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CASE REPORT

Intradural-extramedullary haemangioblastoma with paraspinal extension in a dog

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Keywords dogs; haemangioblastoma; immunohistochemistry; magnetic resonance imaging; spinal neoplasm

Abbreviations αSMA, α-smooth muscle actin; ABC, avidin-biotin-peroxidase complex; CT, computed tomography; GFAP, glial fibrillary acidic protein; INHα, anti-human inhibin-α; MRI, magnetic resonance imaging; NSE, neuron-specific enolase; PAS, periodic acid-Schiff; STIR, short tau inversion recovery

Case report An 8-year-old spayed female cross-breed dog was evaluated following a 2-month history of thoracic limb weakness. Neurological examination revealed a spinal cord lesion between C1 and C5 segments. Magnetic resonance imaging (MRI) revealed that almost 70% of the spinal canal between C1 and C2 was occupied by an intradural extramedullary mass that was connected to a paraspinal mass from the cranial aspect of C2 to the cranial aspect of C3. The dog was anaesthetised and a dorsal, right-sided hemilaminectomy was performed. A durotomy was performed to expose a multilobular mass located principally along the right dorsal-lateral aspect of the spinal cord. The mass did not appear to infiltrate the cord parenchyma. The abnormal tissue was removed as completely as possible using gentle dissection and submitted for histological evaluation. The histological findings were consistent with an intradural-extramedullary haemangioblastoma with paraspinal extension. Following surgery, no neurological deterioration was detected. A metronomic-dosing chemotherapy protocol was administered to prevent progression or recurrence of the tumour. Follow-up MRI studies were performed 3, 6 and 12 months after the surgery, confirming complete tumour removal and the absence of recurrence.

Conclusion Haemangioblastoma is an extremely rare neoplasm in animals and only two cases of this tumour have been reported, but in other anatomical locations. Haemangioblastomas in human patients are more commonly located in the cerebellum and intradural-extramedullary growth is extremely rare. The dog in this study responded favourably to combined surgery and metronomic chemotherapy and was clinically normal 1 year after surgery.

Haemangioblastoma is an uncommon neoplasm in humans, usually located in the cerebellum but can occur in all parts of the central nervous system.[1-3] Spinal cord localisation of a haemangioblastoma is unusual; most clinical reports of the disease in humans describe intramedullary tumours, whereas intradural extramedullary growth is extremely rare.[1,4-6] These tumours represent approximately 2-10% of all spinal cord neoplasms in people[1,5] and can occur sporadically or as a part of the Von Hippel-Lindau syndrome.[2,3,7] In the veterinary literature, only two cases of haemangioblastoma have been reported,[8,9] both in dogs. In one case, the neoplasm was diagnosed as intramedullary and was located at the level of the first thoracic vertebra[8] and in the other case the tumour had an intracranial location.[9] We report the clinical, neurological, and magnetic resonance imaging (MRI) findings, as well as pathological examinations and the clinical outcome of a spinal intradural-extramedullary haemangioblastoma in a dog that was successfully treated with a hemilaminectomy and metronomic chemotherapy.

Case report

An 8-year-old spayed female cross-breed dog was evaluated following a 2-month history of thoracic limb weakness. Physical examination revealed abrasions on the dorsal aspect of the distal thoracic limbs. Neurological examination revealed mild ataxia and delayed conscious proprioception in all limbs. Spinal reflexes and the cranial nerves were normal. In addition, a mild hyper-reflexia of limb reflexes was noted. No neck pain was observed during the clinical examination. Neurological signs were consistent with a spinal cord lesion between the C1 and C5 segments. Haematology and serum biochemistry analyses were within reference limits.

MRI of the cervical spine was performed with the dog under general anaesthesia using a 0.2-T magnet (Vet-MR Esaote SpA, Genoa, Italy). T1-weighted (pre- and post-contrast), T2-weighted and STIR sequences were performed in the sagittal, transverse and dorsal planes.

On the transverse plane at the level of the intervertebral foramen between C1 and C2, the MRI revealed that almost 70% of the entire transverse section of the spinal canal was occupied by a protruding mass (10 mm in diameter). The mass severely compressed the spinal cord on the right

side, inducing a spinal cord displacement (Figure 1A, D). Moreover, a paraspinal multilobular mass (20 mm in diameter) was observed adjacent to the right side of the column, extending from the cranial aspect of C2 to the cranial aspect of C3 and involving the obliquus capitis caudalis muscle (Figure 1B, C, E, F). Both masses showed moderate hyperintensity on T1-weighted images (Figure 1A-C), as well as discrete and heterogeneous hyperintensity on both T2-weighted and STIR sequences. After administering the contrast medium, gadodiamide (0.2 mL/kg; Omniscan 287 mg/mL, GE Healthcare, Milano, Italy), the masses showed strong and homogeneous enhancement (Figure 1D-F). In addition, at the dorsomedial margin of the paraspinal mass, small areas that appeared hypointense in all sequences were detected (Figure 1B, E). A schematic of the MRI findings is presented in Figure 2. Based on the MRI findings, the differential diagnoses included paravertebral intradural extramedullary neoplasm as a peripheral nerve sheath tumour, lymphoma, a myxoma/myxosarcoma, haemangioblastoma or metastatic tumours.

The dog was anaesthetised, and a right-sided hemilaminectomy was performed from the C1 to the C2 vertebrae, with an incision into the dural sac and arachnoid membrane. An extramedullary, firm, reddish, multilobular mass with several serpiginous draining vessels was found (Figure 3). No intramedullary extension of the mass was observed. The intradural mass was connected to an extradural paravertebral mass with the same macroscopic aspect. The paraspinal mass was surgically removed with wide margins of normal tissue (2 cm margins of grossly normal tissue around the mass). After excision, both masses were submitted for histopathological examination. The dog recovered well after surgery and no neurological deterioration was observed. At a clinical examination performed 30 days after surgery, no neurological abnormalities were detected and the animal was discharged.

The excised masses were fixed in 10% buffered formalin, processed routinely and embedded in paraffin wax for histological and immunohistochemical examinations. Sections were stained with haematoxylin and eosin and periodic acid-Schiff (PAS). Immunohistochemistry was performed using the avidin-biotin-peroxidase complex (ABC) method (Vectastain ABC Kit, Vector

Laboratories Inc., Burlingame, CA, USA). The primary antibodies used included rabbit polyclonal antibodies against glial fibrillary acidic protein (GFAP, 1:2000, Dako Denmark A/S, Glostrup, Denmark), S-100 protein (1 : 10,000, Dako) and factor VIII-related antigen (1 : 200, Dako), and mouse monoclonal antibodies against vimentin (clone 3B4, 1:1000, Dako), neuron-specific enolase (NSE, 1:25, Dako), pan-cytokeratin (AE1/AE3, 1:2000, Zymed Laboratories Inc., San Francisco, CA, USA), α-smooth muscle actin (αSMA, clone 1A4, 1:2000, Dako), anti-human inhibin-α (INHα, clone R1, 1:40, Serotec Co., Oxford, UK) and CD31 (clone JC/70A, 1 : 20, Dako).

Histological evaluation of the resected tumour revealed an encapsulated, densely cellular, highly vascularised neoplasm composed of haphazardly arranged small capillaries lined by plump endothelial cells and a small number of thin-walled blood vessels. Capillaries were separated by numerous large pleomorphic polygonal and spindle cells that were 40-100 μm in diameter and had indistinct cell borders, a low nuclear to cytoplasmic ratio and abundant lightly eosinophilic cytoplasm, often containing optically empty vacuoles (Figure 4A). Nuclei were round to oval and 20-40 μm in diameter; they also had marginated to finely granular chromatin and one central magenta nucleolus. In a small portion of the mass, large polygonal cells were arranged in larger sheets or clusters with fewer capillaries. Severe anisocytosis and anisokaryosis, as well as scattered multinucleated cells, were occasionally present. There were 4-5 mitoses were observed per 10 high-power fields (×400). The PAS reaction strongly highlighted the basement membranes of vessels, but stromal cells were negative for uptake (Figure 4B). Histological findings were consistent with a diagnosis of an intradural-extramedullary haemangioblastoma.

Immunohistochemical staining with factor VIII-related antigen and CD31 antibodies was positive in all endothelial cells, confirming the presence of many capillary vessels, whereas neoplastic stromal cells were uniformly negative. Stromal cells did not exhibit immunoreactivity to αSMA, but vessel walls were diffusely positive (Figure 4C). Stromal cells stained strongly and diffusely positive with vimentin (Figure 4D) and were multifocally positive for GFAP (Figure 4E), NSE (Figure 4F) and

S-100, with a highly variable pattern throughout the tumour. Both endothelial and stromal cells were negative for pan-cytokeratin and INHα. The immunohistochemical examination confirmed the diagnosis of haemangioblastoma, as the observed characteristics closely resembled those reported in humans.

After the diagnosis, a metronomic-dosing chemotherapy protocol was planned to prevent progression or recurrence of the tumour. Endoxan/cyclophosphamide (Baxter Healthcare, Deerfield, IL, USA), which is a drug with antiangiogenic effects, was administered at the dose of 9 mg/day for 180 days. The treatment was well tolerated and no adverse effects were observed. Follow-up MRI studies were performed 3, 6 and 12 months (Figure 1G-I) after the surgery, confirming complete tumour removal and absence of recurrence.

Discussion

Haemangioblastoma is considered to be a benign tumour in humans, despite the morbidity and mortality caused by compression by the mass.[10] In the present veterinary case, although the mass occupied nearly the entire vertebral canal, causing severe compression of the spinal cord, the dog had an excellent recovery after surgery, with a return to normal neurological status and no recurrence after 1 year. Complete surgical excision of the neoplastic mass, therefore, appears to be the procedure of choice, but an accurate preoperative diagnostic imaging evaluation of the tumour location is important to ensure a good surgical outcome. Moreover, the present report suggests that pairing surgery with metronomic chemotherapy may be a treatment option for dogs with a haemangioblastoma. Metronomic chemotherapy is a treatment characterised by low doses of chemotherapeutic drugs and is administered to the patient to prevent or delay the progression of cancer.[11,12] The positive effects of metronomic chemotherapy are well known in humans, resulting in a delay of tumour growth. This therapy has also recently been evaluated for the treatment of various types of tumours in dogs.[13-16]

Cyclophosphamide has a well-known antiangiogenic effect without any high-grade toxicity.[14,15] During the treatment of haemangioblastoma, which is a highly vascularised neoplasm, continuous

administration of low doses of this drug can inhibit mobilisation of endothelial progenitor cells. In addition, metronomic chemotherapy stimulates the production of thrombospondin-1, a potent endogenous angiogenesis inhibitor.[14,15] Although haemangioblastoma is considered to be a benign tumour in humans, continuous dosing with cyclophosphamide could be beneficial in cases of an incompletely resected tumour. A similar therapeutic approach that relies on antiangiogenic drugs has been reported in humans with haemangioblastomas.[17]

Spinal neoplasms are categorised according to the anatomical area involved, relative to the dura and spinal cord.[18] In the present case, the tumour developed within the dura, but outside the proper spinal cord, with a final diagnosis of intradural-extramedullary neoplasm.[18] In human medicine, distinguishing an intramedullary and extramedullary mass with large extramedullary tumour components that compress the spinal cord from an intradural-extramedullary tumour may not be easy, despite the use of a high-field MRI system. Failure to detect an intramedullary tumour may lead to misdiagnosis and the potential for complications during surgery.[1]

In veterinary medicine, where high-field MRI systems are not widely used, identifying the exact location of a spinal tumour can be extremely difficult, especially to discern between intramedullary-extramedullary and intradural-extramedullary tumours.[1,19] The most important features detected on MRI that can be used to differentiate an intramedullary-extramedullary tumour from an intradural-extramedullary tumour are the presence of a syrinx or oedema that has spread lengthwise along the craniocaudal sides of the tumour and the presence of the 'snowman sign' on transverse images. An intramedullary-extramedullary tumour provides strong signals for both the extramedullary and intramedullary components; this finding, referred to as the 'snowman sign', is considered to be a characteristic that can be used to discern between intramedullary-extramedullary tumours and tumours limited to the extramedullary region (Figure 2). Haemangioblastoma is a relatively soft tumour and, in the case of a purely extramedullary neoplasm, the spinal cord is displaced only from the extramedullary side, with a smooth boundary line between the tumour and the spinal cord. A similar MRI finding was observed

in the present case (Figure 1D), but MRI did not reveal the presence of syrinx and/or oedema or findings suggestive of the 'snowman sign.' These findings allowed the correct diagnosis of the tumour as an intradural and extramedullary tumour.

Other techniques that can be used to diagnose an intradural-extradural spinal mass include myelography, alone or in combination with computed tomography (CT). In veterinary medicine, myelography is a widely used radiographic technique that indicates the accurate location of compression sites along the neuraxis.

Haemangioblastomas typically exhibit an intradural/extramedullary pattern (golf-tee sign) with a minority of extradural, intramedullary, or indeterminate patterns.[1] In general, CT is no more successful than the original myelogram at characterising the tumour location with respect to the dura, but a CT myelography is necessary to define the location precisely.[20]

The most common differential diagnosis for tumours in this location is nerve sheath tumours, but other tumours with intradural and extramedullary locations and paravertebral extension reported in dogs include neuroepithelioma, nephroblastoma, lipoma/liposarcoma, myxoma/myxosarcoma, glioma, mixed germ cell tumour, lymphoma and other metastatic tumours.[18,21] The presence of small hypointense, round, well-defined areas, mainly at the dorsomedial margin of the tumour, suggest prominent vessels located directly adjacent to the tumour. These findings indicate draining vessels and are often observed in a haemangioblastoma.[19,22]

Histologically, a haemangioblastoma is a capillary-rich neoplasm characterised by the presence of two main components: large lipid-containing polygonal interstitial cells (stromal cells) and an abundant, randomly oriented capillary network lined by normal to plump endothelial cells.[23]

Stromal cells are considered to be the neoplastic component of the tumour, although their histogenesis is still debated.[23] Two histological variants of this tumour have been recognised in human medicine: the reticular variant, in which the stromal cells are evenly distributed around the vascular network and the less common cellular variant, in which the stromal cells are arranged

in larger sheets or nests.[23] Both of these variants were observed in the present case; most of the tumour was characterised by a typical reticular pattern, although a small cellular area contained large, pleomorphic stromal cells arranged in sheets and incompletely surrounded by small capillaries.

Haemangioblastomas exhibit a typical histological pattern and haematoxylin-eosin staining is crucial for diagnosis, although several immunohistochemical studies have explored a variety of markers to identify antibodies that are highly sensitive to, and specific for, haemangioblastomas. However, none of them has been considered to show independent diagnostic value.[24] Recent studies reported that stromal cells show variable patterns of immunoreactivity for numerous markers expressed in the neuroectoderm, such as NSE, S-100, GFAP and vimentin, whereas stromal cells are invariably negative for cytokeratin.[23,25] These findings are in accordance with the results obtained in the present study and a similar immunohistochemical panel of markers could help confirm the diagnosis.[3,24] Recently, INHα immunoreactivity was observed in a haemangioblastoma, with cytoplasmic expression in the stromal cells.[2,3,25,26] INHα is typically expressed in sex cord-stromal tumours, but recent studies have shown that this antibody is also a useful marker for the confirmation of haemangioblastomas.[2,3,24] In the veterinary literature, only two cases of haemangioblastoma have been reported and neither included INHα in the immunohistochemical panel. In the present study, stromal cells were negative throughout the tumour. Although this finding was unexpected, it has previously been reported that a subset of haemangioblastomas should be negative for this marker.[3,24,25] The present study is the first report to examine the expression of INHα in haemangioblastoma and further study is needed to better understand the expression of specific markers in this rare tumour.

Conclusion

Haemangioblastoma is an extremely rare neoplasm that should be considered as a differential diagnosis in dogs with intradural-extramedullary masses. A detailed neurological examination

and imaging are necessary to detect this rare disease. Moreover, a thorough histopathological examination of the mass is required to make a definitive diagnosis of haemangioblastoma.

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Legends

Figure 1. Magnetic resonance imaging (MRI) of the upper tract of the cervical spine. Right is to the left side of the images. (A-F) MRI examination performed prior to surgery. (G-I) MRI performed 12 months after surgery. Transverse T1-weighted images (A, B) and transverse T1-weighted post-contrast images (D, E) were acquired at the C1-C2 junction. Images C and F are dorsal T1-weighted pre- and post-contrast administration, respectively; image I is dorsal T1-weighted acquired 12 months after surgery. Dotted lines represent 'references lines' indicating the position of the transverse images on the dorsal plane. On T1-weighted pre- (A-C) and post-contrast images (D-F), the vertebral canal is almost completely occupied by an intradural, ill-defined and slight hyperintense mass (* A, C) that shows strong homogeneous contrast enhancement (* D, F). Dural enhancement was also present (black arrow; D). A smooth boundary line between the mass and the spinal cord, suggestive of intradural-extramedullary pattern, can be seen (arrowheads; D). Caudally, the mass becomes wider and is located outside of the spinal canal into the m. obliquus capitis caudalis (white arrows; B, E, C, F). Note the hypointense areas at the dorsomedial margin of the paraspinal mass (open arrow; B, E) suggestive of tumour vessels. In the postoperative images (G-I), there is no evidence of tumour recurrence.

Figure 2. Schematic of MRI images along the transverse plane for various locations of haemangioblastoma. Intramedullary-extramedullary tumours are characterised by a 'snowman sign,' whereas a smooth boundary line between the tumour and the spinal cord indicates an extramedullary tumour. MRI, magnetic resonance imaging.

Figure 3. Intraoperative photograph during hemilaminectomy from C1 to C2 through an incision into the dural sac and arachnoid membrane, revealing an extramedullary, firm, multilobular mass with several serpiginous draining vessels.

Figure 4. Histopathological features of a canine intradural-extramedullary haemangioblastoma. (A) Numerous large, pleomorphic cells with optically empty vacuoles can be seen, separated by numerous thin-walled blood vessels (H&E; bar = 150 μm). (B) Positive periodic acid-Schiff

reaction strongly highlights the basement membranes of the vessels, whereas stromal cells are negative (bar = 75 μm). (C-F) Immunohistochemistry using the avidin-biotin-peroxidase complex detection method with Mayer's haematoxylin counterstain. The vessel walls are diffusely positive to smooth muscle actin, but stromal cells are diffusely negative; bar = 75 μm (C). Stromal cells stain strongly and are diffusely positive for vimentin (D), glial fibrillary acidic protein (E) and neuron-specific enolase (F); bar = 150 μm (D-F).

Figures

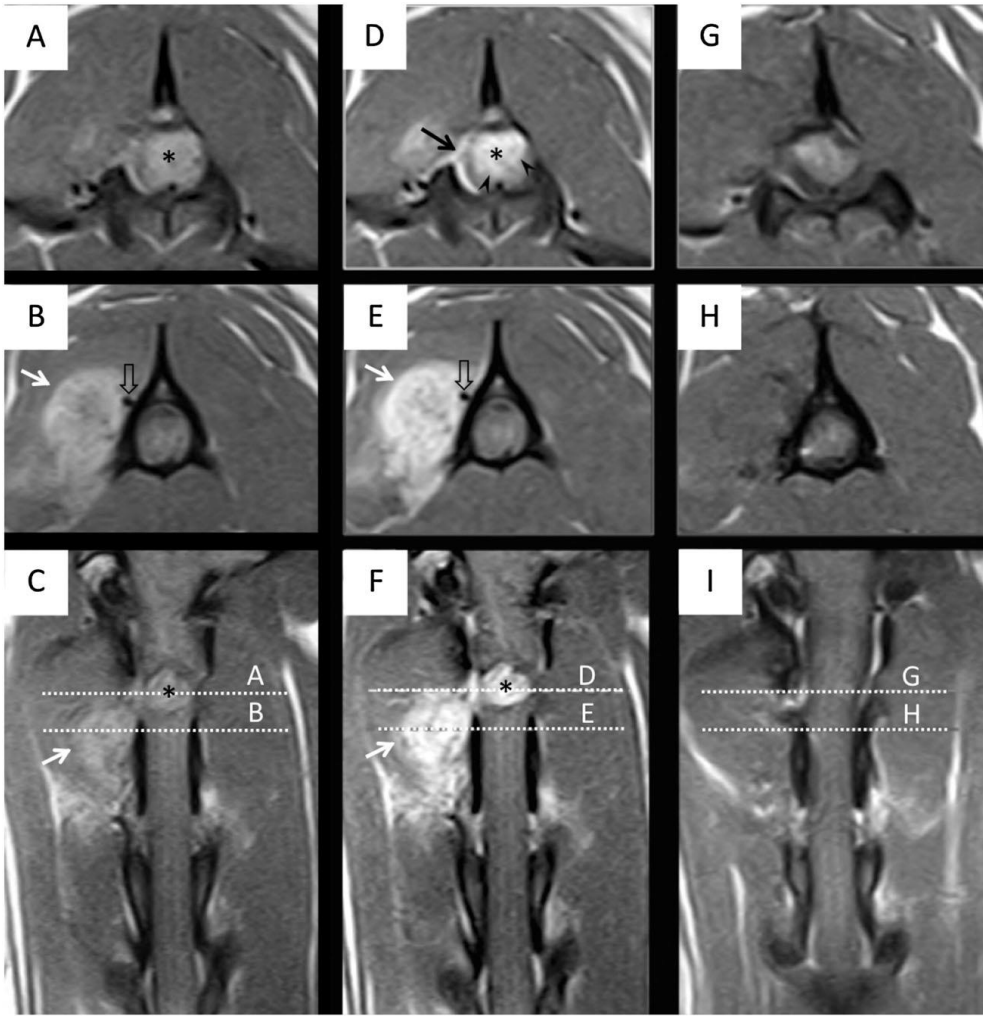

Figure 1

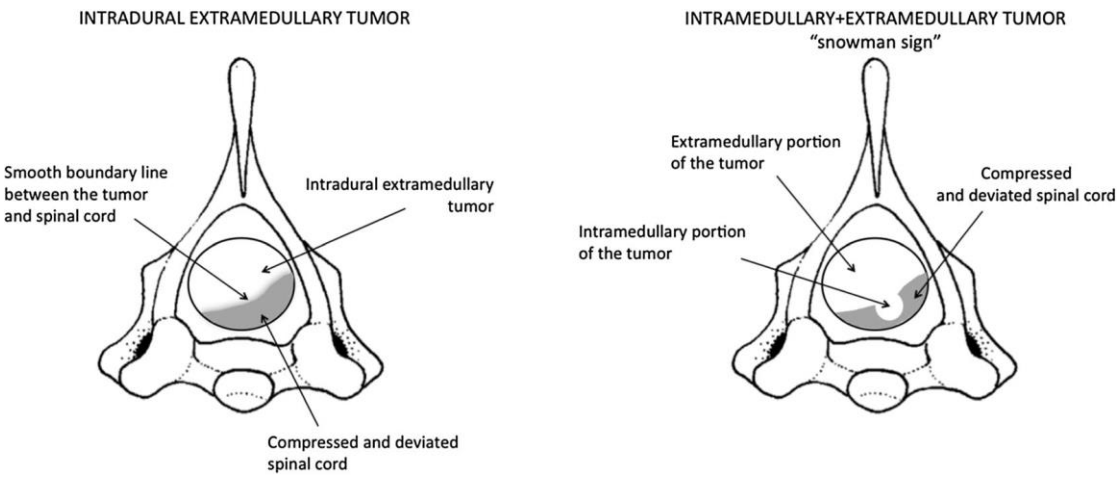

Figure 2

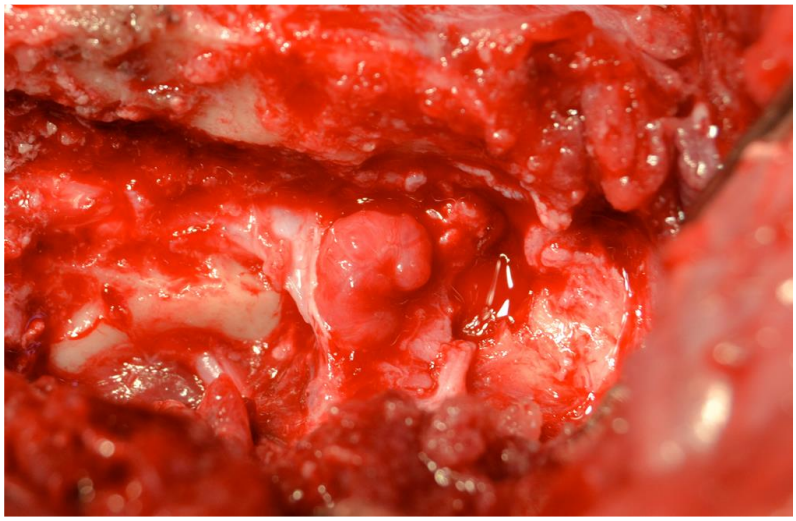

Figure 3

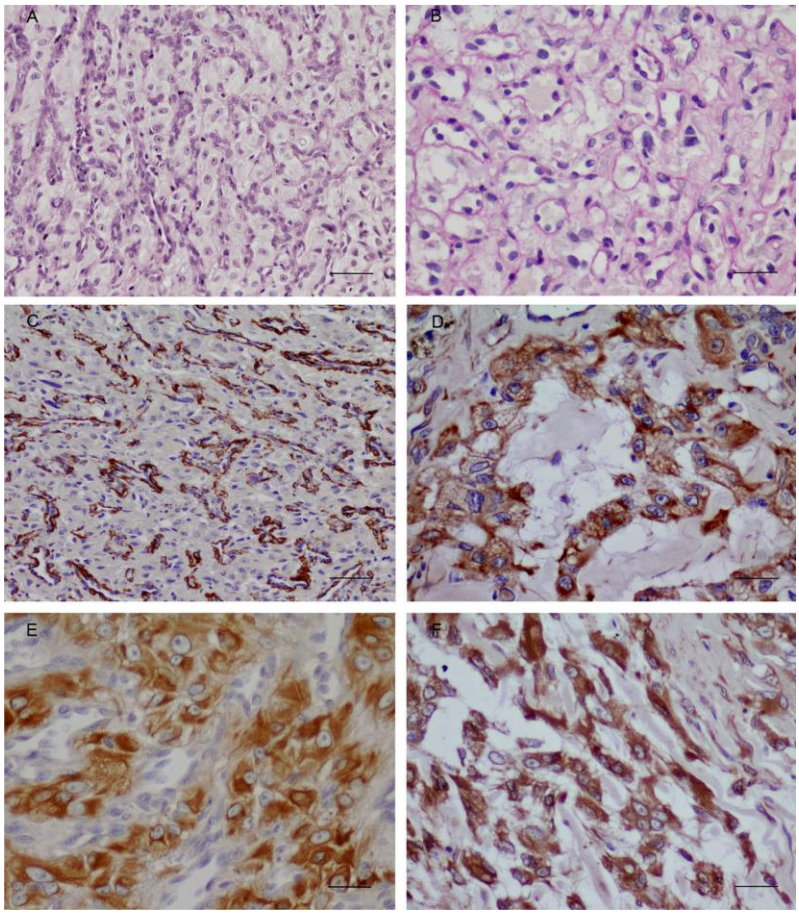

Figure 4