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Cerebrospinal fluid and smears of a lumbar subarachnoid lesion in a dog.

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Signalment:
'Jasper', 7 years old neutered male Miniature Schnauzer.

History:
Jasper presented to the University of Edinburgh's Neurology service for investigation of rapidly progressive paraplegia of approximately 2-week duration. Initially, Jasper presented to the primary veterinarian for sudden onset of hyporexia, reduced mobility and stumbling with right pelvic limb. The primary veterinarian noted that Jasper was pyrexic, but failed to report his body temperature in the clinical notes. Jasper was treated with meloxicam, ranitidine and amoxicillin-clavulanate. The following day the primary veterinarian noted an improvement in Jasper's appetite and normalization of the body temperature, however, 72 hours later Jasper started dragging the right pelvic limb. One week later Jasper presented with paraparesis and hyperaesthesia on palpation of the thoracic spine. Haematology and biochemistry profiles performed by the referring veterinarian were mostly unremarkable, apart from mild leukopenia (5.34×10^9/L, RI: 5.50-16.90), and mild increase in ALP (219 U/L, RI: 23-212) and moderate increase in ALT (331 U/L, RI: 10-100) most likely secondary to treatment with meloxicam.

Physical and neurological examination:
On presentation at the R(D)SVS Neurology service Jasper had mild hyperthermia (T = 39.1°C), mild bilateral submandibular lymphadenopathy and periodontal disease, and a body condition score of 6/9. The rest of the general physical examination was normal. On neurological examination Jasper was paraplegic, with increased extensor tone in both thoracic limbs and the left pelvic limb. Postural reactions were normal in the thoracic limbs and absent in the pelvic limbs. Spinal reflexes were normal in the thoracic limbs and exaggerated in both pelvic limbs, except for the right pelvic limb withdrawal reflex, which was reduced. The cutaneous trunci reflex was cut off at L4. Perineal reflex and tail tone and movements were normal. Pain was elicited on palpation of the lumbar spine.

Based on the Schiff-Sherrington posture and exaggerated pelvic limb spinal reflexes, the lesion was localized to T3-L3 spinal cord segments, however, due to the reduced muscle tone and withdrawal reflex in the right pelvic limb and cutaneous trunci cut off, a second lesion was suspected on the right side affecting the L6-S1 spinal cord segments or nerve roots.
Differential diagnoses included an infectious or immune-mediated meningomyelitis, multifocal intervertebral disc disease or neoplasia.

Diagnostic procedures:
Due to the rapid progression of the clinical signs and immediate unavailability of MRI, a CT scan of the thoracic and lumbar spine was initially performed to rule out intervertebral disc disease. Only mildly compressive extradural lesions (compatible with intervertebral disc protrusions) were observed at the level of T13-L1, L5-L6 and L7-S1. These findings could not explain the severity of the clinical signs, therefore a cerebrospinal tap was attempted at the lumbar location, first at L5-6 and when this did not yield any fluid, a second attempt was made in a more caudal site at the L6-7 space with less risk of penetrating the spinal cord. The needle was inserted three times in the

subarachnoid space and since no cerebrospinal fluid (CSF) could be collected, the content of the spinal needle was expelled on a slide and submitted for cytological examination. A cisterna magna CSF tap was then performed and the sample was submitted for analysis.

An MRI scan of the thoracic and lumbar spinal cord was performed 4 days after admission to better characterize the lesion. Brain and cervical spinal cord appeared normal. Along the lumbar spinal cord there were scattered multifocal, sharply marginated lesions showing marked solid enhancement post contrast medium administration, with associated cord swelling and oedema (Figure 1). The right hemicord appeared more extensively involved, and all the lesions involved the surface of the cord, with a dorsal meningeal component. The largest lesion was at the level of L2, affecting two thirds of the spinal cord width (Fig 2). At the level of the conus medullaris located over the L6-7 vertebral joint, there was marked intradural/extramedullary enhancement.
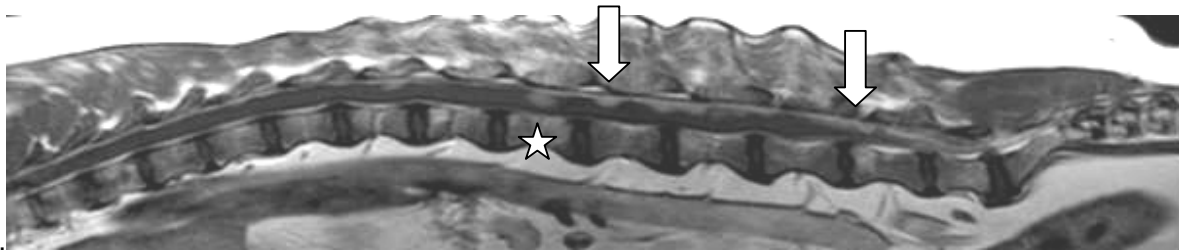

Figure 1.
Sagittal magnetic resonance image of the T3-S3 vertebral column T1W post contrast. Arrows indicate contrast enhancing meningeal lesions. The star indicates the L2 vertebra.
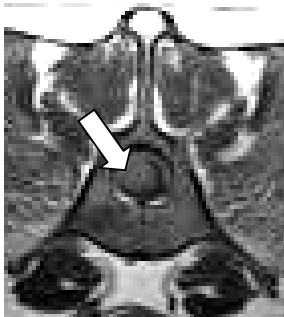
Figure 2.
T1W post contrast transverse magnetic resonance image at the level of the second lumbar vertebra. The arrow indicates the intramedullary contrast enhancing lesion that occupies ¾ of the spinal cord.

The diagnostic imaging diagnosis was multifocal solidly enhancing masses affecting the lumbar cord, with associated cord swelling and oedema, and distribution indicative of leptomeningeal/haematogenous spread.

CSF (cisterna magna) numeric results:

Nucleated cell count: 450 cells/μL (RI: <8)
Protein concentration: 207 mg/dL (RI: <36)

Images of CSF and L6-7subarachnoid space smears:

Figure 3:
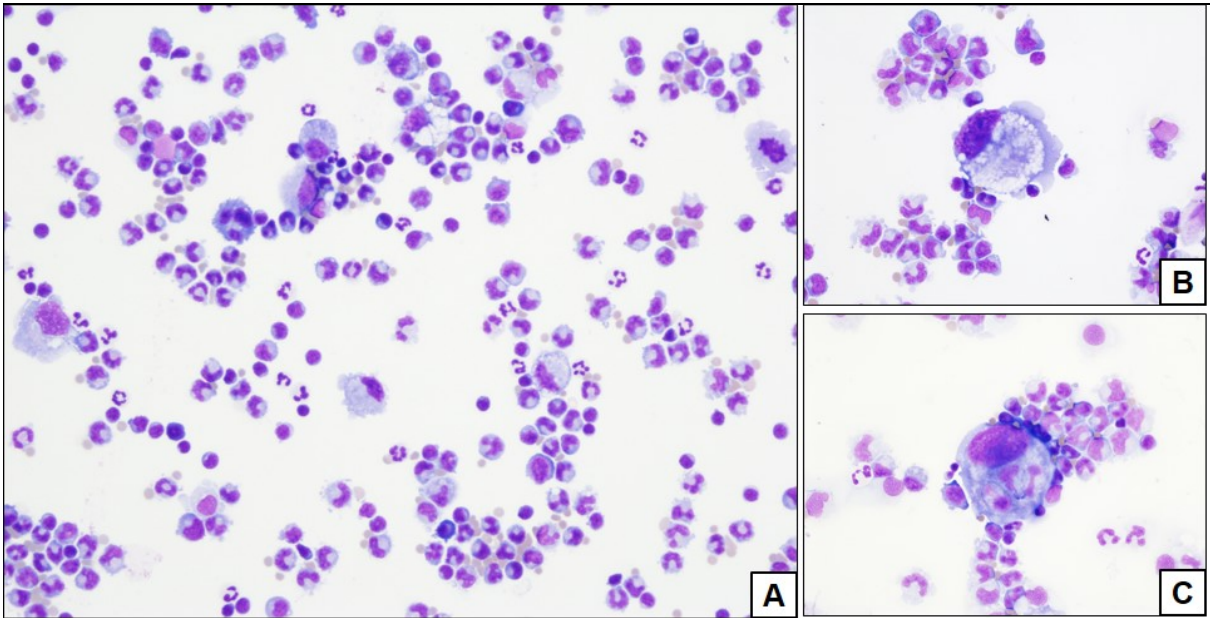Cerebrospinal fluid, cytospin preparation, May-Grünwald Giemsa. **(A)** x20 objective; **(B)** and **(C)** x40 objective.
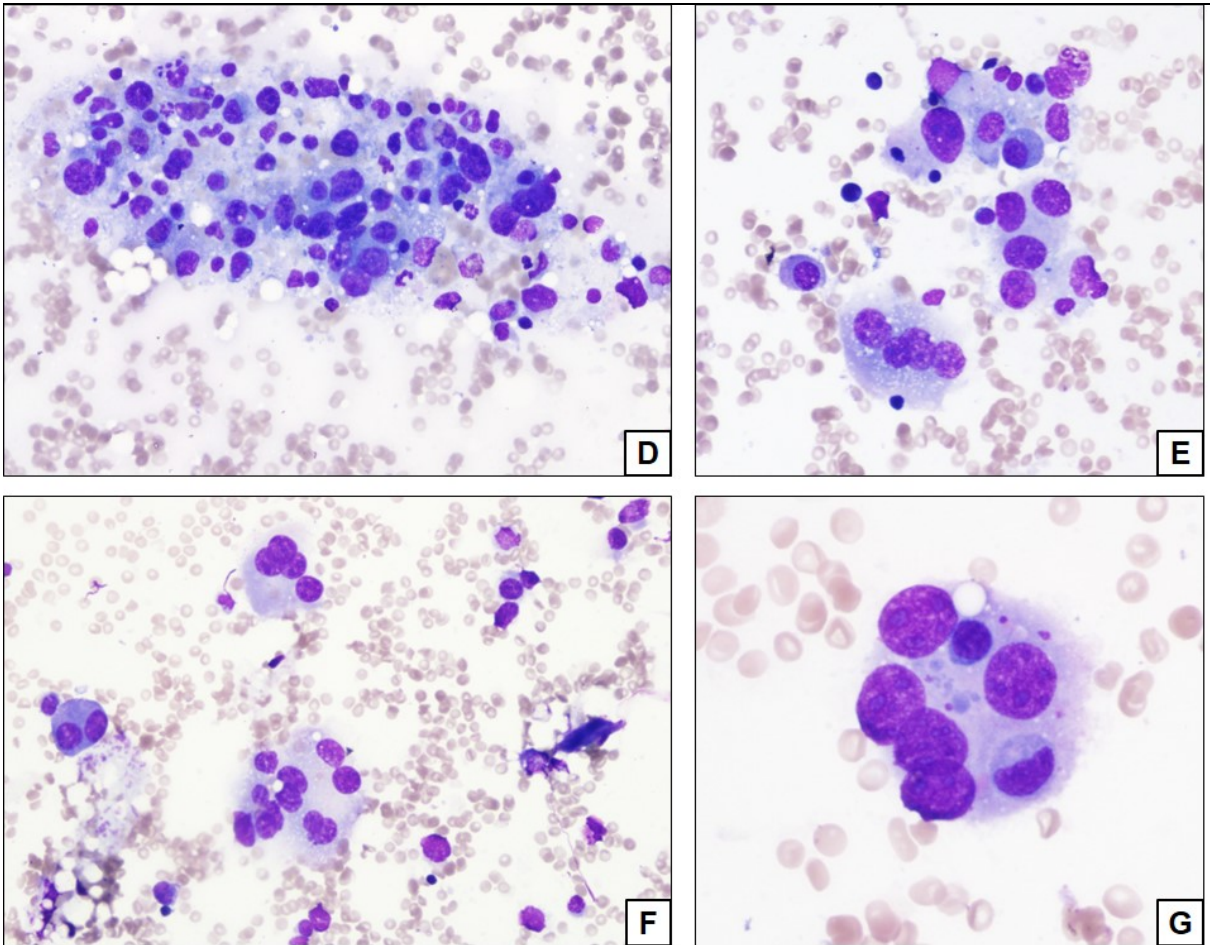

Figure 4:
Smears from the L6-7subarachnoid space, May-Grünwald Giemsa. **(D)**, **(E)** and **(F)** x20 objective; **(G)** x40 objective.

Questions:
1. What is your cytologic description and what are your main differential diagnoses?
2. What additional tests could be performed to further investigate the process?

Cytologic description:
The smears from the subarachnoid lesion were moderately cellular with a moderate background of erythrocytes and a few vacuolated macrophages. A population of medium-sized to very large atypical cells was observed. They were individualised or occasionally arranged in small, loose clusters, and varied from round to polyhedral with variably distinct cell borders. The cytoplasm was abundant, grey to medium basophilic, occasionally finely vacuolated and engulfed cellular debris and leukocytes. The nuclei were eccentric, round to oval to indented, with stippled to coarse chromatin and indistinct to multiple, variably sized and shaped nucleoli. Anisocytosis and anisokaryosis were marked, and many binucleated and multinucleated cells were observed, together with rare mitotic figures.

Cytospin preparations of the cerebrospinal fluid showed marked pleocytosis, with the nucleated cells composed of approximately 65% macrophages, 22% lymphocytes and 13% neutrophils. Small numbers of large atypical cells, similar to those described in the smears from the subarachnoid lesion, were also observed.

The cytologic diagnosis for the subarachnoid lesion was malignant poorly differentiated neoplasia. The main differential diagnosis was considered to be histiocytic sarcoma but poorly differentiated meningioma or metastatic carcinoma were also considered possible, due to the presence of clusters. Other differential diagnoses included lymphoma or tumours of neuroglial origin. The diagnosis for the CSF was marked mixed pleocytosis with presence of atypical cells.

Clinical outcome:
Given the diagnosis of neoplasia and suspicion of histiocytic sarcoma, treatment with lomustine and prednisolone (that might have resulted in improvement of histiocytic sarcoma and lymphoma) was started. After three days, due to the lack of response, the treatment was changed to cytarabine. Unfortunately, there was no improvement in the clinical signs, and the owners elected for euthanasia.

Gross necropsy:
On gross examination the most relevant findings involved the spinal cord. The lumbar spinal cord from L2 to the cauda equina was diffusely pale and swollen; focal wider areas were also identifiable at L2-3, L4-5 and L6-7. There were also 1-2 mm diameter, firm white raised lesions protruding dorsally over the disc spaces caudal to L3, and protruding ventrally at the L7-sacrum level (intervertebral disc disease). The liver was moderately enlarged, with a uniformly orange-red and friable parenchyma and the renal cortex appeared granular and friable on cut section. No other significant abnormalities were evident in the cadaver, including the brain and pituitary gland.

Histopathology:
On histopathological examination of the liver there was moderate vacuolar hepatopathy, likely to be consistent with glycogen accumulation secondary to corticosteroid therapy. The kidney had lesions of chronic membranous glomerulopathy and tubular degeneration.
Sections of lumbar spinal cord were taken caudal to the L5 nerve root, and cranial to the L4 and L2 nerve roots. The subarachnoid space, leptomeninges and locally extensive regions of the spinal cord were infiltrated by a population of pleomorphic atypical cells. These cells were round to

polygonal, with round to oval or indented, irregular nuclei. The chromatin was finely granular to clumped, and hypo- to hyperchromatic, with frequent multiple, prominent nucleoli. Many multinucleated cells were seen. The cytoplasm was eosinophilic to amphophilic, granular to finely vacuolated, and occasionally contained leukocytes, erythrocytes or cell debris. Anisocytosis and anisokaryosis were marked, and 9 mitotic figures per 10 HPF were seen. Moderate numbers of lymphocytes and plasma cells were admixed with these cells, and occasionally formed perivascular aggregates.

The atypical cells infiltrated the dorsolateral subarachnoid space and effaced the leptomeninges; they also surrounded the nerve roots and infiltrated the white and grey matter of the spinal cord, occupying up to two thirds of the section at the L2 level.

Sections of the thoracic spinal cord were also taken cranial to the T16 and T1 nerve roots. In these sections there was an infiltrate of atypical cells in the subarachnoid space surrounding the spinal cord and nerve roots, but no obvious invasion of the white matter of spinal cord or nerve roots.

Based on H&E-stained sections, the diagnosis was of poorly differentiated malignant neoplasia affecting the meninges and spinal cord. Differential diagnoses considered were histiocytic sarcoma and meningeal sarcomatosis, but T-cell lymphoma or meningioma were also considered to be possibilities.

Immunohistochemistry:
A panel of markers composed of CD18, MHC II, MAC387, PAX5, CD3, vimentin, pancytokeratin, smooth muscle actin, S-100 and GFAP was utilised to further characterise the tumour, using sections of the spinal cord at the level of L2.

Approximately 90-95% of the neoplastic cells strongly expressed CD18, vimentin and MHCII. The neoplastic cells were negative for all the other markers examined; PAX5 and CD3 confirmed the presence of a scattered mixed population of T and B lymphocytes admixed with the neoplastic cells and occasionally arranged in perivascular aggregates, where B cells predominated.

The final morphological diagnosis was of primary, central nervous system (CNS) histiocytic sarcoma.

Discussion:
Diseases arising from proliferation of histiocytic cells in dogs include reactive disorders (cutaneous and systemic histiocytosis, originating from interstitial dendritic cells), neoplastic disorders arising from Langerhans cells (cutaneous histiocytoma and Langerhans cell histiocytosis) and neoplastic disorders originating from interstitial dendritic cells (dendritic cell leukemia and histiocytic sarcoma). A particular form of histiocytic sarcoma is the haemophagocytic variant that arises from macrophages of the splenic red pulp. Histiocytic sarcoma can occur as a localised neoplasia, when it originates in a single tissue or organ, or be disseminated, when it spreads beyond the draining lymph nodes to distant sites (9).

Histiocytic sarcoma involving the CNS in dogs is reported infrequently. The CNS can be involved as a site of metastatic spread in disseminated forms of histiocytic sarcoma or be affected as a primary location. Interstitial dendritic cells are present in the meninges and choroid plexus; it arises most frequently in the leptomeninges, and the involvement of white and grey matters occurs subsequently. The lesions in the CNS can be present as focal subdural masses, but diffuse meningeal infiltrates are also observed (8, 9). One report (3) described the presence of two distinct patterns of lesions (focal mass lesion or diffuse leptomeningeal involvement) in a series of 15 cases. However, in a recent series (8), cases with coexistence of solid lesions and a diffuse leptomeningeal infiltrate are described.

In the present case, both patterns were present; there were solid lesions in the lumbar area, arising from the leptomeninges and invading the white and grey matter of the spinal cord. However, a diffuse meningeal infiltrate in the absence of concurrent solid masses was also observed, with a widespread diffuse meningeal infiltrate extending at least to the T1 level and possibly further proximally. Histopathological examination of the cervical spinal cord and brain would have been required to fully investigate the extent of the meningeal infiltrate. In the present case, the presence of neoplastic cells in the CSF sample from the cisterna magna supported the hypothesis of possible spread of the neoplastic cells through the subarachnoid space via cerebrospinal fluid circulation (8) to areas of the CNS cranial to the most proximal spinal cord section (T1) examined.

The presence of neoplastic cells in CSF has been reported in a variety of primary and metastatic tumours involving the CNS, including lymphoma, plasma cell tumour, choroid plexus carcinoma, medulloblastoma, mammary carcinoma, and histiocytic sarcoma (1). In a report that reviewed the characteristics of cisternal CSF in 56 dogs with intracranial meningioma (2), no neoplastic cells were observed. The presence of neoplastic cells in CSF in cases of histiocytic sarcoma has been recorded in several reports (11, 13), but is not a constant finding. In a recent case series (8) of 19 cases of histiocytic sarcoma with CNS involvement, CSF analysis was performed in 6 patients; pleocytosis was present in all the samples, but in none of them were neoplastic cells observed.

In the present case, atypical neoplastic cells were observed both in the smears obtained from the subarachnoid lesion at the L6-L7 level and the CSF sample from the cisterna magna. The morphological appearance of the cells, with prominent cytological atypia and presence of leukophagocytosis, was suspicious for histiocytic origin; however, in the smears from the lesion, the cells occasionally showed a mild degree of cohesiveness, and a few small epithelial-like clusters were observed. Therefore, the possibility of an atypical, malignant form of meningioma or metastatic spread of a carcinoma were still considered to be possible differential diagnoses. These aggregates could have represented accidental sampling of residual normal meningeal tissue during aspiration.

The results of the immunohistochemistry panel confirmed the diagnosis of histiocytic sarcoma. As expected for histiocytic sarcomas, the neoplastic cells were diffusely and strongly immunoreactive for CD18, MHC II and vimentin (9, 13). Lack of expression of PAX5 and CD3 ruled out the possibility of a lymphoma, while negative staining for pancytokeratin, S-100 and GFAP excluded the possible presence of an atypical form of tumours arising from neuroglia, meningiomas or metastatic carcinomas.

Based on histopathologic findings, meningeal sarcomatosis was considered another possible differential diagnosis in this case; the location and distribution of lesions, morphological appearance of the neoplastic cells and signalment of the patient overlapped with the described features of this tumour (5). Neoplastic cells in meningeal sarcomatosis are reported to sparsely and moderately express CD18 and actin (7). In this case the neoplasia showed strong and diffuse positive staining for CD18, and showed no immunoreactivity for actin, indicating a histiocytic origin of the cells. Reviewing the veterinary literature, reports of meningeal sarcomatosis date back to the 1980s (6, 10), and IHC was not performed as it was a less routine procedure at that time. Moreover, in one early paper (6), the neoplastic cells were described as 'histiocytes' despite the final diagnosis of meningeal sarcomatosis. The term 'meningeal sarcomatosis' could therefore reflect a different terminology used before the advent of reliable IHC techniques, and may indeed refer to neoplastic entities that nowadays would be classified as histiocytic sarcoma.

We also tested the expression of MAC387 (Monoclonal Mouse anti human Myeloid/Histiocyte antigen). This antibody highlights the intracytoplasmatic antigen calprotectin and has been shown to cross react with dog tissues. It is expressed in granulocytes, monocytes and macrophages, including early stages of monopoiesis and granulopoiesis (12). To our knowledge, there are no references to the use of this marker for histiocytic sarcoma in dogs; in a report of sarcoma arising from interdigitating dendritic cells in people, the neoplastic cells expressed this marker (4). In the present case, however, the neoplastic cells did not express this marker.

In this case, the spinal cord lesions were considered most likely to be the primary lesions: from imaging and gross examination there was no evidence of lesions or involvement of the brain, peripheral and visceral lymph nodes, and spleen. Histopathology in the liver confirmed absence of involvement in the neoplastic process although the vacuolar hepatopathy might have explained the mild increase in liver enzymes. However histopathology of brain, spleen, lymph nodes and bone marrow would have been required to completely rule out their involvement.

Survival times appear to be very short with currently utilised therapies for histiocytic sarcomas with CNS involvement, and the prognosis is very poor (8). In the present case, two different chemotherapy protocols were unsuccessful, and euthanasia was elected for a few days after presentation.
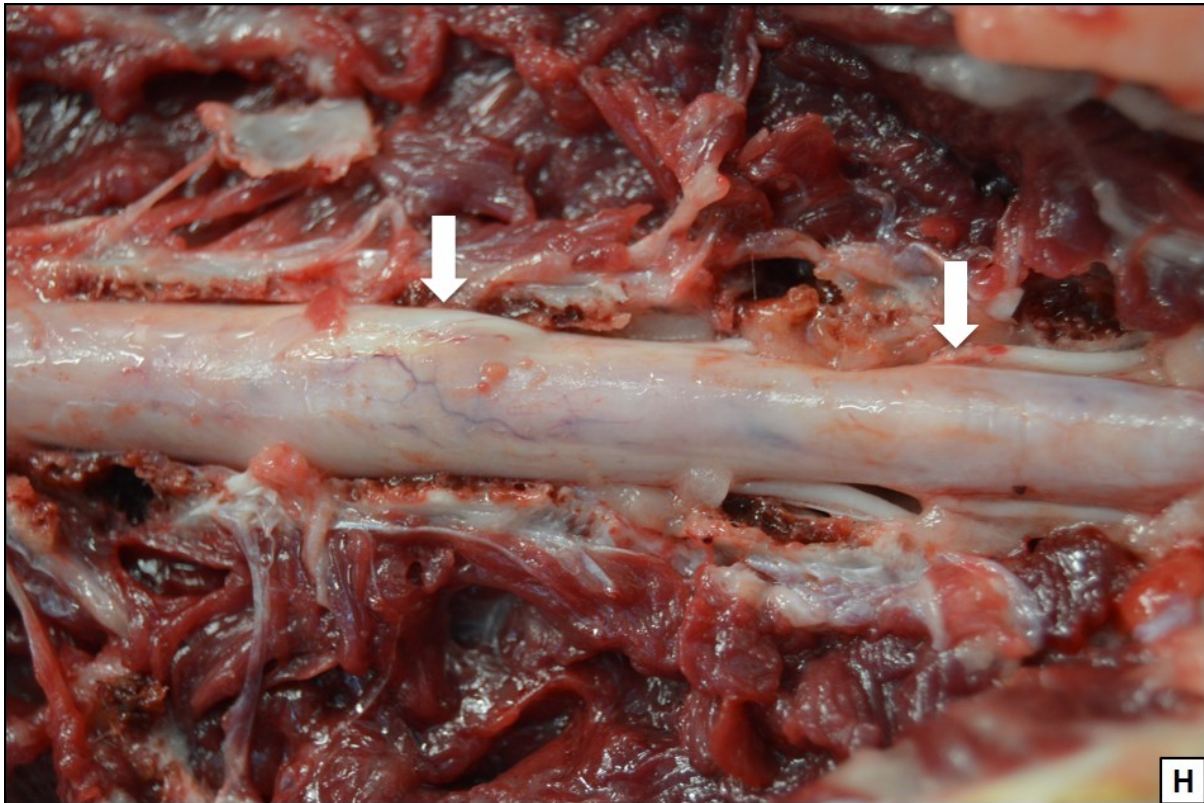
Additional pictures:

Figure 5:
Photograph of the spinal cord at the L3-L6 level. The spinal cord appears diffusely swollen and pale. Further enlargement is appreciable at the L4 and L6 level (arrows).
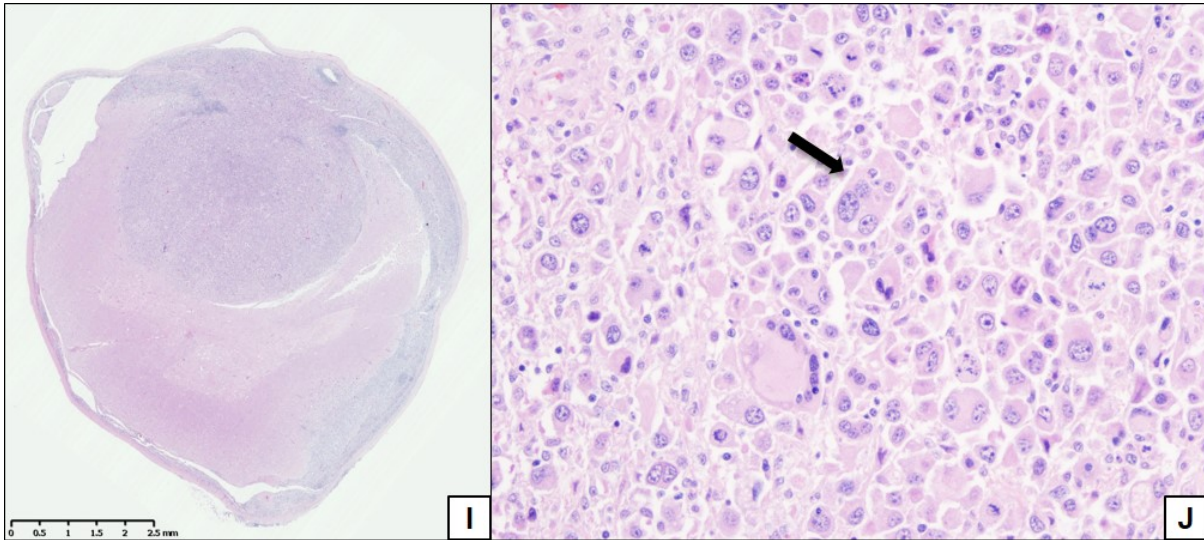
Figure 4:
Histological section of spinal cord cranial to L2 nerve root, H&E.
(I) x1 objective. An expansile infiltrate originating from the dorsolateral subarachnoid space extends around the whole meningeal layer and infiltrates the white and grey matter.
(J) x20 objective. The pleomorphic neoplastic cells show marked anisocytosis and anisokaryosis; many multinucleated cells are present, and they exhibit leukophagocytosis (arrow).
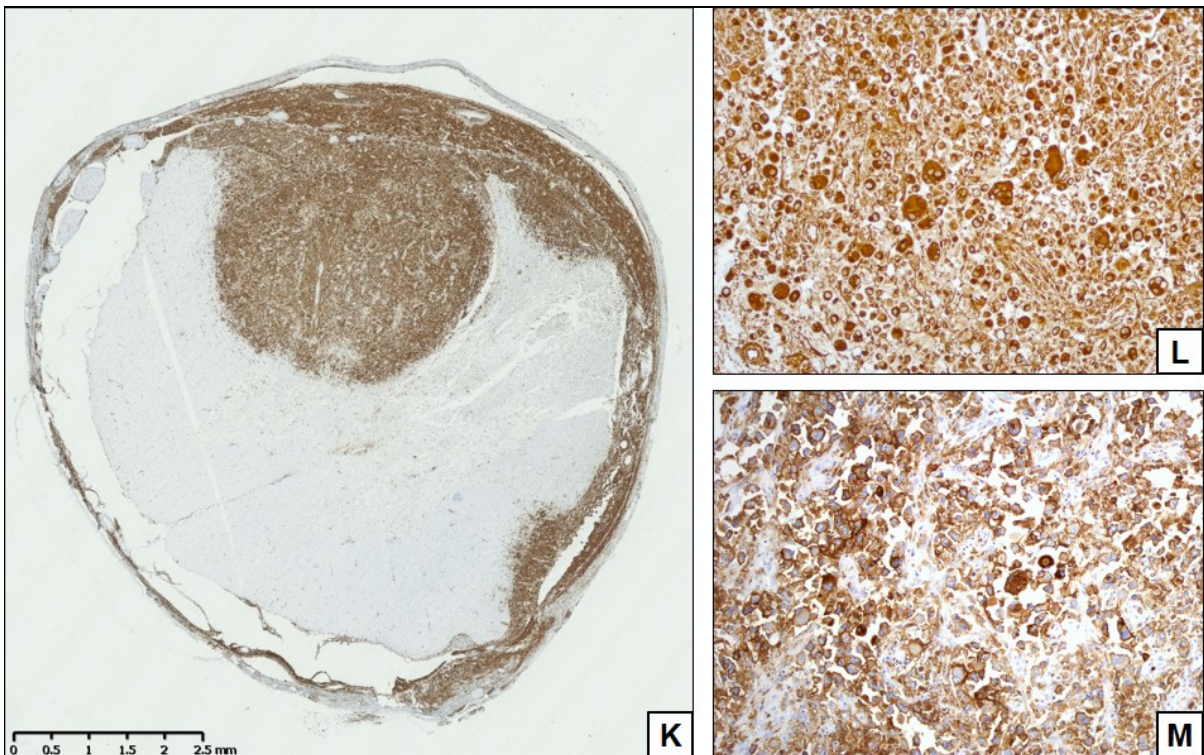

Figure 5:
Histological section of spinal cord cranial to L2 nerve root, positive immunohistochemical staining.
(K) MHCII, x1 objective. Presence of strongly immunoreactive neoplastic cells in the meninges surrounding the whole spinal cord section.
(L) Vimentin, x20 objective.
(M) CD18, x20 objective.

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