Opis przypadku *Case report*

Kaposi-like vascular tumor of the cardiac muscle in a dog: morphological and immunohistochemical study

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Summary

An 8-year-old crossbred dog died suddenly with signs of shock. Necropsy revealed a cardiac tamponade caused by a perforating tumor in the right atrium. A similar tumor was found in the abdominal cavity. The histopathological and immunohistochemical examination showed that both tumors were composed of well-differentiated pseudo-vascular structures, with endothelial cells that were positive for vimentin and von Willebrand factor, pericytes that were positive for vimentin and smooth muscle actin, and various amounts of collagen and reticulin fibers. The mitotic activity of tumor cells was low to moderate. Both tumors were consistent with Kaposi's sarcoma that occurs in humans. On the basis of the histological features and immunohistochemical examination findings, both tumors were classified as Kaposi-like vascular tumors. This is the first report of a Kaposi-like vascular tumor with a unique, multicentric location and a fatal outcome in a dog.

Keywords: sarcoma, kaposiform hemangioendothelioma, hemangiosarcoma, immunohistochemistry

Kaposi-like vascular tumor, with morphological features of Kaposi's sarcoma and kaposiform hemangioendothelioma found in humans, is an extremely rare and controversial entity, with only a few reports in dogs (2). The first case of Kaposi's sarcoma in a non-human species was described in an immunodeficient dog after phosphate poisoning (11). Human Kaposi's sarcoma is a low-grade vascular tumor of endothelial origin associated with human herpesvirus 8 (HHV8) infections. The tumor is composed of abnormal vessels forming slit-like spaces surrounded by larger, ectatic vessels. In contrast to hemangiosarcoma, Kaposi's sarcoma lacks a significant number of mitoses, marked pleomorphism, or areas of necrosis (8). Immunohistochemical analysis has shown that the tumor cells of Kaposi's sarcoma express vimentin, vascular endothelial markers (factor VIII, CD31, CD34), and a selective lymphatic endothelial marker, podoplanin (4). Human kaposiform hemangioendothelioma possesses some of the histopathologic features of Kaposi's sarcoma, but is not associated with herpesvirus infections (9). Previous reports of canine Kaposi-like vascular tumors described the tumor as single or multiple dermal or submucosal masses. Central slits with peripheral cavernous spaces give the tumor an appearance dissimilar to any other canine vascular tumor (2). This is the first report of a Kaposi-like vascular tumor situated in the right atrium in a dog with a simultaneous presence of a tumor in the abdominal cavity.

Case description

An American Staffordshire terrier–crossbred dog (male, 8-years-old) was presented for examination because of clinical signs including loss of consciousness, pallor, decreased internal temperature (34.2°C), and a thready pulse. A complete blood count and biochemical panel revealed values within their reference ranges. Despite immediate physiological saline infusions and hydrocortisone administration, the patient died suddenly, approximately 40 minutes after presentation. Necropsy revealed cardiac tamponade caused by a perforating tumor located in the cardiac muscle in the area of the right atrium (Fig. 1). The tumor was poorly circumscribed and non-encapsulated, invaginating slightly into the lumen of the right atrium. The tumor had perforated the epicardium, but the internal, endocardiac surface was continuous. Moreover, necropsy also revealed concentric cardiac hypertrophy of the left ventricle and edema of the atrioventricular and semilunar valves. A similar tumor was located in the peritoneal adipose tissue in the midline of the abdomen, just caudal to the sternum. Other major gross findings were the consequences of shock (focal pulmonary

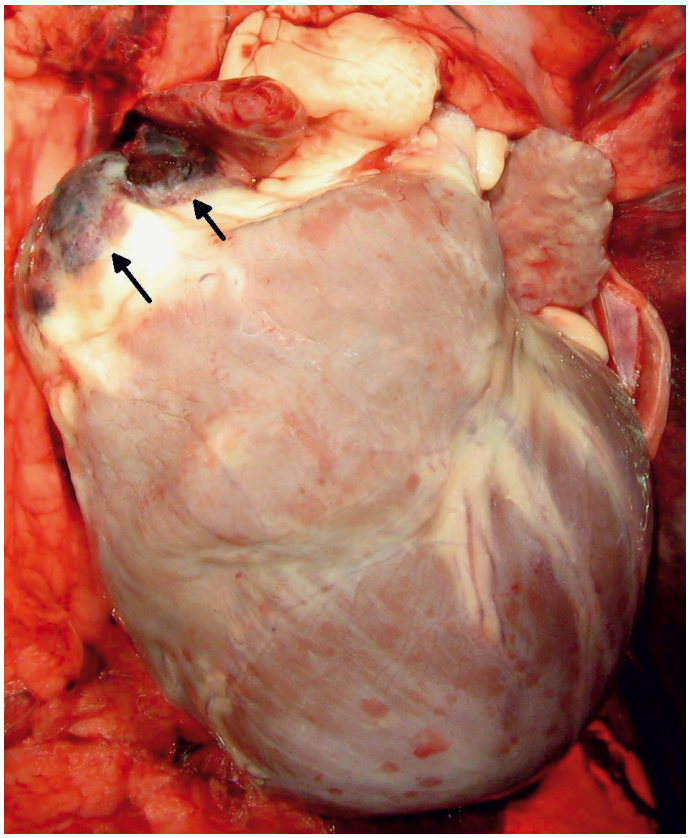

Fig. 1. Tumor located in the cardiac muscle of the right atrium, perforating the epicardium (arrows)

atelectasis and edema; pulmonary, hepatic and splenic congestion; and acute cortical necrosis with arcuate vessel congestion in the kidneys). The necropsy also revealed benign prostatic hyperplasia, catarrhal enteritis, gallbladder wall edema, chronic focal nephritis, and pleural adhesions.

Both tumors were sampled, fixed in 10% buffered formalin, processed by the routine paraffin technique, and stained with hematoxylin and eosin (HE). The histochemical stains were performed using Mallory trichrome (staining kit, Bio-Optica, Italy), silver impregnation for reticulum (staining kit, Bio-Optica, Italy), Perls' Prussian blue method (1), and the periodic acid-Schiff technique according to McManus (1). The immunohistochemical examination was performed using the following monoclonal mouse antibodies (DAKO, Denmark): anti-vimentin (dilution 1 : 100, clone 3B4, anti-bovine), anti-cytokeratin (dilution 1 : 50, clone AE1/AE3, anti-human), anti-desmin (dilution 1 : 50, clone D33, anti-human), anti-smooth muscle actin (SMA) (dilution 1 : 50, clone 1A4, anti-human), anti-major histocompatibility complex class II (MHCII) (dilution 1 : 20, clone TAL.1B5, anti-human), anti-Ki67 (dilution 1 : 75, clone MIB-1, anti-human), anti-CD79αcy (dilution 1 : 25, clone HM57, anti-human) and the following polyclonal rabbit anti-human antibodies (DAKO, Denmark): anti-factor VIII-related antigen/von Willebrand factor (dilution 1 : 100) and anti-CD3 (dilution 1: 50). The primary antibodies were detected using a common immunoperoxidase method and 3,3-diaminobenzidine (DAB) as a chromogen. The specimens were counterstained with Mayer's hematoxylin. Appropriate normal canine tissue sections were used as positive controls. For the negative control, the primary antibody was omitted. The Ki67 expression was evaluated quantitatively in five randomly selected areas of the section (magnification 400 ×), avoiding large cystic spaces and areas where only single neoplastic cells were Ki67 positive. The Ki67 index is expressed as the percentage of positively stained neoplastic cells. The expression of vimentin, cytokeratin, desmin, SMA, MHCII, CD79αcy, CD3, and von Willebrand factor was evaluated qualitatively in a whole slide.

The tumor of the right atrium was poorly circumscribed and non-encapsulated. There was a large tumor mass with centrally located, blood-filled cystic spaces, as well as small neoplastic foci located circumferentially. There were some well-developed, hyperemic blood vessels at the tumor periphery (Fig. 2). The tumor consisted of pseudo-vascular structures lined with neoplastic endothelial cells and various amounts of collagen fibers and surrounding spindle cells (Fig. 3). The neoplastic pseudo-vascular channels were filled with blood, but in some areas these were empty

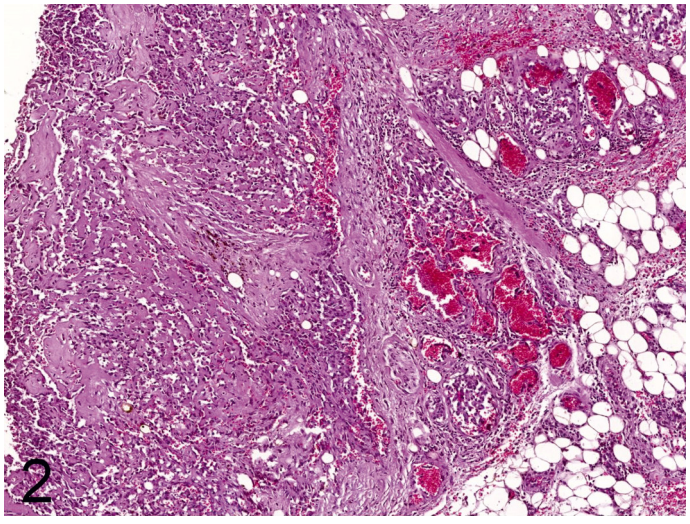

Fig. 2. Tumor of the right atrium. The tumor was composed of slit-like pseudo-vascular structures with some large, ectatic vessels at the periphery. HE. Magnification 50 ×

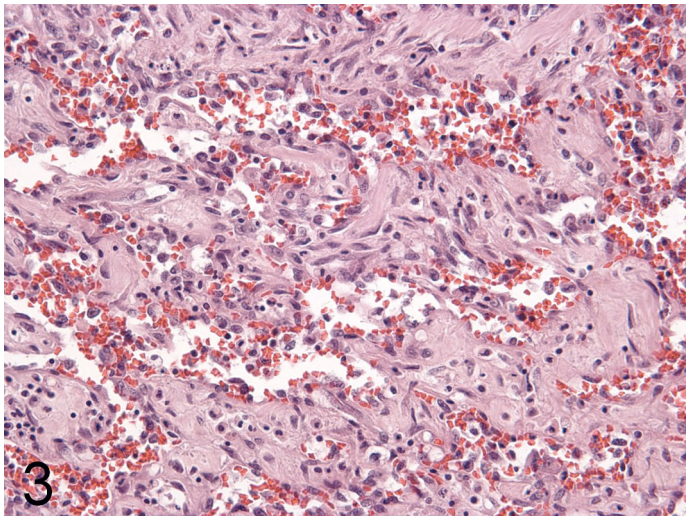

Fig. 3. Tumor of the right atrium. The pseudo-vascular structures were composed of neoplastic endothelial cells and spindle cells, and contained various amounts of collagen fibers. HE. Magnification 400 ×

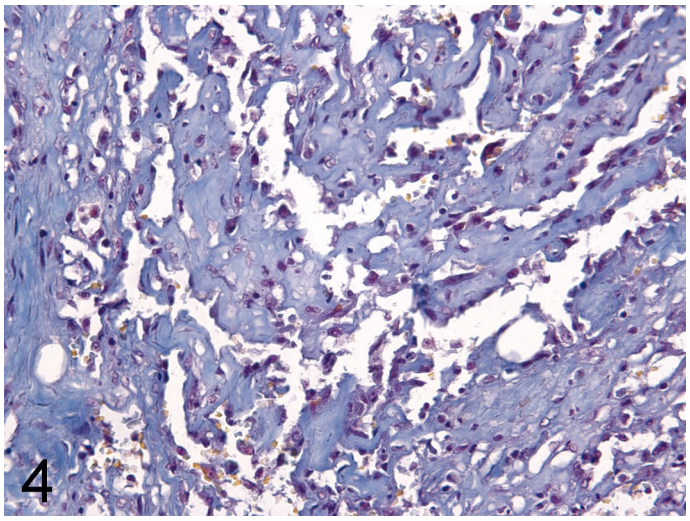

Fig. 4. Tumor of the right atrium. The collagen fibers formed a distinct layer in the pseudo-vascular structures. Mallory trichrome. Magnification 400 ×

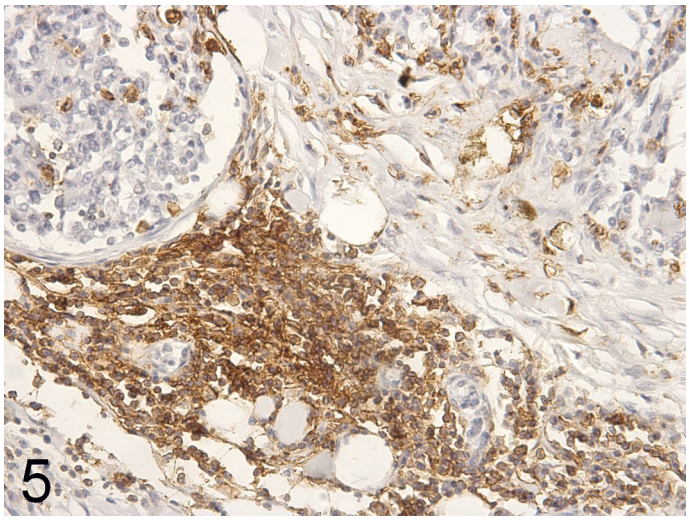

Fig. 5. The tumor of the right atrium. The MHC II positive lymphocytes infiltrated the tumor diffusely and formed distinct foci at the tumor periphery. MHC II immunostaining. Magnification 400 ×

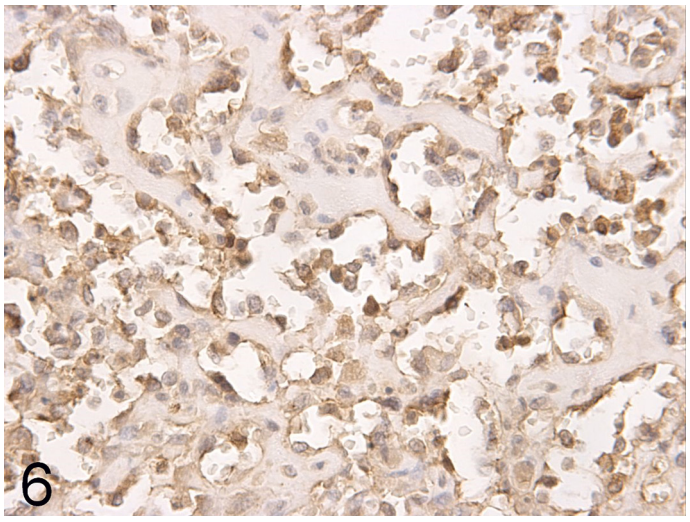

Fig. 6. Tumor of the right atrium. The neoplastic endothelial cells showed weak to moderate expression of von Willebrand factor, whereas the accompanying spindle cells were negative. Von Willebrand factor immunostaining. Magnification 600 ×

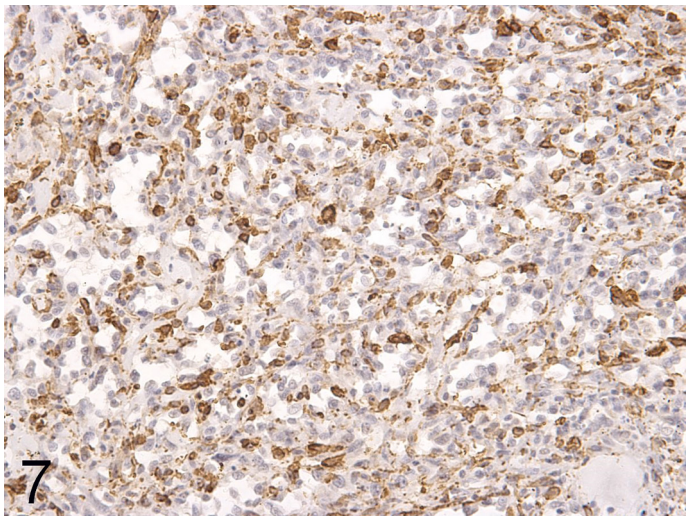

Fig. 7. Tumor of the right atrium. The spindle cells were SMA positive and surrounded the endothelial cells. SMA immunostaining. Magnification 400 ×

or filled with numerous lymphocytes. Neoplastic endothelial cells were moderately polymorphic, polyhedral, or slightly elongated, with moderate amounts of eosinophilic cytoplasm. The nuclei were oval and sporadically indented with coarse chromatin and inconspicuous nucleoli. The collagen fibers occasionally formed a distinct, thick, sclerotic layer (Fig. 4) and were absent in some areas. The spindle cells were distributed irregularly and were situated at the periphery of the vascular slits in some areas. These cells were monomorphic and did not show cellular or nuclear atypia. The apoptotic bodies were numerous, and mitotic figures were only sporadically observed. There was moderate lymphocytic infiltration within the tumor; lymphocytes were scattered randomly in neoplastic pseudo-vascular structures or formed distinct foci at the tumor periphery (Fig. 5). The reticulin fibers formed a fine meshwork in the tumor stroma. Ferric salt deposits were observed focally within the tumor. The cardiac muscle adjacent to the tumor mass was focally atrophic, with distinct areas of damage infiltrated by lymphocytes and macrophages.

The neoplastic endothelial cells and the spindle peripheral cells expressed vimentin in the cytoplasm. The neoplastic endothelial cells showed a weak to moderate cytoplasmic expression of von Willebrand factor (Fig. 6); strong expression was observed in well-developed blood vessels at the tumor periphery. The neoplastic endothelial cells were SMA negative, but the spindle peripheral cells showed strong cytoplasmic SMA expression (Fig. 7). The tumor cells (neoplastic endothelial cells and spindle peripheral cells) were constitutively negative for desmin, cytokeratin, MHCII, CD3, and CD79. Variable cytoplasmic desmin expression was confirmed in the cardiac muscle and in the myocytes of the normal arterioles. The Ki67 expression of the neoplastic cells was nuclear and variable (Fig. 8), the Ki67 index ranged from 6% to 14.5% with a mean value of 10.5%, but there were also areas with no neoplastic cells or only single

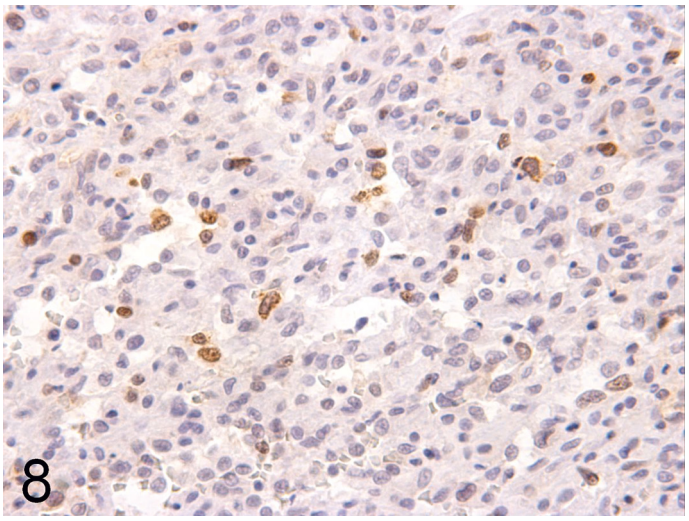

Fig. 8. Tumor of the right atrium. A small percentage of tumor cells showed Ki67 expression. Ki67 immunostaining. Magnification 600 ×

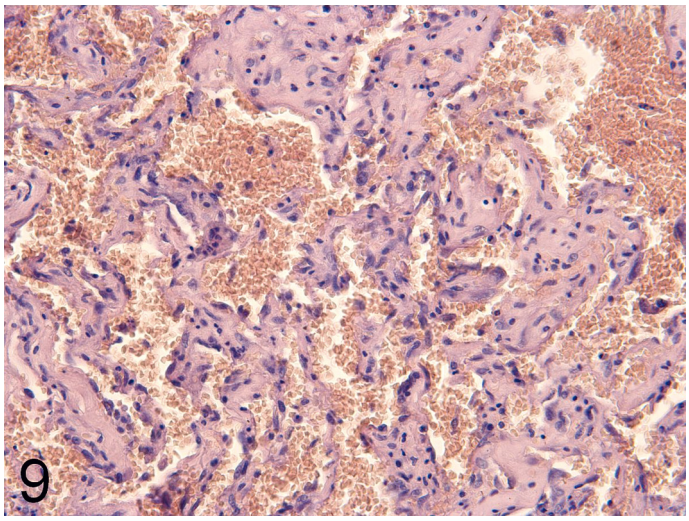

Fig. 9. Tumor of the abdominal cavity. The tumor was composed of collagen-rich pseudo-vascular structures that were highly hyperemic. HE. Magnification 400 ×

Ki67-positive neoplastic cells. Some lymphocytes within the tumor were also Ki67-positive. The lymphocytes were MHC II positive (Fig. 5). T (CD3-positive) and B (CD79--positive) lymphocytes were scattered in the tumor mass or in the adjacent heart muscle, but only T lymphocytes formed distinct foci at the tumor periphery.

The tumor of the abdominal cavity was highly hemorrhagic, and only small areas with a pattern similar to that described above were observed (Fig. 9). The neoplastic pseudo-vascular structures were heavily hyperemic, and there were well-developed cystic blood vessels at the tumor periphery. In some areas, the collagen layer of the pseudo-vascular structures was thick. The immunophenotype of the tumor cells was similar to that described above, but the expression of von Willebrand factor was weak in the neoplastic endothelial cells, and only single cells were Ki67 positive.

Discussion

Canine Kaposi-like vascular tumor has previously been described solely as a dermal or submucosal mass (2). Epithelioid haemangioendothelioma, morphologically resembling Kaposi-like vascular tumor, has been previously described in the lung of the dog (5). In the present report, vascular tumor occurred in the right atrium, which is the predilection site for hemangiosarcoma (2). The second tumor, located in the abdominal fat, could have been primary, metastatic, or simultaneous. Kaposi-like vascular tumor is believed to possess intermediate malignancy (3), and metastasis has never been reported. Kaposi's sarcoma in humans is regarded as a low-grade tumor (8). Therefore, in this case a multicentric origin for the tumor seems to be likely.

The vascular tumor described in this manuscript was composed of moderately polymorphic endothelial cells and monomorphic spindle cells with varying amounts of collagen and reticulin fibers between these populations. This pattern resembles the histological structure of normal capillaries, which are composed of endothelial cells and pericytes sharing a basement membrane (10). The endothelial cells of the tumor expressed von Willebrand factor, one of the determinants associated with endothelial differentiation (13). Previous reports of Kaposi-like vascular tumor in dogs revealed that only rare neoplastic cells were positive for von Willebrand factor, in contrast to hemangiosarcoma (2, 3). However, further investigations have shown that some hemangiosarcomas do not express this marker (2), and there is a report of a von Willebrand factor--positive Kaposi-like vascular tumor from a bovine urinary bladder (7). Therefore, the expression of von Willebrand factor cannot differentiate between hemangiosarcoma and Kaposi-like vascular tumor. The tumor cells of human Kaposi's sarcoma express von Willebrand factor as well as podoplanin, a selective lymphatic endothelial marker (4). Although podoplanin expression was not investigated in this study, the neoplastic pseudo-vascular channels were empty or filled with lymphocytes in some areas of the tumor, suggesting focal lymphatic endothelial differentiation of the tumor. The spindle cells of the tumor expressed SMA, but lacked desmin expression. The pericyte-derived tumor in dogs, hemangiopericytoma, can be either SMA positive or SMA negative, but only rarely shows expression of desmin (6). Therefore, the spindle cells of the tumor morphologically and immunohistochemically corresponded to pericytes.

The tumor was diffusely infiltrated with T and B lymphocytes, but lacked PAS-positive hyaline globules. Both chronic inflammatory cells and PAS-positive hyaline globules are features of human Kaposi's sarcoma (8), but occur variably in Kaposi-like vascular tumor in animals (2, 7, 12). The Ki67 index in the tumor was low to moderate, similar to those described in a previous report (7).

In conclusion, Kaposi-like vascular tumor is not an exclusively cutaneous or submucosal neoplasm. Despite its low proliferative potential and a well--differentiated morphological pattern, any tumor

located in the cardiac muscle should always be regarded as clinically malignant and life threatening. It can be suggested, that Kaposi-like vascular tumor is not a separate entity, but a well-differentiated morphological pattern of hemangiosarcoma. This is the first report of a Kaposi-like vascular tumor with a unique, multicentric location and a fatal outcome in a dog.

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