require informed consent. The study was performed in accordance with the principles stated in the Declaration of Helsinki.

Statistical analysis. Descriptive statistics were used. The statistical program SPSS version 20 was used.

RESULT

From 2016 to 2022, 4715 CNS surgical specimens were studied, of which, 36 were lymphomas. Ten cases were excluded: Seven cases were secondary CNS infiltration by systemic lymphoma; three cases of PCNSL were excluded because insufficient material was available for immunohistochemical analysis. Therefore, of a total of 29 primary CNS lymphomas, 26 were studied.

The patients, 13 of which were women and 13 of which were men, came from 11 different states/provinces in the country. Age range was 33-71 years (mean 58.16 years; median 59 years; SD 10.2 years).

Immunosuppression: Three patients (12 %), a man and two women were HIV+ and had large cell B-lymphoma. A woman also had hepatitis C virus

infection (HCV). Two patients had large solitary lesions (occipital and parietal lobes lesions). Patient with HCV infection showed multifocal cortico-subcortical lesions (Figure 1, A, B).

Pregnancy: A 33-year-old woman started with personality and behavior changes in the fifth month of pregnancy. A month later, she went to another hospital due to progressive right hemiparesis. At 26 weeks of gestation, she was referred to our hospital with a diagnosis of probable cerebral venous thrombosis. CT angiography showed white matter hypodensities in the frontal and parietal lobes. MRI showed confluent parenchymal lesions in the frontal and parietal lobes, basal ganglia and thalamus, with preservation of the cortical and subcortical fibers (Figure 1, C, D). At 32 weeks of gestation, she experienced premature rupture of membranes and required emergency caesarean section and intubation. Subsequently, a brain biopsy showed lymphoma and she was sent to a cancer hospital for treatment.

Tumor location: 24 tumors were supratentorial. Frontal lobe was the most frequently affected (nine cases, 34.6%); also, in three cases (11.5%), frontal lobe and other regions (parietal, insular, and temporal regions) were involved. Other locations included occipital and parietal lobes, and thalamic region. Two cases were located in cerebellum. The majority of the lesions showed by MRI a hypointense tumor on T1 and T2 sequences on MRI, with homogeneous uptake of the contrast material, which gave the appearance of a snowball image (Figure 1, E-F).

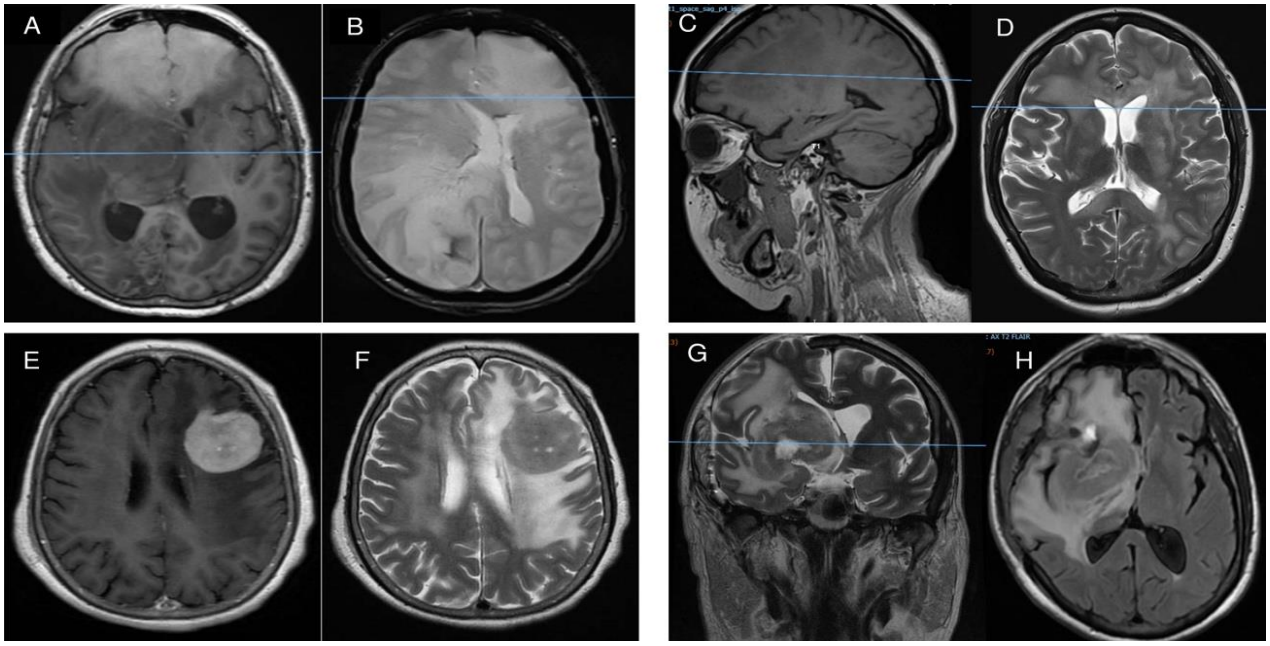

Figure 1. A-B. Patient with HIV+ & HCV infection showed multifocal cortico-subcortical lesions. C-D. 26 weeks of gestation woman with confluent parenchymal lesions in the frontal and parietal lobes, basal ganglia and thalamus. E-F. Typical snowball image. G-H. Patient diagnosed with cerebral vasculitis of 8 years of evolution who presented with right frontal and insular lesion

Time elapsed between onset of symptoms and hospital admission: The range was 1-96 months: One month (34.6%), two months (30.8%), three and six months (7.7% each one). However, a 46-year-old patient reported that 8 years earlier, he had started with repeated episodes of loss of vision, right hemicranial headache, and paresis of the right hand. At that time, according to the patient, the MRI study showed an image "compatible with neoplasia" and a brain biopsy was performed; the histological diagnosis was vasculitis. With this diagnosis, the patient was treated repeatedly over the years, with both, bolus and oral steroids, and saw improvement. Finally, he came to our hospital due to weakness of the pelvic limbs of two months of evolution, severe right hemicranial headache and paresis of the right thoracic limb that no longer responded to steroids. On admission, a right frontal and insular lesion was documented (Figure 1, G-H), corresponding to a T cell lymphoma.

Signs and symptoms: Almost all patients started with headache; however, reasons for consultation included: Severe headache (38.5%), motor and/or sensory disturbances (26.9%), seizures (15.4%), altered alertness (7.7%), hallucinations (7.7%), and vertigo (3.8%).

Histological analysis: All cases were positive for common leukocyte antigen stain (CD45). 24 cases were B-cell lymphomas, CD20+ (92%). 23 cases were diffuse large B-cell Non-Hodgkin Lymphoma, and one case was a Burkitt-type lymphoma. Large B cell lymphomas showed vesicular nucleus with notches, prominent nucleolus, clumpy chromatin, and scant cytoplasm. In 7 cases, perivascular reactive T lymphocytes (T-cell-rich lymphoma) were observed. In the other hand, T lymphomas were made up of small cells. Hyalinized blood vessels in the tumor were a frequent finding in both B and T lymphomas.

Nestin: All cases showed strong cytoplasmic staining in some large astrocytes. Despite the fact that, in the tumor area, GFAP staining showed a diffuse pattern of positive processes, surrounding clusters of tumor cells, and blood vessels (Figure 2, A); the nestin staining revealed few astrocytes with frank cytoplasmic positivity that made their radiated processes evident. (Figure 2, B). These hypertrophic astrocytes (Figure 2, C) formed patches in relation to blood vessels that presented also positive cytoplasmic staining in endothelium and pericytes (Figure 2, D-F). 5-10% of the intra-tumoral arterioles showed endothelial cells with strongly positive staining for nestin. Also, giant endothelial cells with vesicular oval nuclei were observed (Figure 2-F, arrows).

These findings were seen in both B and T lymphomas.

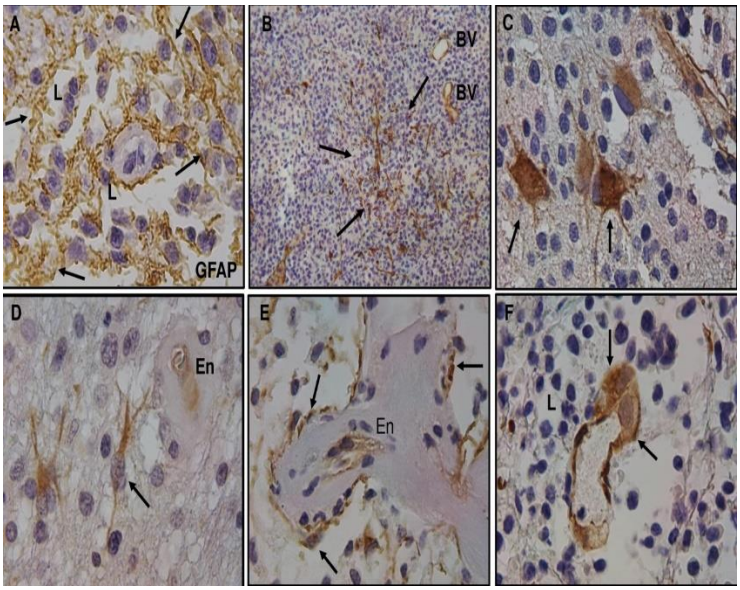

Figure 2. A. GFAP. An interconnected network of GFAP+ astrocytic processes is observed (arrows), surrounding clusters of lymphoid cells (L). B-F. Nestin. B. Patches (arrows) made up of hypertrophic astrocytes and blood vessels (BV). C. Hypertrophic astrocytes with radial processes (arrows). D. + Bipolar cell and endothelial cells (En). E. Numerous pericytes (arrows) and endothelial cells (En). F. Giant endothelial cells (arrows).

OCT 4: Brain parenchyma cells did not show OCT4 nuclear expression; this was only observed in five large B-cell lymphomas; expression was negative in Burkitt's lymphoma and also, in the two T-lymphomas.

In cases without OCT-4 nuclear expression (n=21), the range of mitoses/HPF was 2-5 mitoses, with a median of 2.0; this contrasted with the range of OCT4+ lymphomas, which had 2-8 mitoses/HPF with a median of 6 mitoses/HPF. However, the Mann Whitney test (24.50) showed a p=0.054.

Variability in Oct4 expression was observed. As described, 5 cases of B lymphoma showed nuclear

positivity (Figure 3, A). This nuclear expression was identified in 10-80% of the nuclei. In the other hand, in 6 cases, diffuse (Figure 3, C) or focal, condensed cytoplasmic positivity (Figure 3, D) was observed in 50-70% of the lymphoma cells including two of the cases with nuclear staining (Figure 3, B).

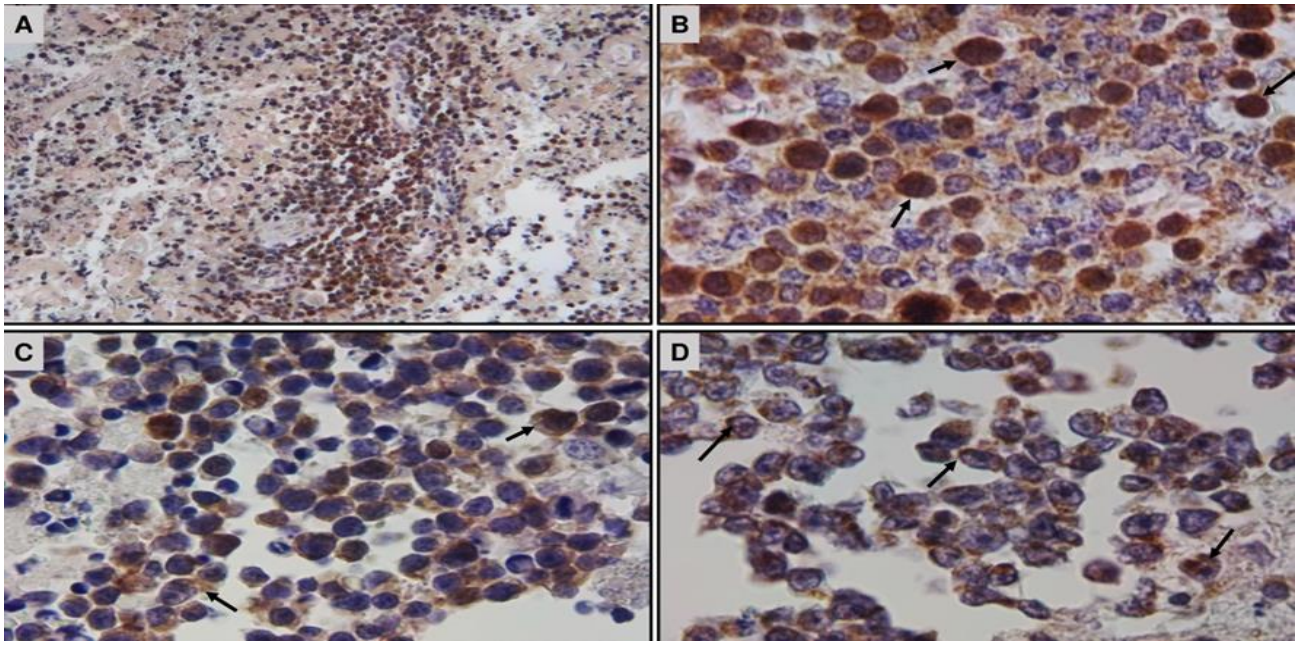

Figure 3. Oct 4. A. Positive nuclear staining in 80% of neoplastic cells. B. Lymphoma cells show both, nuclear and cytoplasm expression (arrows). C. Cells with diffuse cytoplasmic staining (arrows). D. Neoplastic cells with positive cytoplasmic bars (arrows)

Clinical evolution: Given the characteristics of our Institution, once the diagnosis has been obtained, patients with neoplastic problems are referred to the corresponding Cancer Institute for treatment; however, they remain with an open appointment for their review at any time, and a recommendation for a scheduled appointment once they finish their oncological treatment scheme. Of these patients, at least 8 (30.7%) died within 2-14 months of the onset of symptoms, with an average post-diagnosis survival of 5.25 months. Unfortunately, we do not have information on the rest of the patients.

DISCUSSION

In this sample of primary CNS lymphomas, 7.7 % were of T lineage. This percentage coincides with that reported in the literature, which estimates that T-lymphomas represent 2-8.5% of primary CNS lymphomas. In our country, there are no extensive population studies of PSNCL. Generally, the studies are case series in concentration hospitals, in which T-lymphomas have been reported from zero[11] to a maximum of 19% in the northeast region of the country[12]. In the present study, as expected, predominance of large B-cell lymphomas was observed, and also a Burkitt-type lymphoma was documented.

Only 12% of the cases were associated with HIV infection. Primary CNS lymphoma accounts for up to 15% of non-Hodgkin lymphomas in human immunodeficiency virus (HIV) patients, usually Epstein-Barr virus (EBV)-related[13].

The clinical and radiological findings were very similar to those described in the literature, although there were very interesting cases, such as that of the pregnant woman and the man with a previous history of cerebral vasculitis. However, the most novel findings were observed in the immunohistochemical study.

The presence of intra-tumoral vessels with nestin-positive giant endothelial cells, as well as nestin + pericytes and hypertrophic astrocytes forming

patches, suggests stem cell activation in relation to angiogenesis, probably induced by neoplastic cells. This finding had not been previously described in CNS lymphomas. It is known that stem/progenitor cells are located in the 3 layers of blood vessel walls. Physiologically, these cells participate in endothelial repair and regeneration[14]; in the tumor context, a small percentage (1-13%) of mesenchymal stem cells (MSCs) have been described in some CNS neoplasms, especially glioblastoma. The glioma associated MSCs [GA-MSCs] have MSC-like antigen profile CD105+/CD73+/ CD90+, but they do not co-express CD133 antigen, typical for cancer stem cells. Therefore, they are non-tumorigenic. However, in co-culture and *in vivo* GBM model-based experiments, GA-MSCs increased the aggressiveness of GSCs, stimulating their proliferation and self-renewal, thus acting pro-tumorigenically. It is known that tumors cannot grow beyond the size of 2-3mm^3 without forming new blood vessels[15]. Nestin, in addition to being a marker of neural stem cells, is also considered a marker of newly formed vessels[16]. In fact, in pulmonary arterial hypertension there is an uncontrolled proliferation of endothelial cells. These cells express Nestin. Nestin overexpression promotes proliferation and expression of angiogenic factors such as CXC chemokine ligand 12, which increases angiogenic tube formation *in vitro*[17]. In murine models, stimulation of microvascular endothelial cells with transforming growth factor-β_1 (TGF) and epidermal growth factor induced nestin expression[18]. Non-Hodgkin lymphomas express TGF and the expression values are higher in the high-grade lymphomas[19]. It appears that there is communication in the tumor microenvironment between neoplastic cells and stem cells to promote angiogenesis. Nestin, which under normal physiological conditions is absent in adult subjects, could be a therapeutic target.

Octamer binding transcription factor (OCT) 3/4 is a transcription factor encoded by *POU5F1* gene. Together with SOX2, it is essential for reprogramming somatic cells into pluripotent stem cells; also, they are needed for pluripotency and self-renewal of stem cells [20]. OCT4 has at least two isoforms, “A” with nuclear localization and “B” with cytoplasmic localization[21]. The nuclear expression (considered the “active” form) of OCT-4 is essential to maintain an undifferentiated state of the cell; oncogenic functions are attributed to it in the development of cancer, and the phenotype that expresses it, is considered an adult stem cell[22]. The “inactive” form appears to be characterized by its cytoplasmic localization; however, the OCT4-B isoform has been shown to increase proliferation and tumor formation, due to anti-apoptotic activity. To make the interpretation more complex, OCT4 has various transcript variants, protein isoforms, as well as pseudogenes[23]. Therefore, it is important to highlight that OCT4 expression was observed only in lineage B tumor cells; of the total lymphomas, only 19% expressed nuclear positivity and there seemed to be some relationship between this expression and a higher number of mitoses/HPF in those lymphomas, compared with those that did not express it, although the statistical analysis did not show a difference between the two groups. Other cases showed cytoplasmic positivity, either diffuse and discrete, or focal, in the form of a "bar" in close relationship with the cytoplasmic membrane. Finally, there were other lymphomas that showed neither nuclear nor cytoplasmic positivity for OCT4. A prospective study with a larger number of cases is required to evaluate the clinical significance of these findings.

CONCLUSION

In conclusion, CNS lymphomas are heterogeneous neoplasms in terms of the expression of certain markers such as OCT4 and this should be investigated in prospective studies. It appears that there is a dialogue between neoplastic cells and vascular stem cells that promotes the development of angiogenesis, as evidenced by the presence of newly formed vessels with positive labeling for nestin in endothelial cells, pericytes, and some hypertrophic astrocytes in contact with them.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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