Case report: generalized haemangiosarcoma in a cat

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SUMMARY
This case report presents a generalised haemangiosarcoma in a 12 year-old male mongrel cat. A voluminous tumour mass with a soft consistency and a dark red-blackish colour at the cross section was found in the scapular area. Numerous other masses with variable dimensions were also seen in many subcutaneous areas as well as in the cardiac right atrium, kidneys, lymph nodes and cerebellum. Histopathological examination revealed capillary or cavernous vascular channel structures into masses. In addition, some papillary structures consisting in hyalinoid collagen fibres and fibrous bands extended into the vessel lumens. The neoplastic endothelial cells generally belonged to the epitheloid type. Immunohistochemically, neoplastic endothelial cells strongly reacted with anti-CD31 antibodies whereas the intensity of the CD34 and factor VIII positive immunolabelling was weaker and fluctuated according to the anatomical sites. This case showed the presence of numerous haemangiosarcoma metastases into subcutaneous tissues and various organs, particularly in cerebellum, although this tumour type is known to be rare in cats and that localisation into the central nervous system remains exceptional in this species.

Keywords: Cat, haemangioepitheliosarcoma, metastasis, cerebellum, subcutaneous tissue, endothelial cells, immunohistochemistry, factor VIII, CD31, CD34.

Introduction
Haemangiosarcoma (HSA) is a malignant tumour that derives from the endothelial cells of blood vessels [3, 7]. Haemangiosarcomas are very common in dogs, and particularly in the Golden retriever and German shepherd breeds, whereas they are encountered less frequently in cats [1, 3, 7]. In cats, haemangiosarcomas constitute less than 2% of all non haemapoietic neoplasms [9]. Subtypes, namely visceral and non visceral, are classified according to their respective localisation. Non visceral haemangiosarcomas are localised in cutaneous and subcutaneous regions, whereas visceral neoplasms develop either as primary or secondary tumours resulting from metastases mainly in spleen, liver and heart [1, 3, 4, 7, 9, 10, 14, 17]. Primary and secondary haemangiosarcomas of the central nervous system have also been reported to be rare in cats [3, 13-15]. The present study describes the case of a haemangiosarcoma in the central nervous system in a cat and its characterisation by pathological and immunohistochemical analyses.

Materials and Methods
A 12 year-old male mongrel cat was submitted to the Department of Pathology, Faculty of Veterinary Medicine, University of Ankara (Turkey) for necropsy. After gross examination, various tissue samples, particularly from brain, heart and lymph nodes, were fixed in 10% buffered formaldehyde, subjected to the routine pathology processes, and embedded in parafin. Tissue sections with 5 μm thickness were stained with the specific Haematoxylin-Eosin (HE), and also with the Masson’s trichrome and the Gridley’s stain [11].

When applying immunohistochemical staining, tumour markers (von Willebrand factor (VIII), CD31, CD34) were used for the determination of endothelial cell characteristics. The cross sections were firstly incubated in 3% H2O2 for 20 minutes for blocking endogenous peroxidases, then, after washing with PBS, they were treated with 1.5% goat serum at 40°C for 20 minutes in order to prevent non-specific protein binding. Subsequently, the sections were incubated with
the different primary polyclonal antibodies diluted in PBS to 1:100 (Mouse anti-human F VIII-RAg, mouse anti-human CD31 and mouse anti-human CD34) at 37°C for 30 minutes. After again washing with PBS, sections were incubated for 30 minutes with biotinylated goat anti-mouse immunoglobulin G (dilution: 1: 200) at room temperature (23°C). After washing again, the immune complexes were detected by the avidin-peroxidase complex using 3-amino-9-ethylcarbazole (AEC substrate-chromogen, DAKO, Denmark) as chromogen. Counterstaining was performed with Mayer’s haematoxylin. Negative control sections were treated as described above except that primary antibodies were substituted by the PBS solution (DAKO, Denmark) [6, 9].

**Results**

**MACROSCOPIC FINDINGS**

At necropsy, a dark red-blackish coloured subcutaneous mass (5 x 4 x 2.5 cm³) with a soft consistency was observed on the left scapula (figure 1a). Numerous similar structures subcutaneously localised were also observed in various parts of the body (thoracic, abdominal and lumbar regions). The mass sizes ranged from 1.5 x 1 x 0.8 cm³ to 0.3 x 0.7 x 1 cm³. The cut surfaces of these structures appeared yellow to red and contained a multitude of cystic structures filled with blood or necrotic material.

The left popliteal lymph node and mesenterial lymph nodes were rather swollen and soft in consistency. A mass, soft in consistency, which measured 2.6 x 1.9 cm² was observed in the right atrium of the heart and had a red blackish cut surface. Small white foci, with a pinhead size, were found in both kidneys. The sagittal cut of the cerebellum revealed the presence of a dark red coloured mass, soft in consistency, in 3.7 x 2.2 cm² size which was embedded within the interior of the organ. Haemorrhages were evidenced within the cut surface of this mass (figure 1b).

![Figure 1: (a) Dark red-blackish coloured mass (arrow) on the left scapula; (b) Dark red coloured mass (arrow) inside the cerebellum.](image)

**MICROSCOPIC FINDINGS**

Capillary and cavernous vascular structures composed of neoplastic endothelial cells enclosed in a thin stroma made of connective tissue were observed within subcutaneous regions, in the right atrium of the heart, in lymph nodes, kidneys and cerebellum (figure 2a). Furthermore, tubular vascular structures branching between partially hyalinised collagen fibres and connective tissue bands were observed in some regions and several papillary extensions extended into the lumen were evidenced (figure 2b). In certain parts, aggregated or scattered neoplastic cells and necrotic and haemorrhagic areas of varying sizes were present among the vascular structures. In some areas, the neoplastic cells were mainly endothelial cells belonging to the epitheloid type, characterized by a hyperchromatic nucleus and a vacuole-enriched cytoplasm (figure 2c). Moreover, mitosis was common in well-differentiated neoplastic cells. The Masson’s trichrome staining demonstrated evident mesenchymal stroma (figure 3a) and the reticular threads (brown stained with the Gridley’s reticulum stain) surrounding the vessels allowed easy identification of these endothelial structures (figure 3b).

On the other hand, the neoplastic endothelial cells of the epitheloid type were positively immunolabelled with anti-CD31 and anti-factor VIII antibodies. However, the intensity of the immunolabelling varied according to the tissue nature and to the marker nature. Globally, the positive reaction was the most intense with the anti-CD31 antibody and throughout the recognition of this membrane antigen, the membranes of the neoplastic endothelial cells were correctly visualised (figure 4). Cerebellum and subcutaneous tissues have given a strong positive reaction with CD31 antibodies, while heart, lymph nodes and kidney tissues were less positive. The strongest reaction was determined in cerebellum.

**Discussion**

Haemangiosarcomas are encountered in cats above the age of 8-10 years, without sex or breed predisposition [3, 9]. The present case concerns an old male cat (12 year old) and is in agreement with previous reports. Tumours may develop in more than one region and are frequently solid and rarely multicentric. In the present report, the main tumour localised within the cerebellum is reddish black in colour and presents a haemorrhagic appearance. However, the other tumours found in subcutaneous regions are soft and appear as spongy masses with weak borders, varying from 1 cm to 10 cm in length. In addition, the dissociation of these masses from their environment is difficult. The cut surface colour ranged from greyish white to yellowish red and numerous cystic structures filled with blood or necrotic debris are included within the tumour masses. The neoplastic endothelial cells, which histologically display frequent pleomorphism, contain a swollen and hyperchromatic nucleus which takes various forms (from spindle to polygonal or oval). These cells, which frequently exhibit mitotic figures, are found either in the large cavernous structures or in solid cell groups containing numerous small capillary beds or fissures [1, 3, 7, 10, 13]. The macroscopic and microscopic findings obtained in the present case are in agreement with the haemangiosarcoma characteristics found in the literature.

The primary location of this tumour in cats is mostly the spleen. Metastases may occur in liver, kidneys, intestines,
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heart (right atrium), omentum, pancreas, diaphragm, muscles, bones, nasal cavity, eyes, lymph nodes, mediastins and mesenteries, skin and in central nervous system. In the present report, tumour lesions are evidenced in the subcutaneous tissue, in heart (right atrium), lymph nodes, kidneys and in cerebellum. Due to its multiple locations, the determination of the primary origin site generally proves to be a difficult task [1, 3, 4, 6, 10, 13, 14, 17]. Nevertheless, primary or secondary haemangiosarcomas located in the central nervous system and in the periphery nervous system are rare in both animals and humans [2, 5, 13-15]. For example, cerebral haemangiosarcomas in cats are limited to only a few cases [1, 3, 14]. The

![Figure 2: (a) Neoplastic endothelial cells formed blood-filled cavern (arrow) within the cerebellum, Haematoxylin - Eosin, Bar: 56 µm; (b) Papillary extensions (arrows) extended into the lumen, Haematoxylin - Eosin, Bar: 56 µm; (c) Neoplastic endothelial cells belonging to the epitheloid type (arrowhead), Haematoxylin - Eosin, Bar: 22 µm.](image1)

![Figure 3: (a) Mesenchymal stroma (arrows), Masson’s trichrome, bar: 40 µm; (b) Reticular threads (arrowheads) surrounding the vessels, Gridley’s reticulum stain, Bar: 40µm.](image2)

![Figure 4: CD31 antigen positive membranes (arrowheads) of the neoplastic endothelial cells lining vascular structure, Avidin-biotin-peroxidase method, Bar: 22 µm.](image3)
clinical interest of the present case is based on the evidence of haemangiosarcoma metastasis in a rare location, i.e. the cerebellum.

The CD31 and CD34 membrane antigens as well as the factor VIII are considered as classical vascular endothelium markers [6, 9]: positive immunolabelling with the both 3 markers is usually obtained, particularly in benign vascular tumours whereas the CD34 membrane protein and the coagulation factor are not always expressed in the case of malignant tumours [8]. Warren and Summers [16] have reported that some canine haemangiosarcomas do not react with anti-factor VIII antibodies and they stated that the degree of atypical behaviour of neoplastic cells may be inversely proportional to the factor VIII-positive reaction. Although the present case was morphologically and histologically diagnosed as a generalised haemangioendothelioma, all organs have not always given immunopositive reactions for the 3 markers; the heterogeneity of the immunohistochemical labelling according to tissues, appears to be relatively common. Indeed, it was reported that the expression of these 3 markers in normal human vascular endothelial cells may depend from the anatomical localisation and from the blood vessel type [12].

As a conclusion, a generalised haemangioepithelioma is diagnosed in an old cat based on histological and immunohistochemical criteria. This clinical case is out of interest because of the rarity in the cat of this type of malignant tumour and of this large dissemination to subcutaneous tissues and various organs, particularly within the cerebellum.

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**References**