

## Cytologic and histologic features of spinal cord ependymoma in a young dog: a case report

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**ABSTRACT:** A case of intramedullary ependymoma in a young dog is reported. A two year old dog was presented with paralysis of the forelimbs. At myelographic examination, an intramedullary pattern, blocking the progression of contrast medium, was observed. At necropsy, a 3 × 2 cm white-greyish mass was found extending from the 3<sup>rd</sup> to 5<sup>th</sup> lumbar levels. At cytological and histological examination, the mass was highly cellular and was comprised of ovoid cells with indistinct borders, elongated eosinophilic cytoplasm and round to oval vesicular nuclei. Cytoplasmic processes formed a fibrillar network wherein true rosettes and many pseudorosettes around a fibrovascular stroma were observed. Immunohistochemistry for vimentin and GFAP gave strong positive results in the neoplastic cells, especially around pseudorosettes and confirmed the diagnosis of intramedullary spinal ependymoma.

**Keywords:** ependymoma; spinal cord; tumor; immunohistochemistry; dog

Ependymomas are rare tumours arising from the ependymal cell lining of the ventricle system and spinal cord central canal. These tumors are slow-growing, circumscribed, expansile masses that have been reported in dogs, cats, cattle and some rodents where they develop mainly within lateral ventricles, less often in the third and fourth ventricle, and rarely within the central canal of the spinal cord (Summers et al., 1988; Rittinghausen and Deerberg, 1989; Cordy, 1990; Carrigan et al., 1996; Kraft et al., 1997; Lantos et al., 1997; McKay et al., 1999; Simpson et al., 1999; Wiestler et al., 2000; Koestner and Higgins, 2002; Michimae et al., 2004; Vural et al., 2005). According to the WHO histological classification of animal tumours there are two major histological subtypes of ependymomas, namely the ependymoma and anaplastic ependymoma (Koestner and Higgins, 2002). In recent years even in veterinary pathology cellular, papillary and tanycytic subtypes of ependymoma have been described (Koestner and Higgins, 2002). Ependymomas are composed of well differentiated ependymal cells arranged in rosettes with well defined central lumina, perivascular pseudorosettes and papillary-tubular formations. In detail, pseudorosettes are comprised of nuclear-free perivascular zones formed by tumour cell processes; rosettes

result from a radially arranged pattern of tumour cells centered around a miniature ependymal lumen; papillary-tubular formations are composed by a vascular core forming papillae whose surfaces are covered by single or multiple layers of columnar cells arranged in a pseudorosette pattern. The differential diagnosis between ependymomas and other CNS tumours showing pseudorosette patterns, is made easily by the finding of true ependymal rosettes along with a uniform and consistent GFAP (glial acidic fibrillary protein) and Vimentin immunohistochemical stain (Koestner and Higgins, 2002). This paper describes the clinical, cytological, pathological and immunohistological findings in a dog with spinal cord ependymoma.

### Case presentation

A two year old male mixed-breed dog was presented with paralysis of forelimbs. Serological tests for infectious diseases were negative. At myelographic examination, an intramedullary pattern, blocking the progression of contrast medium, was observed. The dorsal and ventral of contrast medium were not clearly observed in the subdural



Figure 1. A 3 × 2 cm white-greyish mass extending from the 3<sup>rd</sup> to 5<sup>th</sup> lumbar segments

space forward to the lumbar 5<sup>th</sup> (L5) level, which implied marked swelling of spinal parenchyma lines. Cerebrospinal fluid (CSF) collected by lumbosacral puncture was colourless and clear. Due to the severity of the clinical signs, the owners requested euthanasia. At necropsy, a 3 × 2 cm white-greyish mass was found at the lumbar segment extending from 3<sup>rd</sup> to 5<sup>th</sup> lumbar segments (Figure 1). From the CSF and the excised mass smears were prepared and stained with May Grunwald-Giemsa (MGG) for cytological examination. The affected spinal cord was removed, fixed in 10% neutral buffered formalin, embedded in paraffin wax and sectioned at 5 µm. Haematoxylin and eosin and immunohistochemistry (IHC) were performed. For IHC the slides were steamed in 0.01 mol/l sodium citrate buffer, pH 6, in a microwave oven for 15 min. Endogenous peroxidase activity was quenched by 0.3% hydrogen peroxide in methanol while aspecific protein reactions were blocked by incubation with 2.5% BSA for 30 min. Slides were then incu-

bated over night at 4°C with anti-GFAP (mouse monoclonal; clone 10D11; Novocastra, 1 : 150 dilution) and anti-Vimentin (mouse monoclonal; clone V9, Scytek laboratories, Logan, Utah, USA) primary antibodies, followed by an incubation at room temperature with an horse anti-mouse biotinylated IgG (BioSpa, Milan, Italy). The reaction was developed by an avidin peroxidase complex (BioSpa, Milan, Italy), revealed with Vector Nova Red (Vector Laboratories, Burlingame, CA) and counterstained with hematoxylin.

For each sample negative controls were also included by omitting the primary Ab, substituting the primary Ab with normal mouse IgGs and substituting the primary Ab with an indifferent mouse primary Ab. Immunohistochemical stains were interpreted by assessing the intensity of staining. Cytoplasmic and/or membrane immunoreactivity was considered positive. The cytological examination from the CSF was negative while smears showed a cellular specimen composed of cells with

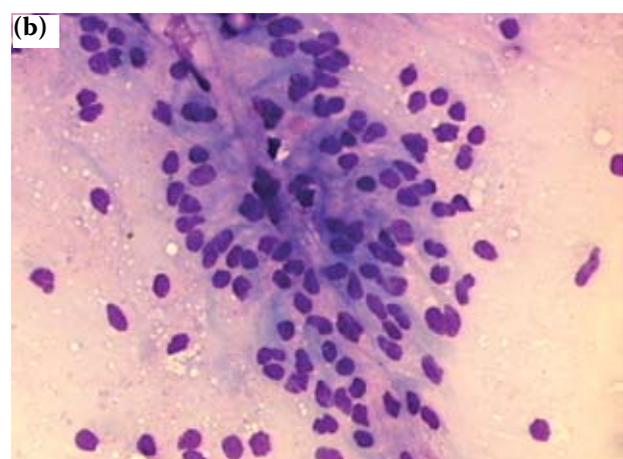
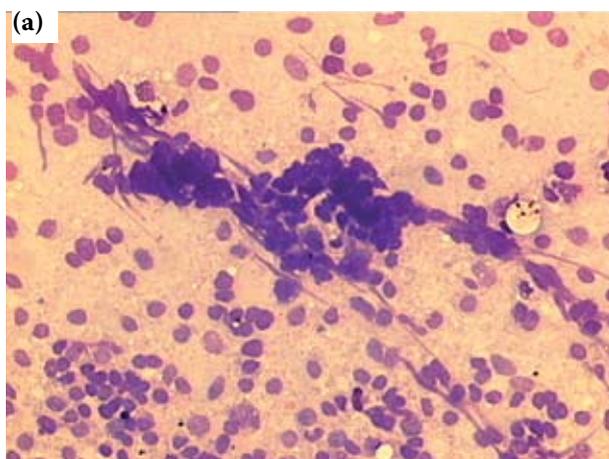


Figure 2. Cytological smear preparation showing small and round nuclei with lacy or clumped chromatin (a); cells with no clear cytoplasm, forming loosely cohesive aggregates sometimes around vessels (b)

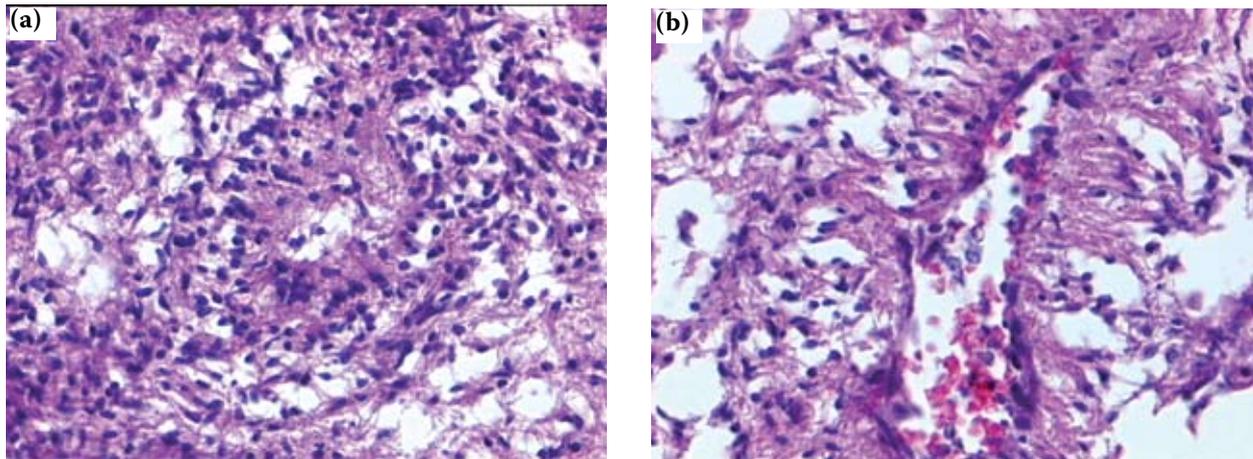


Figure 3. True ependymal rosette characterized by a radially arranged pattern of tumor cells centered around a miniature ependymal lumen (a); a pseudorosette around a fibrovascular stroma (b)

no clear cytoplasm, sometimes forming loosely cohesive aggregates around vessels (Figure 2a). The nuclei were small and round with lacy or clumped chromatin (Figure 2b). Haematoxylin-eosin histological examination showed a well vascularized and highly cellular mass that was mainly constituted by ovoid cells with indistinct borders, elongated eosinophilic cytoplasm and round to oval vesicular nuclei. Cytoplasmic processes formed a fibrillar network wherein rare true ependymal rosettes (Figure 3a) and many pseudorosettes around a fibrovascular stroma were observed (Figure 3b). No mitotic figures were detectable despite the high concentration of cells and local invasion. Foci of necrosis and haemorrhage were present inside the mass. Around the neoplasm, the spinal cord showed signs of malacia, axonal swelling and haemorrhage. Normal spinal cord architecture

was detectable only in the spinal cord segments proximal and distal to the mass, where neoplastic cells were focally located around the ependymal canal. Immunohistochemically, GFAP (Figure 4a) and vimentin immunoreactivity (Figure 4b) was uniform and intensely expressed in the neoplastic cells especially around pseudorosettes.

### DISCUSSION

Based on cyto-histopathological and immunohistochemical results, a diagnosis of ependymoma was made. We found both pseudorosettes and true rosettes that, along with the Vimentin and GFAP stain, are considered to be useful in differential diagnosis between ependymomas and other tumours (Koestner and Higgins, 2002). However,

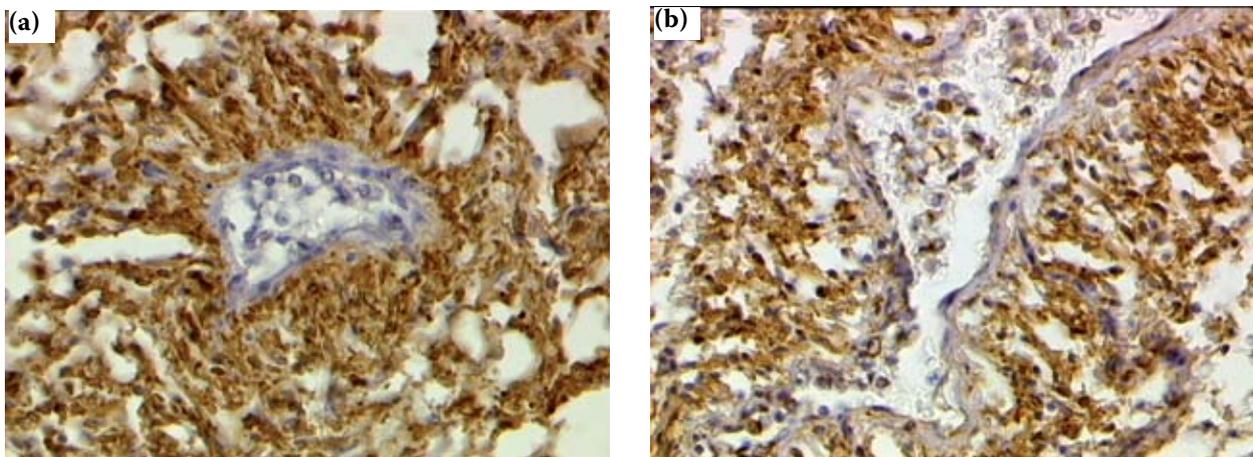


Figure 4. GFAP (a) and Vimentin immunoreactivity (b) was uniformly and intensely expressed in the neoplastic cells especially around pseudorosettes

even if ependymoma is a well known tumour, to the authors' knowledge, detailed cytologic, histologic and immunohistochemical features on a single case of spinal cord ependymoma are rarely reported. Fernandez et al. (1997) described the cytologic, histologic and immunocytochemical features of a poorly differentiated intramedullary glioma, most likely an anaplastic ependymoma. Vernau et al. (2001) reported cytological patterns of canine intracranial ependymoma smears suggesting that smear preparations of the various major categories of brain tumors each have distinctive cytologic profiles and that this technique, as with human CNS tumors, can provide an accurate, rapid intraoperative diagnosis. The study reported here provides a post-mortem evaluation of a spinal cord ependymoma in a very young dog. Since intramedullary ependymoma occurrence only rarely, more detailed cytological descriptions of ependymomas affecting the spinal cord, along with haematoxylin-eosin and immunohistochemical stains, could help in the clinical and histologic differential diagnosis of this rare neoplasm often confused with spinal thoracolumbar tumours and neuroblastomas.

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