Gliomatosis of the spinal cord in a cat: a case report

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ABSTRACT: Gliomatosis cerebri situated in the thoracolumbar and lumbosacral segments of the spinal cord was diagnosed in a Persian 10.5-year old tom cat. Clinical manifestation consisted of rump ataxy, weakened posture in the hind limbs, paraparesis, weakened spinal reflexes, no spinal hyperaesthesia or pain, and hypotonia of the urinary bladder. Magnetic resonance imaging revealed a diffuse hyperintense signal in the thoracolumbar junction and in lumbosacral segments (L6–S1) of the spinal cord. Normal size, form and colour of the spinal cord were apparent at autopsy; however, changes in grey matter shape were recorded on some transversal sections of the spinal cord. Dense, diffuse infiltration of the grey matter and surrounding white matter by glia-like neoplastic cells was histologically demonstrated. Immunohistochemical examination did not enable specification of the origin of the neoplastic cells but their neuroectodermal histogenesis can be assumed. On the basis of MRI, histological and immunohistochemical examinations gliomatosis of the spinal cord was diagnosed.

Keywords: feline; medulla; neoplasia; clinic; pathology

Gliomatosis cerebri is a rare neoplasia both in humans and animals. Maxie and Youssef (2007) and Vandevelde et al. (2012) have described gliomatosis as a widespread diffuse infiltrating disease of dogs, predominantly of brachycephalic breeds, characterised by an infiltrative cell type more reminiscent of astrocytes than of the formation of a distinct tumour mass. The infiltrates involve the hemispheric lobes, cerebellum and brainstem, often bilaterally but assymetrically. Discontinuous areas of gliomatosis have also been described in the spinal cord. There is no tumour mass, rather a diffuse enlargement of the affected regions; the neoplastic cells insinuate among normal structures that remain intact with only slight damage to axons and neurons. The tumour cells usually have elongated, enlarged, often twisted mildly hyperchromic nuclei without detectable cytoplasm. Formation of secondary Scherer structures is common (Vandevelde et al. 2012). Oligodendrocyte-like cells, cells of transitional character, and small unclassified cells can also be present (Maxie and Youssef 2007). The origin of the neoplastic cells is still controversial, as they can be immunoreactive for GFAP but in many cases do not stain with glial markers such as GFAP. Molecular genetic studies in humans have confirmed their astrocytic origin (Vandevelde et al. 2012). A neoplastic lesion with gliomatosis features located in the spinal cord of a cat is described in this case report.

Case description

The owner of an indoor/outdoor Persian 10.5-year old tom cat reported that his cat suddenly could not jump on the armchair and that his hind limbs appeared week. Over the next 12 days the owner observed that some attempts of similar jumps were successful and some of them not. In this period micturition habits also changed. Instead of the former two or three urinations per day, the frequency increased up to nine but the quantity of urine markedly decreased per each micturition. Seven days after the onset of the motion disorder, biochemical and haematological examinations were carried out. These examinations revealed only mild monocytosis (7%) and mild renal azotaemia (creatinine 157 µmol/l). All remaining haematological and biochemical parameters were within the physiological range. Twelve days after appearance of the first symptoms, the cat could not rise to his hind limbs and was referred to the veterinary clinic for a specialized examination.

Normal consciousness, plantigrade posture of hind limbs, paraparesis, rump ataxy, weakened posture in the hind limbs, weakened spinal reflexes of the pelvic right flexor and both perineal flexors, no spinal hyperaesthesia or pain, and hypotonia of the urinary bladder were diagnosed by neurological examination. Magnetic resonance imaging revealed a diffuse hyperintense signal in the thoracolumbar junction and in lumbosacral segments (L6–S1) of the spinal cord. Herniation or degeneration of the vertebral disc/s or compression of the spinal cord was not detected. On the basis of neurological and magnetic resonance imaging examinations a noncompressive disease of the spinal cord with secondary hypotonia of the urinary bladder was diagnosed. The disease continuously progressed, the cat could not use his hind limbs anymore and spontaneous micturition and defecation disappeared. For urination Myocholin was administered and after 30 min it was necessary to manually compress the urinary bladder. This was done at least twice a day. For stimulation of defecation Lactulosa was used. In spite of this, the health status of the animal progressively deteriorated and for compassionate reasons the cat was euthanised, 20 days after the onset of the illness.

Necropsy revealed only acute moderate hyperaemia and oedema of the lung, mild dilatation of the right cardiac chamber and hyperaemia of the liver. The lesions in the lung and the heart were, very probably, related to euthanasia. All remaining internal organs, including the brain, were without any macroscopically apparent pathological changes. From the vertebral channel the thoracolumbar and lumbosacral segments of the spinal cord were removed. They were of normal size, form and colour. The segments of the spinal cord were split up by perpendicularly oriented sections into ten parts. In four of them, changes in the form of the grey matter were recorded. Instead of the normal butterfly-like formation of the grey matter, a large round formation of grey colour, surrounded by a rather narrow rim of white matter, was visible. In two other parts of the spinal cord, a small round or wedge-shaped grey substance was located in the white matter.

Samples of all parenchymatous organs, transversal sections of the brain at the levels of the thalamus and hypothalamus, at mesencephalon, and at cerebellum with pons as well as all the above mentioned samples of the spinal cord were fixed in 10% neutral buffered formalin, subsequently paraffin-embedded using routine methods, sectioned at 4 to 5 µm, and stained with haematoxylin and eosin. Sections of the kidneys were additionally stained with blue trichrome and with PAS. Immunohistochemistry (IHC) was performed in order to demonstrate the following antigens: Cytokeratins, glial fibrillary acidic protein (GFAP), S100 protein, vimentin, neuron specific enolase (NSE), neurofilament protein (NF), synaptophysin calretinin, growth associated phosphoprotein 43 (GAP 43), glutamate receptor 2 (GLUR 2), cyclooxygenase 2 (COX 2) and CD3. Clone, source and dilution of the antibodies are specified in Table 1.

Table 1. Specification of the antibodies and their dilution

Antibody	Clone	Source	Dilution
Cytokeratin	MNF	DakoCytomation	1 : 50
Cytokeratin 8	polyclonal	Diag. BioSystems	1 : 50
Glial fibrillary acidic protein	polyclonal	DakoCytomation	1 : 200
S100 protein	polyclonal	DakoCytomation	1 : 300
Vimentin	V9	DakoCytomation	1 : 25
Neuron specific enolase	BBS/NC/ViH14	DakoCytomation	1 : 100
Neurofilament protein	2F11	DakoCytomation	1 : 50
Synaptophysin	SY38	DakoCytomation	1 : 100
Calretinin	polyclonal	Diag. BioSystems	1 : 50
Growth associated phosphoprotein 43	1G7	Novocastra	1 : 20
Glutamate receptor 2	1B5	Novocastra	1 : 10
Cyclooxygenase 2	CX-294	Diag. BioSystems	1 : 50
CD3	polyclonal	DakoCytomation	1 : 25

Tissue antigens, except for cytokeratin, were umasked by boiling the slides for 10 min in citrate buffer (pH 6.0, 0.1M). Cytokeratin was unmasked using Pronase (DakoCytomation) for 10 min at room temperature. Endogenous peroxidase activity was blocked with 3% H_2O_2 for 15 min. Binding of the primary antibodies was detected by means of the Streptavidin-Biotin Universal Detection System (Immunotech, Marseille, France) and with the DAB Chromogen Kit (Immunotech, Marseille, France). The slides were counterstained with haematoxylin.

Acute congestion and moderate oedema in the alveoles and bronchioles were well apparent in the lung. Centrilobular dilatation of the sinusoids, intracellular and extracellular cholestasis with disseminated small lipogranulomas was present in many hepatic lobules. Several large veins were surrounded by zones of hepatocytes in the stage of recent necrosis without any reaction in the live liver tissue. Membranous glomerulonephritis with dystrophy or necrosis of the tubular epithelium was diagnosed in the kidneys. Only suspect oedema of the choroid plexus in the lateral ventricles and suspect subependymal oedema in the fourth ventricle were noted in the brain and cerebellum.

Spinal cord. Minimal lesions consisted of an irregular shape of the spinal canal lined with discontinuous ependym. In one sample an infiltration of subpendymal tissue and ependym by neoplastic cells was observed, which included invasion of these cells into the central canal. Ependymal cells appeared as tanycytes that are specialized ependymal cells with basal processes extending between the astrocytic processes to form an endfoot on blood vessels (Kierszenbaum 2007). In one half of the grey matter of this sample, mild diffuse gliosis was present, in the opposite half gliosis and mild infiltration by the neoplastic cells were observed (Figure 1). With the exception of moderate brown pigment deposits in perikarykaria, motor neurons appeared intact. Some small foci of glial cells were disseminated in the white matter. Ballooning of some myelin sheaths was also present. Macroscopically visible grey foci in the white matter consisted of neoplastic cells infiltrating the original tissue. Perivascular lymphocytic cufts were present around regions infiltrated by neoplastic cells (Figure 2). Fully developed process were characterised by dense, diffuse infiltration of the grey matter and surrounding white matter by neoplastic cells (Figure 3). It was clearly evident that the neoplastic cells insinuated among normal structures that were slightly or moderately damaged (Figure 4). Adjacent normal tissue was not compressed by neoplasia; on the contrary, neoplastic cells merged gradually into the surrounding parenchyma causing indistinct margins between neoplastic and normal tissue. In these samples the central canal was not apparent. The neoplastic cells were arranged in thin strips, plugs or suspect pseudorosettes. Two types of neoplastic cells could be distinguished. Larger, with round, oval, elongated

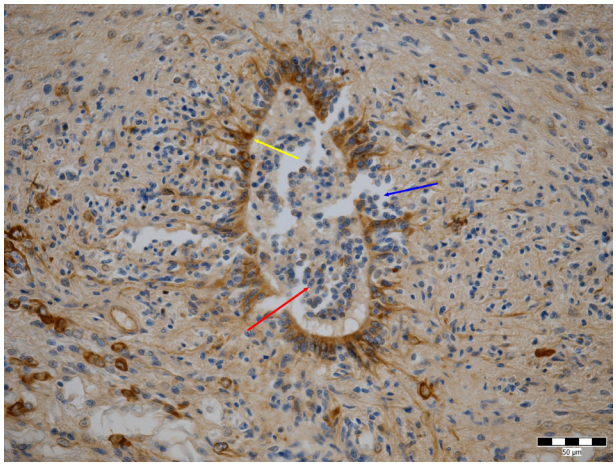

Figure 1. The central canal lined with discontinuous ependym, infiltration of subpendymal tissue and ependym by neoplastic cells (blue arrow), invasion of these cells into the lumen of the central canal (red arrow). Ependymal cells have the appearance of tanycytes (yellow arrow); Vimentin, immunoperoxidase method

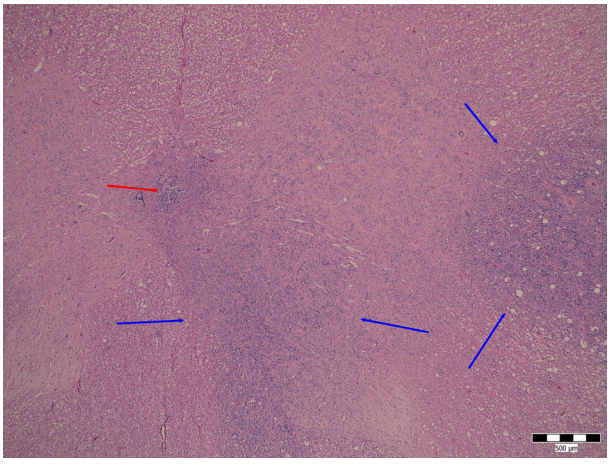

Figure 2. Regions of grey and white matter infiltrated with neoplasitc cells (blue arrows). Alteration and infiltration of the central canal with neoplastic cells (red arrow); HE

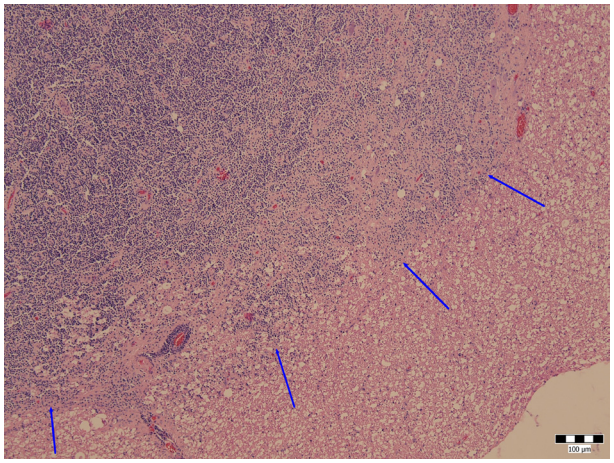

Figure 3. Extensive infiltration of the spinal cord with neoplastic cells (blue arrows). Only the narow rim of the white matter is unaffected; HE

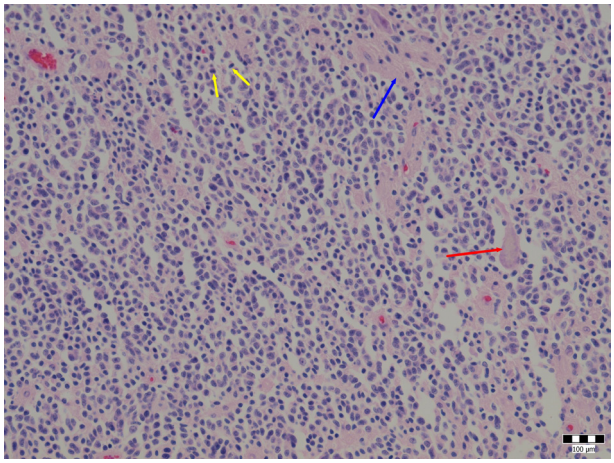

Figure 4. The neoplastic cells are arranged in thin strips or suspect pseudorosettes. Two types of neoplastic cells – one larger and one smaller could be distinguished. An intact neuron (red arrow) and neuropil (blue arrow) are apparent. Mitotic figures (yellow arrows); HE

or slightly irregular nuclei containing fine, stippled chromatin and inconspicuous nucleoli. The mitotic activity of these cells was moderate with a mitotic index of 2.8. Cytoplasm was narrow or moderately voluminous, slightly basophilic or eosinophilic. Smaller cells had round to oval nuclei with dense chromatin and inconspicuous cytoplasm. In the regions densely infiltrated by neoplastic tissue numerous small blood vessels surrounded by narrow perivascular lymphatic cufts were observed. Segments of arachnoidea and leptomening were slightly infiltrated by the lymphocytes.

Table 2. Summary of the results of immunohistochemical examinations

Antibody	Reaction in neoplastic cells
Cytokeratin	–
Cytokeratin 8	–
Glial fibrillary acidic protein	–
S100 protein	– to +
Vimentin	–
Neuron specific enolase	+ to ++
Neurofilament protein	– to +
Synaptophysin	–
Calretinin	– to +
Growth associated phosphoprotein 43	– to +
Glutamate receptor 2	+ to ++
Cyclooxygenase 2	– to +
CD3	–

Results of immunohistochemical examination (Table 2). Pancytokeratin and cytokeratin 8 staining were negative both in the normal and neoplastic cells. GFAP was present in the ependym, in astroglia and neuropil. Cells in the lumen of the central canal and neoplastic cells were negative (Figure 5). S100 protein staining was positive in the ependymal and glial cells. Neoplastic cells were negative or slightly positive. Vimentin gave strong positivity in the ependymal cells that had the appearance of tanycytes. Positive were also astrocytes, including those in areas of gliosis, and glial cells in the white matter. The neoplastic cells were negative; however, in regions densely infiltrated by the neoplastic

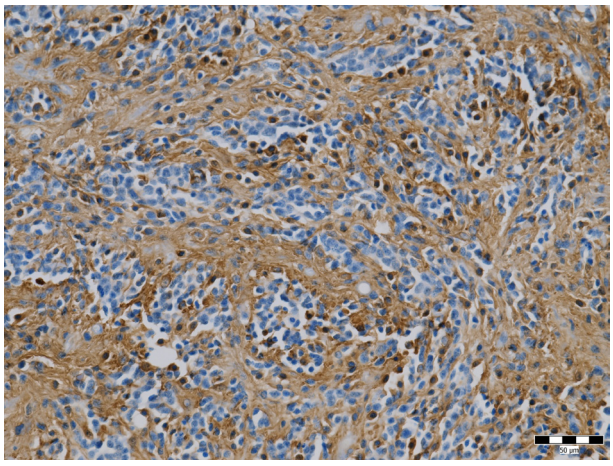

Figure 5. Original structures are GFAP-positive (brown colour), neoplastic cells are negative; immunoperoxidase method

cells, strongly positive cells with the appearance of gemistocytes were disseminated. Positive fibrillary astrocytes were present in foci of gliosis located in the white matter and surrounded by neoplastic cells. Ependymal cells were moderately positive for neuron-specific enolase. Perikarya of neurons, neuropil in the grey matter and myelinated nerve fibres in the white matter and glial cells yielded moderate to strong positivity. A slight or moderate positive reaction was observed in the neoplastic cells. Smaller cells were more positive than the larger ones. An antibody against neurofilaments gave strong positivity in perikarya, neuropil in the grey matter and nerve fibres in the white matter. A negative or mildly positive reaction in the neoplastic cells and moderate positivity of the ependym were noted. Marked positivity for synaptophysin in neurons and neuropil was apparent but neoplastic cells were negative. Calretinin was positive in the normal tissue. In neoplastic cells the reaction was negative or slightly positive. Moderate GAP43 positivity was observed in the ependym of the central canal, glial cells and neuropil, while the perikarya of motoric neurons were markedly positive. In the foci of gliosis disseminated in the white matter cells of glial appearance were positive. Suspect or mild positivity was seen in the neoplastic cells. Large neurons and neuropil in the gray matter were positive for GLUR 2. In the cytoplasm of neoplastic cells mild to moderate positivity was observed. The reaction to cyclooxygenase-2 was moderately positive in the ependym and in the activated astrocytes. In neurons, neuropil and glial cells there was mild to moderate positivity and in neoplastic cells it was negative or mildly positive. The great majority of the lymphocytes in the perivascular cufts exhibited strong CD3 positivity. Nonspecific binding of this antibody was recorded in the neurons and ependym. Neoplastic cells were negative. Immunohistochemical examination did not allow specification of the origin of the neoplastic cells but their neuroectodermal histogenesis can be assumed on the basis of this examination.

Based on magnetic resonance imaging, gross pathology and histological examination, gliomatosis of the spinal cord was diagnosed.

DISCUSSION AND CONCLUSIONS

It is generally accepted that gliomatosis is a rare neoplasia. In some textbooks, both of veterinary and human pathology, and even in specialised books (Koestner and Higgins 2002; Bolon and Butt 2011) this diagnosis is not even mentioned.

Gliomatosis is macroscopically poorly defined and the only helpful clue is a diffuse enlargement of the infiltrated structures (Koestner et al. 1999). Porter at al. (2003) recognise two forms of gliomatosis: Type I is the classical form, in which there is diffuse infiltration without the formation of grossly visible mass. In type II gliomatosis, the diffuse infiltrate is accompanied by a mass lesion.

Microscopically gliomatosis cerebri is characterised by diffuse and widespread infiltration of the central nervous system by neoplastic cells without destruction of pre-existing structures. Neoplastic cells seem to migrate along blood vessels and myelinated pathways in the white matter (Koestner et al. 1999). Gliomatosis cerebri is distinguished from astrocytomas by the more widespread neuroaxial involvement and relative preservation of brain architecture. Tumour cells are mostly elongated with long processes. Some tumours consist of oligodendrocyte-like cells, cells of transitional character, and small unclassified cells (Maxie and Youssef 2007). Peretti-Viton et al. (2002) characterised gliomatosis in humans as a heterogeneous glial proliferation with various degrees of anaplasia. Prognosis of gliomatosis is variable but for at least 50% of patients it is as poor as for glioblastoma (Herrlinger et al. 2002). The poor outcome for these patients and low survival rates was also mentioned by Bruna and Velasco (2010). Hilbig et al. (2006) demonstrated that most tumour cells were negative for GFAP even though there were foci of positivity for this marker. However, the authors detected the presence of many cells positive for nestin and vimentin. This may indicate that the tumour cells originate from undifferentiated cells with a high degree of mobility.

Tumoral infiltration in the brain characteristically involves mainly the white matter, basal ganglia and thalamus, brainstem and less often hypothalamus (Peretti-Viton et al. 2002). In humans gliomatosis cerebri involving the brain and lumbosacral spinal cord (Yaguchi et al. 2003) or even affecting the entire neuraxis (Yip et al. 2003), has been diagnosed. Primary gliomatosis in the leptomeninges of the brain was described by Havlik et al. (2001), and gliomatosis in the leptomeninges of the brain and over the spinal cord was reported by Baborie et al. (2001). The same tumour in the leptomeninges adjacent to the lumbar spinal cord was observed by Bilic et al. (2005).

Porter at al. (2003) described gliomatosis cerebri in six dogs. Histologically there was a remarkable diffuse infiltration of the white and grey matter of the brain by small numbers of elongated neoplastic cells. Areas of greater cellularity that formed grossly visible lesions were described in four cases. Neoplastic cells were negative in immunohistochemical stains for GFAP and leukocyte markers. Gruber et al. (2006) described gliomatosis cerebri in a 9-year-old, male, flat-coated retriever. Galan et al. (2010) have diagnosed oligodendroglial gliomatosis in a 6-year-old male Poodle dog. Sant'Ana and Barros (2011) published a case of gliomatosis in a dog. No gross findings were made in the brain but numerous neoplastic glial cells were observed throughout the thalamus, midbrain, pons and medulla oblongata. Severe multifocal proliferation of glial cells was also observed in the leptomenninges and white matter of the cerebellum. Plattner et al. (2012) observed gliomatosis cerebri in two dogs. Neoplastic cells were present in the medulla oblongata and cranial cervical spinal cord in one dog and in the T13 to L2 spinal cord segments in the second one. Woolford et al. (2013) reported the examination of ventricular and extraventricular ependymal tumours in 18 cats. The tumours formed predominantly intraventricular masses and usually arose from the lining of lateral or third ventricles, followed by the fourth ventricle, mesencephalic aqueduct, and spinal cord central canal. Histologically, 15 tumours were classified as ependymomas and three as subependymomas. Six ependymomas infiltrated the adjacent neural parenchyma. Although in this case the neoplasia surrounded and damaged in some segments the central cord canal, it did not form any masses and the histological structure was quite different from an ependymoma. Moreover, immunohistochemistry gave no evidence to suggest that the cells were neoplastic cells from the ependym. Boozer et al. (2012) investigated the presence of a variety of immune cell subsets within canine intracranial meningiomas. They demonstrated that immune cell infiltration was evident in all samples, with a predominance of $CD3^+$ T cells. In our case the perivascular cuffing also consisted of CD^{3+} T cells. This reaction very probably consists in the recognition of modified molecular structures on neoplastic cells by the innate immune system.

It is intriguing that gliomatosis in the brain primarily involves the white matter and structures surrounding the ventricular system but that in the spinal cord primarily grey matter which is situated around the central canal is affected. Gliomatosis of the leptomeninges also suggests some relation of this tumour to the ventricular system and the cerebrospinal fluid in the CNS.

To the author's knowledge this is the first report of gliomatosis of the spinal cord in a cat.

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