BJVP
Brazilian Journal of Veterinary Pathology

Case Report

Canine cerebellar protoplasmic astrocytoma: clinical, histopathological and immunohistochemical features

Elisângela O. da Silva[1]; Antônio C. F. dos Reis[1]; Ana Paula F. R. L. Bracarense[1]

[1]Laboratório de Patologia Animal, Universidade Estadual de Londrina (UEL), PR, Brazil.
Corresponding author: Ana Paula F.R. L. Bracarense, Departamento de Medicina Veterinária Preventiva, UEL, Londrina, CEP 86051-990, PR, Brazil.
E-mail: anauel02@yahoo.com.br

Submitted August 20th 2010, Accepted September 11th 2010

Abstract

Protoplasmic astrocytomas are uncommon in humans and animals. A 14-year-old, intact female German Shepherd dog presented with a history of ataxia of the pelvic limbs, left-side head torsion and hypermetric response of the right side for approximately 15 days. Neurological examination revealed the presence of cerebellar syndrome and paradoxical vestibular syndrome. Medical therapy was initiated, but neurological signs were progressive, and the owner opted for euthanasia. Gross examination showed no cerebellar lesion. Microscopic features were characterized by prominent background microcystic degeneration and the presence of spindle cells with scant cytoplasm and delicate glial fibers. The immunohistochemical assay showed positive staining for GFAP (glial fibrillar acidic protein), vimentin and S100, and negative staining for factor VIII. A definitive diagnosis of protoplasmic astrocytoma was made on the basis of the histological and immunohistochemical findings.

Key Words: dog, neoplasia, cerebellum, central nervous system.

Introduction

Gliomas are the most common type of neuroectodermal-origin tumor in humans and animals (12, 14, 24). Glioma is a term including ependymoma, oligodendroglioma, glioblastoma, astrocytoma, and their various subtypes and combinations (19). Astrocytomas comprise the majority of canine gliomas, but are uncommon in cats (20, 31). Astrocytomas are well-differentiated, diffusely infiltrative neoplasms of fibrillary astrocytes and occur most commonly in the pyriform lobe, convexity of the cerebral hemisphere, thalamus and hypothalamus, midbrain and rarely in the cerebellum and spinal cord (4, 14, 30, 31).

Astrocytes are derived from the neuroectoderm and are characterized by a star-like appearance and broad feet on their processes. They contribute to the blood-brain barrier, make up about 85% of the basal lamina of all blood vessels within the CNS (central nervous system) and are involved in guiding neurons during embryonic development. If a group of neurons along the neuroaxis dies, the astrocytes multiply to form a glial scar. They help to control the contents of the intracellular space by taking up excess potassium and also take up neurotransmitters from synaptic zones, thereby facilitating synaptic activities (4, 21).

Astrocytes are heterogeneous cells. Two types have been identified, the fibrillary and the protoplasmic, both of which exhibit the classic star shape. Fibrillary astrocytes are more common in white matter. Protoplasmic astrocytes are predominantly found in grey matter and are characterized by processes that branch more frequently than those of fibrillary astrocytes. Other important subtypes of astrocytes include the pilocytic astrocytes of the periventricular region, cerebellum, and spinal cord and the Bergmann astrocytes distributed in a narrow lamina between the cell bodies of Purkinje neurons in the cerebellar cortex (4, 10).

Protoplasmic astrocytomas are an unusual and rare variant of diffuse astrocytomas composed of cells resembling protoplasmic astrocytes of gray matter. The neoplasm is characterized by the presence of astrocytes with a more stellate shape, with short and delicate processes and a prominent background of microcysts. The astrocytomas show positive immunostaining for GFAP, protein S100 and vimentin (4, 10). There are few reports of protoplasmic astrocytoma in dogs (14, 20).

Environmental, genetic, chemical, viral and immunological factors may contribute to the development of CNS tumors in humans as animals (9, 16). CNS tumors have nonspecific clinical signs that depend on the location of the tumor (16).

The definitive diagnosis of a CNS tumor can be made only after histopathological analysis (15). The immunohistochemical assay has become an indispensable technique to establish the diagnosis (14). The aim of this paper is to report a case of a cerebellar protoplasmic astrocytoma in a dog. Clinical signs, histological findings and immunohistochemical aspects are discussed.

Case report

A 14-year-old, intact female German Shepherd dog was admitted to the Veterinary Hospital, Universidade Estadual de Londrina, Londrina/PR, Southern Brazil. The animal had a history of pelvic limb ataxia, left-side head tilt and hypermetric response of the right side for approximately 15 days. Clinical and neurological examinations led to the diagnoses of cerebellar syndrome and paradoxical vestibular syndrome. Cerebrospinal fluid (CSF) analysis was performed, and no alteration was found. Medical therapy was initiated, but the neurological signs were progressive, and the owner opted for euthanasia. Routine necropsy was performed soon after death; collected tissues (cerebellum, brain, spinal cord, lung, kidneys, liver, heart, and spleen) were fixed in 10% buffered formalin solution and routinely processed for histopathological evaluation. Sections were stained with hematoxylin-eosin (HE), periodic acid Schiff (PAS), and Masson's trichrome stain.

Selected paraffin-embedded tissue fragments from the cerebellum were prepared for immunohistochemical (IHC) analysis. Tissue fragments were used to identify GFAP, vimentin, factor VIII and S100 protein. For IHC, the polymer-peroxidase technique was used. The intensity and the distribution of immunoreactivity of the panel of antibodies were subjectively evaluated and combined into grades of – (negative), + (weakly positive), ++ (moderately positive) and +++ (strongly positive) as showed in the Table 1. Fragments of nervous tissue, lymph nodes, and breast tissue were used as positive controls; for negative controls, PBS substituted for the primary antibody.

Table 1- Summary of results of immunohistochemical staining of a canine cerebellar protoplasmic astrocytoma.

Antibodies	Source	Dilution	Result
GFAP	Zymed®	1:100	+++
Vimentin	Dako®	1:100	+++
S100	Zymed®	1:100	++
Factor VIII	Dako®	1:100	-

Gross examination showed no cerebellar lesion. The histopathological exam showed a focal neoplasm in the cerebellar white matter invading the granular and molecular layers (Fig. 1). The tumor was characterized by prominent microcystic degeneration background and clear regions likely caused by the rupture of microcysts (Fig. 2). There was vascular proliferation and vascular congestion. The tumor cells were spindle shaped and presented scant cytoplasm and delicate glial fibers. The nuclei were round to oval, with high atypia and a low mitotic index. The PAS method detected no cells containing protein granules or granular bodies. Masson's trichrome technique stained the vascular walls. IHC analysis showed positive staining for GFAP (Fig. 3), vimentin (Fig. 4) and S100 (Fig. 5) and negative staining for factor VIII.

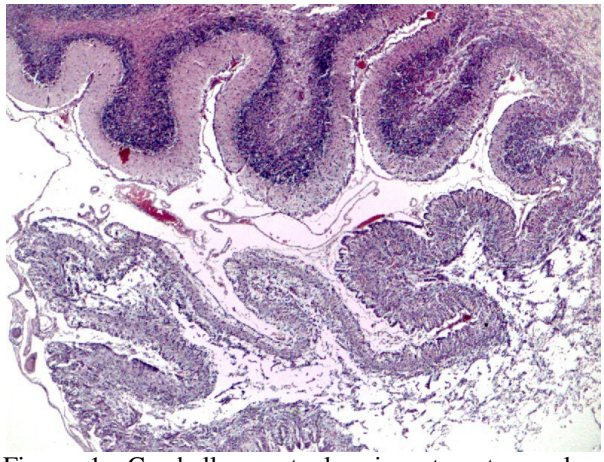

Figure 1- Cerebellar protoplasmic astrocytoma: low magnification showing the affected cerebellar tissue (HE, 2.5x).

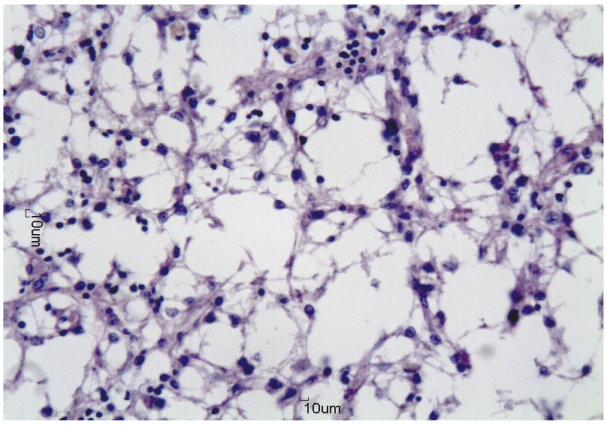

Figure 2- Cerebellar protoplasmic astrocytoma: prominent microcystic back- ground, delicate glial fibers and nuclei round to oval (HE, 40X).

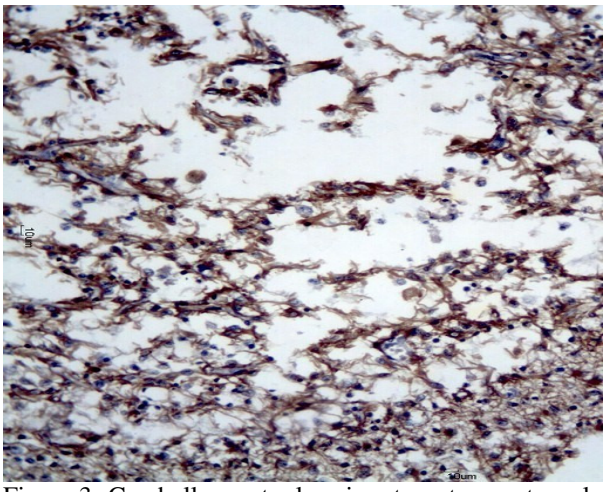
Figure 3- Cerebellar protoplasmic astrocytoma: strongly positive staining for GFAP (HE, 20x).

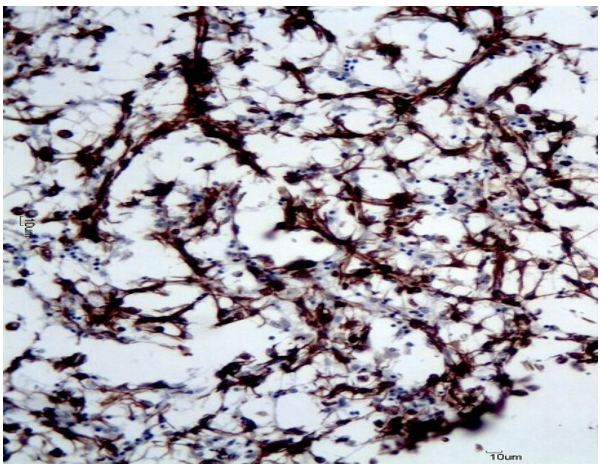
Figure 4- Cerebellar protoplasmic astrocytoma: strongly positive staining for vimentin (HE, 20x).

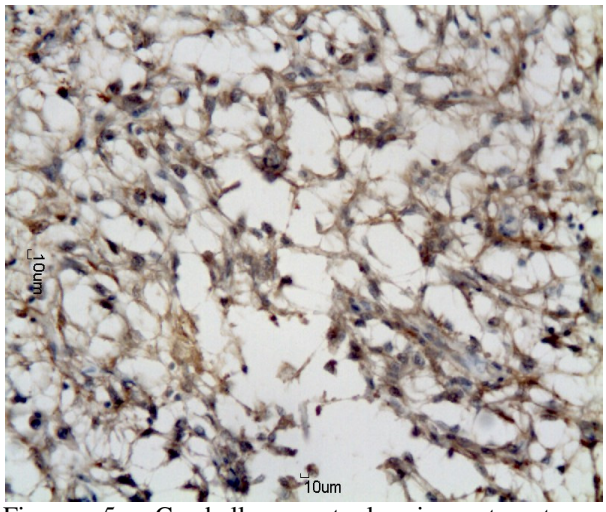

Figure 5- Cerebellar protoplasmic astrocytoma: moderately positive staining for S100 (HE, 20x).

Discussion

Tumors of the CNS are common in humans and constitute the second most prevalent tumor type during childhood (31). Glial tumors are the most common primary tumors of the central nervous system in humans, dogs and cats (2, 14, 24). In humans, astrocytoma most often occurs in the third and fourth decades of life, and males are affected more often than females. Protoplasmic astrocytoma is unusual and is most often found in the cerebrum in children and young adults (18).

Astrocytoma is the most common glial primary tumor in dogs (20). This form of tumor is most frequently diagnosed in brachycephalic breeds, such as Boston Terriers and Boxers, with a greater incidence reported in animals over five years of age (25, 29, 30, 34).

In humans, genetic alterations such as the inactivation of p53, amplification and rearrangement of PDGF-RA (platelet-derived growth factor receptor A) and changes in integrin expression have been associated with the progression of astrocytic tumors from low to high grade. Clonal chromosome abnormalities have been described in human CNS tumors. The most frequent cytogenetic abnormality is the deletion of the long arm of chromosome 22. Radiation can also induce tumors in the CNS, usually after years of radiation therapy, including poorly differentiated sarcomas, gliomas and meningiomas (9, 13, 18, 23, 26). Some reports demonstrate that the occurrence of astrocytomas in dogs and cats can be related to such genetic alterations as the inactivation of p53 and the overexpression of EGFR (*epidermal growth factor receptor)* (1, 28, 29).

Clinical signs in animals with astrocytomas vary depending on the location of the tumor in the CNS. The most common signs include behavioral changes, ataxia, tetraparesis, seizures, circling, and abnormal cranial nerve and proprioceptive reflexes (34). The animal in the present report showed neurological signs compatible with cerebellar syndrome and paradoxical

vestibular syndrome (6). As the tumor showed no amelioration of clinical signs with treatment, the owner opted for euthanasia.

The complementary exams that can be used to confirm the presence of neoplasms in the CNS are plain radiography, myelography, CSF analysis and specialized radiographic techniques such as computerized tomography and magnetic resonance imaging (11, 15, 22, 32, 33). In this case, no imaging exam was performed.

Cerebrospinal fluid findings in animals with lymphoma may be helpful in achieving a diagnosis. However, in animals with primary intracranial neoplasia, a nonspecific inflammatory change in the CSF is usually seen (16, 29). The CSF analysis in this case revealed no changes.

The treatment of astrocytomas is a challenge in veterinary medicine. Corticosteroids are frequently used as palliative therapy and can reduce the vascular permeability, exert cytotoxic effects on tumors, inhibit tumor formation, and decrease CSF production (21). There is controversy concerning the benefits of surgical excision of CNS tumors in animals. However, in humans and in animals, surgical debulking followed by radiation therapy provides the best prognosis for most tumors (11). Postoperative systemic thromboses are the major complication of brain tumor surgery (19).

In veterinary medicine, histopathological examination remains the principal tool for intracranial neoplasia characterization and classification (15, 29). Immunohistochemistry has become an indispensable technique for confirming diagnoses and classifying tumors of the nervous system (3, 7, 14). According to the WHO (World Health Organization), astrocytomas in humans are very diverse and can be classified as low-grade gliomas (grade I or II), diffuse astrocytoma (grade II), anaplastic astrocytoma (grade III) or glioblastoma multiforme (grade IV) (5, 27).

Diffuse astrocytoma has significant potential to diffusely invade the adjacent neuropil or white matter and also has the potential to undergo anaplastic progression. This tumor grade includes astrocytomas of fibrillary, gemistocytic and protoplasmic types (4, 18). According to the WHO classification system, tumors with nuclear atypia alone are considered grade II; those with nuclear atypia and mitotic activity are considered grade III; and tumors with nuclear atypia, mitoses, endothelial proliferation and/or necrosis are considered grade IV. It is important to emphasize that the term "low-grade" that is applied to astrocytoma and others gliomas does not necessarily imply a benign neoplasia or even a favorable prognosis. In this case, the presence of nuclear atypia combined with endothelial proliferation (microvascular hyperplasia) is suggestive of an astrocytoma grade IV according to the literature (19).

Some gliomas are highly vascular, but glioma and angioma can be combined (19). In this case, the tumor exhibits extensive vascular proliferation, but the endothelial cells show no atypia. The antibody anti-Factor VIII can be used to mark endothelial-origin tumors. The present tumor showed negative immunostaining for anti-Factor VIII antibody.

Pilocytic astrocytoma is the most important differential diagnosis of protoplasmic astrocytoma (4, 10). Pilocytic astrocytoma is characterized by the presence of a biphasic pattern, a combination of microcystic and compact areas, and glomeruli-like vascular proliferation. Histological features that greatly facilitate the diagnosis of pilocytic astrocytoma include Rosenthal fibers and cells containing protein droplets or granular bodies that are PAS-positive. Rosenthal fibers appear brightly eosinophilic and assume a hyaline quality. They may be mimicked by tightly stacked erythrocytes in small vessels (4, 10, 18).

As in pilocytic astrocytoma, protoplasmatic astrocytoma may exhibit glomeruloid vessel proliferation, particularly within cyst walls. Unlike pilocytic astrocytomas, protoplasmatic tumors lack a biphasic pattern, Rosenthal fibers and granular bodies. In animals, Rosenthal fibers are less common than in humans but are diagnostic of pilocytic astrocytoma (14). In this case, the histopathological exam and PAS staining showed no presence of Rosenthal fibers, cells containing protein droplets, granular bodies or glomeruli-like vascular proliferation.

According to the literature, protoplasmic astrocytoma immunostaining is heterogeneous. Some authors have found protoplasmic astrocytoma to be weakly reactive to GFAP, although other authors have reported evidence of occasional and multifocal GFAP positivity. This heterogeneous immunostaining may result from a decreased number of intracytoplasmic filaments within the neoplastic cells (10, 18, 19, 22). In this case, we found that strongly positive immunostaining was more evident in paranuclear region of tumor cells. The histopathological and immunohistochemical findings are in accordance with the well-documented diagnosis for protoplasmic astrocytoma in the literature (4, 6, 14, 19, 31).

The prognosis for dogs with cerebellar tumors is poor as such tumors have a relentlessly progressive course (6, 17). Clinical signs and laboratory and imaging exams generally do not yield conclusive diagnoses (8). Histopathological and immunohistochemical methods are fundamental for the definitive diagnosis and classification of cerebellar tumors.

Acknowledgments

The authors thank Raquel B. Ferioli and Giovana W. Di Santis for technical and immunohistochemical assistance, respectively.

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