Case Report

Intravascular Lymphomatosis in the Central Nervous System of Dogs: Immunohistochemical Investigation in Two Cases

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Abstract

We report here two postmortem cases of dogs with intravascular lymphomatosis affecting the central nervous system. Intravascular lymphomatosis is represented by an exclusively intravascular proliferation of neoplastic lymphoid cells. To characterize the origin of the neoplastic cells, we have proceeded with immunohistochemical analysis to identify B and T lymphocytes and endothelial cells. The results showed predominance of cells from the T cell lineage, and no evidence of B cell origin was found. Few cells from one dog also exhibited cytoplasmatic staining for vimentin and Von Willebrand factor. Although in one case some immunophenotype diversity was observed, the massive presence of CD3 positive cells confirmed these neoplasms as intravascular lymphomatosis of T cell origin.

Keywords: cell marker, immunohistochemistry, lymphoproliferative disorder, neoplasia, neuropathology, T cell

Introduction

Intravascular lymphomatosis (IVL) is a rare form of non-Hodgkin lymphoma characterized by neoplastic proliferation of lymphoid cells within the lumen of capillaries, small veins and arteries, with no or minimal involvement of the parenchyma (20). IVL was first described as angioendotheliomatosis proliferans systemisata (13) in a human patient with the cutaneous form of the disease. Initially this term and others, such as malignant angioendotheliomatosis and neoplastic angioendotheliosis, referred to the proliferation of endothelial cells which obliterated the lumen of small vessels with predilection for skin and brain, but possibly present in all organs, such as the adrenal glands, lymph nodes and lungs (3, 4, 19).

Immunocytohistochemical and cytogenetic studies revealed the lymphoid origin of the malignant cells (10). In humans, IVL is recognized as a distinct subtype of extranodal diffuse large-B cell lymphoma (DLBCL) in the World Health Organization (WHO) classification (6). Retrospective reviews of IVL in cats and dogs identified the neoplastic cells as T and B lymphocytes, non-T non-B lymphocytes, and also endothelial cells (9, 14, 16). The diverse origin of the neoplastic cells suggested that some features of this disease remain to be elucidated (15).

IVL exclusively confined to the central nervous system (CNS) is extremely rare, and symptoms are heterogeneous and related to vascular occlusion of the affected tissues, due to thromboses and infarctions (12, 17). The disease is not fully recognized by clinicians because the unspecific symptoms and although the presence of neoplastic cells inside blood vessels, they are not observed in routine blood samples (5, 8). With one exception (2), in all reported feline and canine cases, diagnosis of IVL was made at the necroscopic examination (8, 9, 14, 15, 16, 18, 19).

In the report presented herein, we describe two cases of IVL affecting the CNS of dogs, focusing on the
pattern of histopathological lesions as well as on the immunophenotype of the neoplastic cells.

**Case report**

Case No. 1: A nine-year-old female mixed breed dog, nulliparous, was referred to Franca University Veterinary Hospital with a day history of motor incoordination on the hind limbs and progression to spastic tetraplegia. The dog presented episodes of generalized tonic-clonic seizures, hyporexia, altered behavior, opistotonus and bilateral mydriasis not responsive to the light. A complete blood count revealed normocytic-normochromic anemia, neutrophilic leukocytosis and elevation of the total plasmatic protein. The clinic case rapidly evolved to conscience level depression and death. A necropsy was performed and tissues were collected for histologic examination.

Case No. 2: A brain of a dog was sent to the Department of Veterinary Pathology – UNESP-FCAV-Jaboticabal for histopathological examination and rabies’ differential diagnosis. No day history was provided.

The whole brain of both cases and samples of multiple organs of case No. 1 were formalin-fixed and embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin-eosin (HE). For immunohistochemistry (IHC), paraffin-embedded sections of brains were immunostained using the antibodies mouse anti-canine CD3 (Serotec, MCA1774S), mouse anti-human CD79α (Dako, M7051), rabbit anti-dog IgG (Sigma, A6792), mouse anti-vimentin (Dako, M0725), and rabbit anti-human Von Willebrand factor (formerly factor VIII-related antigen; Dako, A0082). For all antibodies, negative and positive controls (lymph node) were used in conjunction with brain sections. Antigen retrieval was made with microwave and citrate buffer (pH 6.0). Immunolabelling was performed with streptavidin-biotin-horseradish peroxidase (Dako, K0690). The reaction was developed with 3,3´diaminobenzidine (Dako, K3468) and counterstained with Harry’s hematoxylin.

In case No. 1, gross inspection of the brain revealed prominent and congested leptomeningeal vessels and hemorrhages mainly at left cerebral hemisphere and both piriform lobes (Figure 1A-B). In case No. 2 there was no evident gross alteration.

Histopathological examination of both cases demonstrated atypical mononuclear cells filling variably sized cerebromeningeal arteries, veins and capillaries. In both cases, the neoplastic cells are large, with pleomorphic and large nuclei, with one to several nucleoli, variable chromatin pattern, and scant amphophilic cytoplasm, and moderate quantity of mitotic figures. The neoplastic cells are exclusively confined to the lumen of vessels (Figures 2A-B). In some areas adjacent to affected blood vessels there are moderate amounts of macrophages with abundant foamy cytoplasm (Figure 2C), probably consequence of ischemic injury. Vessels, sometimes dilated, show thin walls and thrombosis, mostly within the meninges. Some blood vessels present hypertrophied endothelial cells (Figure 2D). In the affected blood vessels there are scarce erythrocytes along with the neoplastic lymphocytes. Intravascular lymphomatous cells were also found in heart, kidneys and lungs in case No. 1. Bone-marrow involvement was not detected.

**Figure 1.** Brain, dog, intravascular lymphomatosis, case No. 1. A: Dorsal view of the brain showing diffuse meningeal vascular congestion and a focal area of hemorrhage in the left frontal lobe. B: Ventral view of the brain exhibiting a focally extensive area of hemorrhage in the left piriform lobe and multifocal areas of hemorrhages in the right piriform lobe, in the olfactory tract and in the brain stem.

Immunohistochemical analysis showed that the neoplastic cells presented a strong cytoplasmatic CD3 immunostaining (Figure 3A). The immunostaining for CD3 was present exclusively in the cells confined to the vessels lumina. Staining was negative for CD79 (Figure 3B) and IgG. Vimentin was also positive in both cases and presented a strong cytoplasmatic staining of the normal endothelial cells and a pale staining in few intravascular cells (Figure 3C). Immunodetection of the Von Willebrand factor was evident only in case No. 1, in the cytoplasm of rare intravascular cells and in blood vessel walls (Figure 3D).

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leptomeninge presenting the lumen totally occluded by large mononuclear neoplastic cells. There is marked anisokaryosis and anisocytosis, and a great number of mitotic figures. B: Neoplastic cells filling variable sized blood vessels (arrows) in the white matter of the cerebellum. Note that there are no neoplastic cells outside the vessels. C: A cerebral cortex blood vessel dilated by neoplastic cells and surrounded by a discrete lymphoplasmacytic perivascular cuff (arrowhead). Note also the presence of macrophages with abundant foamy cytoplasm (Gitter cells; arrow) in areas of tissue loss. D: An affected blood vessel in the gray matter with marked hypertrophy of the endothelial cells (arrowhead). Hematoxylin-Eosin. Scale bar = 50 µm.

Figure 2. Brain, dog, intravascular lymphomatosis. A: Two blood vessels (arrows) in the cortical

**Discussion**

The histopathological lesions observed in these dogs are consistent with a disease characterized by the proliferation of cells within blood vessels. IVL involving CNS is a rare but important differential diagnosis of encephalopathies and encephalitis. A retrospective review of veterinary medical records identified 17 cases of canine IVL and the clinical presentation was variable including spinal cord ataxia, seizures and vestibular disease (9).

McDonough et al. (9), found T cells positive for CD3 marker (arrow) in the gray matter. B: Section of the gray matter stained with anti-CD79. Observe lack of stain. C: Endothelial cells (arrowheads) and some few intravascular cells (arrows) presenting positive cytoplasmatic staining for vimentin marker in the white matter. D: Case No. 1; however, in a report of IVL in humans, Kano et al. (7), described that 28% of IVLs presented factor VIII positive cells and they suggested that this finding occurs due to a non-specific absorption of factor VIII from the thrombi.

In conclusion, we report two cases of IVL affecting the CNS of dogs, and with IHC, we were able to classify these neoplasms into two subtypes: while case No. 1 was an IVL with predominating T cells; case No. 2 was clearly an IVL of the T cell lineage type. Further, these findings highlight the importance of IHC to aid the correct classification of neoplasms and also emphasize the necessity of IVL to be included in the differential diagnoses of encephalitis and encephalopathies.

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