Widespread epithelioid angiosarcoma with ventricular wall involvement in a dog

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Summary. This paper reports on a canine angiosarcoma, presenting as an "undifferentiated metastasizing tumor". A 14-year-old female Cocker Spaniel was referred to the University of Extremadura Veterinary Clinic for clinical examination after suffering rapid deterioration, with chronic cough, anorexia and cachexia. One week after clinical examination, the dog died of right congestive heart failure and ventricular arrhythmia. Blood counts revealed lymphopenia and platelet depletion. The biochemistry profile was within normal limits, except for a drop in blood urea nitrogen. Cytological evaluation of liver and spleen biopsies revealed clustered anaplastic cells that lacked convincing tissue differentiation. Major findings at necropsy were enlarged spleen and multiple, beige to dark-red nodules ranging from 0.5 to 3 cm in diameter in the heart, lung, liver and spleen. At histological examination, multiple nests of anaplastic epithelioid cells were found in sections from all affected organs. Immunohistochemistry revealed widespread expression of CD31 and Factor VIII-related antigen. The neoplastic cells were negative for CD 18. The diagnosis of epithelioid angiosarcoma, localized in the myocardium, lung, liver and spleen was made. The primary site of the neoplasm could not be determined.

Key words: Dog, Epithelioid angiosarcoma, Immunohistochemistry, CD31, Factor VII-related antigen

Introduction

Spontaneous tumors of blood-vessel endothelial cells have been described commonly in the dog, less frequently in the cat and horse, and sporadically in most other domestic species (Prymak et al., 1988). Canine angiosarcoma (AS) is a malignant and generally-fatal neoplasm of vascular endothelial origin (Robinson and Maxie, 2007), characterized by rapid and widespread metastasis through the hematogenous route. AS is widely thought to represent about 5% of all noncutaneous primary malignant tumors and 12% to 21% of all mesenchymal neoplasms in these species (Brown et al., 1985; Bergman, 2010). AS is generally found in elderly dogs, there appears to be no breed preference, although a number of authors report greater incidence in Golden Retrievers and German Shepherds (Goldschimidt and Hendrick, 2002) and there is no evidence of any predilection for sex. AS is generally associated with hemostatic disorders, including tumor-related hemorrhage, thrombocytopenia and disseminated intravascular coagulation (Hammer et al., 1991; Hargis and Feldman, 1991; Hammond and Pesillo-Crosby, 2008).

AS presents in 2 topographic distribution patterns: visceral and cutaneous (Ward et al., 1994; Warren and Summers, 2007). For visceral AS, the spleen is the most common primary site, but it can occur as a primary tumor in many organs, including lung, liver, heart and prostate (Shor et al., 2009). Two histological variants are recognized: endothelial angiosarcoma, characterized by the arrangement of anaplastic endothelial cells to form vascular structures; and epithelioid or histiocytoid angiosarcoma, distinguished by the formation of nests or cords of spindle-shaped or epithelioid cells within a fibroblastic stroma (Weiss and Enzinger, 1982; Schatteman and Awad, 2004; Shor et al., 2009). Epithelioid angiosarcomas have plump, epithelioid neoplastic endothelial cells with occasional cytoplasmic vacuolation and have been described in the dog, the horse and the cow in several locations, including the heart (Bertazzolo et al., 2005; Warren et al., 2007; Shor et al., 2009). This manuscript describes a canine epithelioid angiosarcoma in the ventricular

myocardium, as well as in the lung, spleen and the heart. In the heart, although the auricles are the most common sites, myocardial AS, either primary or metastatic, has been previously reported (Keene et al., 1990; Hayden et al., 1992; Bertazzolo et al., 2005; Warman et al., 2006; Dennler et al., 2007).

This paper reports on the histopathological features of an epithelioid angiosarcoma located in the ventricular myocardium, spleen, liver and lung of a female Cocker Spaniel, and attempts to ascertain the ontogeny of this neoplasm by studying immunoexpression of the endothelial cell markers Factor VIII related antigen (F VIII RAg) and CD31.

Materials and methods

History, physical examination, abdominal ultrasonography, cytology and laboratory findings

A 14-year-old female Cocker Spaniel was referred to the University of Extremadura Veterinary Clinic for clinicalrapid deterioration, with chronic cough, anorexia and cachexia. Physical examination was followed by thoracic and abdominal ultrasonography, and cytological examination of fine-needle aspirates obtained under ultrasound guidance from heart, lung, liver and spleen masses. Urine analyses, blood counts and blood biochemistry profiles were also obtained.

One week after examination, the dog died of right congestive heart failure and ventricular arrhythmia. Necropsy was performed immediately, and gross, microscopic and cytological examinations were performed.

Gross and microscopic findings

During standard necropsy, samples were taken from the myocardium, liver, lung and spleen masses. Tissues were fixed in 10% neutral buffered formalin and embedded in paraffin; sections 3 μm thick were mounted on glass slides and stained with hematoxylin and eosin (HE) and Masson trichrome (MT).

Immunohistochemistry

Heart, lung, liver and spleen sections were also evaluated immunohistochemically for the expression of Factor VIII-related antigen (F VIII RAg), also known as von Willebrand factor (vWF), CD31 and Cytokeratin 18

(CK 18). Polyclonal rabbit anti-human Factor VIII RAg (Dako, Barcelona, Spain) diluted 1:800, monoclonal mouse anti-human CD31 (Dako, Barcelona, Spain), diluted 1:40 and monoclonal mouse anti-human CK 18 (Dako, Barcelona, Spain), diluted 1:100, in a commercial diluent (Dako Corp., Burlingame, CA, USA), were used for this purpose. Dewaxed and rehydrated sections were subjected to high-temperature antigen retrieval by incubation with 1 mmol/L EDTA (pH 9.0) for 40 min (CD31 antibody), then incubated with the primary antibodies for 18 hours at 4°C. The avidin biotin peroxidase-complex (ABC) method (Vector Laboratories; Burlingame, CA, USA) (F VIII RAg) and the streptavidin biotin peroxidase complex technique (LSAB Peroxidase Universal kit, Dako, Barcelona, Spain) (CD31 and CK 18 antibodies) were applied. The chromogen 0.5% 3,3'diaminobenzidine tetrahydrochloride (Sigma) in 0.05 M Tris containing 0.3% hydrogen peroxide was applied to the slides for 1 min at 20-22°C. Sections were counterstained with Harris' hematoxylin.

Results

Physical examination and abdominal ultrasonography

The dog displayed general deterioration accompanied by dyspnea and chronic cough, anorexia and evident cachexia. Clinical signs included pale mucosa, diminished venous return, cardiac arrhythmia, and enlarged liver and spleen. Abdominal and thoracic ultrasonography confirmed an irregularly-enlarged spleen and the presence of nodules ranging from 0.5 to 3 cm in diameter in the liver, lung and heart.

Cytological examination

Smears from the various masses contained numerous clustered or individualized neoplastic cells, with a background of RBCs, leukocytes, and necrotic cellular debris. Individual neoplastic cells were large and typically contained one nucleus with prominent nucleoli. The cells were pleomorphic, ranging from round to spindle shaped and epithelioid. They were surrounded by a variable amount of eosinophilic cytoplasm with relatively distinct borders. Most cells contained 1-2 large cytoplasmic vacuoles. Marked anisocytosis and anisokaryosis were present, and mitotic figures were common.

Fig. 1. Heart; dog. Reddish areas measuring 0.5 cm in diameter (black arrows) and vascular cavities (white arrow) occupying much of the ventricular surface.

Fig. 2. Lung; dog. Reddish areas of varying sizes, with grayish centres, scattered over the whole surface of lung lobes.

Fig. 3. Liver; dog. The liver is severely enlarged by a beige-brown mass (arrow).

Fig. 4. Spleen; dog. Hypertrophic spleen. Multiple dark-red nodules occupying much of the spleen surface. The rest of the surface has a congestive appearance.
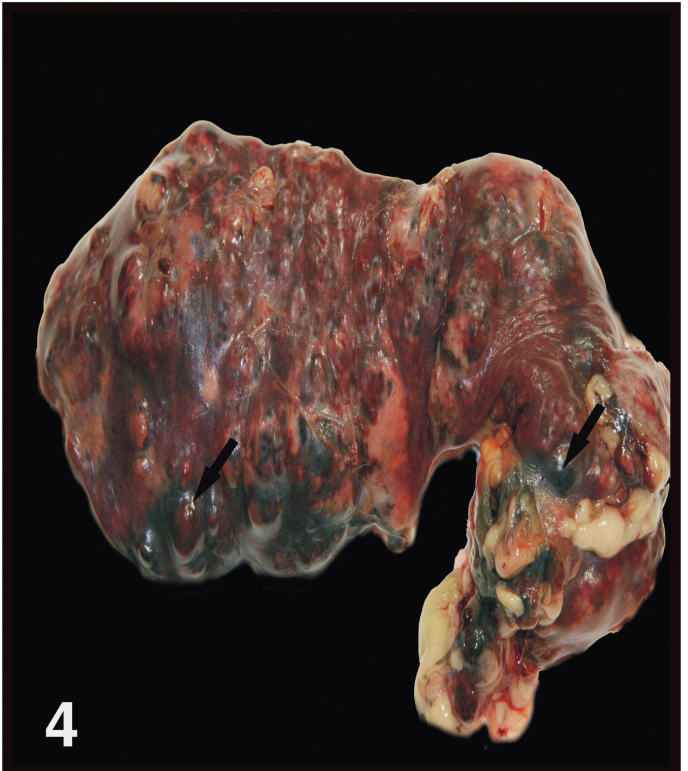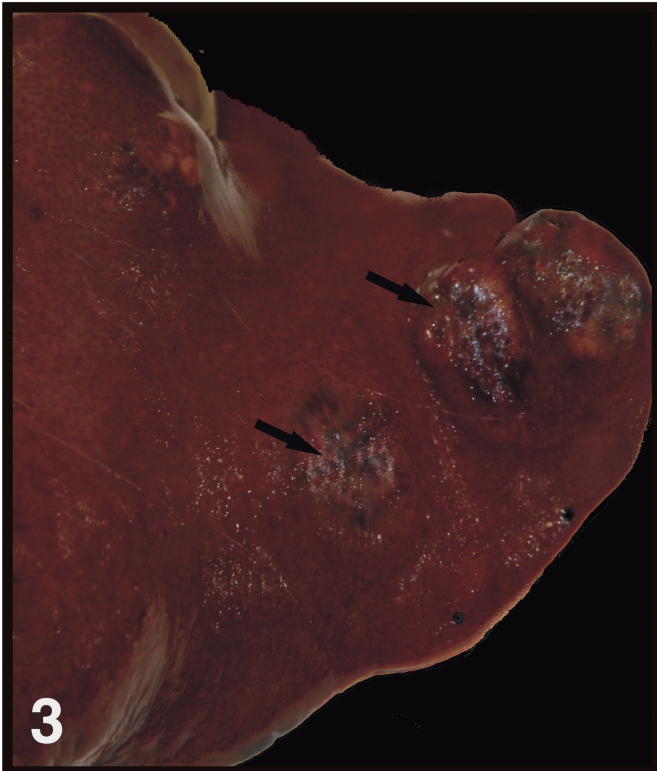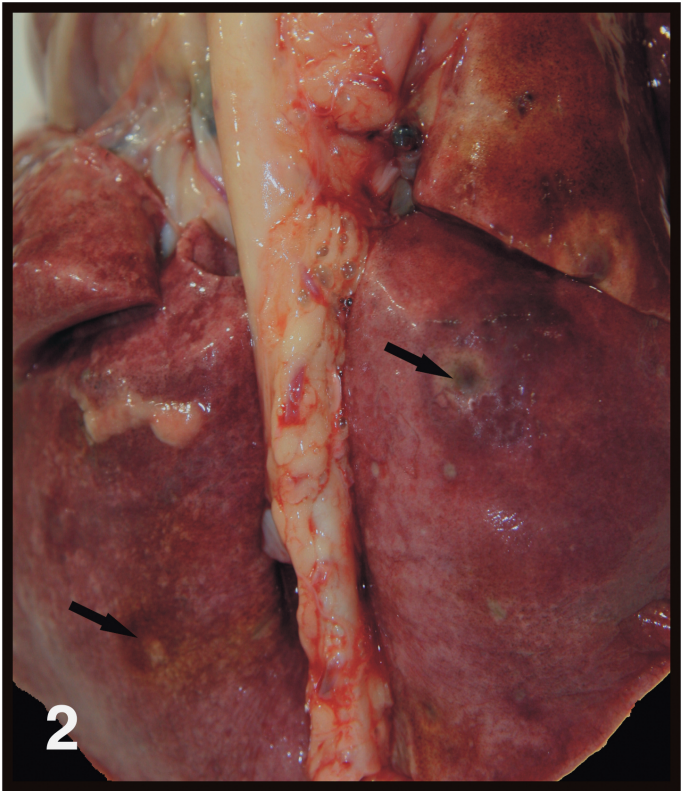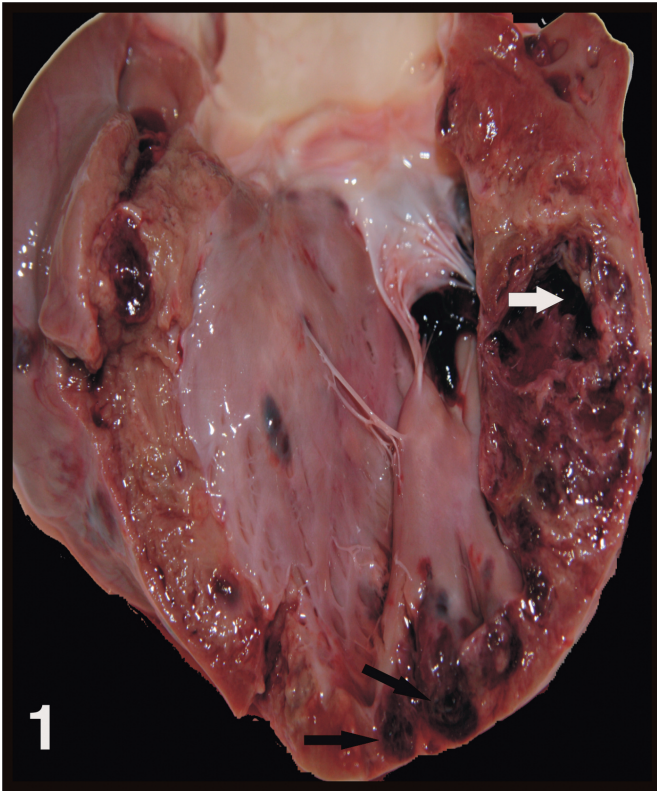
Laboratory results

Fluid obtained by abdominal tap was found to contain blood cells, particularly neutrophils and monocytes. Urine was brown and turbid, and contained numerous red blood cells (>100 cells/hpf) and white blood cells (30-50 cells/hpf). The platelets and white blood cell counts were 7300/μL and 4800/μL respectively. The white blood cell differential count revealed lymphopenia (192/μL). Other blood values were within normal limits. Blood biochemistry analysis revealed only a drop in blood urea nitrogen (6 mg/dL). Alkaline phosphatase, alanine aminotransferase, creatinine and glucose values were within normal ranges.

Gross findings

Most organs displayed hyperemia and edema, and there was evidence of hemopericardium and hemoperitoneum. Scattered reddish, unencapsulated nodules measuring between 0.5 and 3 cm in diameter were observed on the inner wall of the left and right ventricles of the heart; they contained whitish or grayish areas. On sectioning, these nodules exuded a dark red fluid. These lesions were more numerous in the left ventricular myocardium (Fig. 1).

Nodules were observed in both main and accessory lung lobes. Nodules were unencapsulated, rounded, and grayish in color, with a blackish central area (Fig. 2). On sectioning, they were of compact consistency, with no cavity formation, and reddish-brown in color.

Liver parenchyma displayed moderate enlargement; nodules similar in size, shape and coloring to those observed in the lung were observed in all liver lobes (Fig. 3).

The spleen was greatly enlarged (roughly twice its normal size), and displayed total loss of structure. The whole surface was covered in varying-sized nodules ranging in color from whitish-gray to blackish (Fig. 4). On sectioning, some areas were compact and others contained cavities; these were whitish-gray in color and contained focal areas of hemorrhage.

Microscopic findings

Neoplastic lesions were observed in myocardium, lung, liver and spleen. In the myocardium, the neoplasm mostly comprised vascular structures resembling sinusoid capillaries (Fig. 5) or nests of epithelioid or spindle-shaped cells (Fig. 6). Epithelioid cell cytoplasm was eosinophilic and contained vacuoles; single nuclei with one or two prominent nucleoli were observed in areas apparently containing no chromatin, and heterochromatin was peripherally located, close to the nuclear envelope. Neoplastic cell nests displayed marked anisocytosis and anisokaryosis; bizarre mitotic figures and apoptotic bodies were numerous.

In peripheral areas of neoplastic tissue close to unaffected myocardium, neoplastic thrombi were observed in large and, to a lesser extent, small veins (Fig. 7).

Fibroconnective neoplastic stroma displayed abundant ground substance and collagen fibers. In areas close to vascular spaces, neoplastic stroma contained an infiltrate comprising leukocytes and macrophages, many containing hemosiderin granules. Although there was no fibroconnective capsule between the neoplasm and the unaffected myocardial parenchyma, myocardial cells bordering the tumor displayed pressure atrophy; atrophied myocardial cells were separated from tumor areas by amorphous ground substance.

In lung parenchyma, neoplastic cells were arranged in irregular, scattered, non-encapsulated clusters of newly-formed vascular structures. Newly-formed capillaries were located in the interseptal space; vessel walls and lumina were composed of epithelioid cells similar to those observed in myocardium (Fig. 8).

Fig. 5. Heart; dog. Newly-formed vessels lined by cuboidal to columnar endothelial cells (arrow). HE, x 400

Fig. 6. Heart; dog. Neoplastic tissue consisting of narrow vascular spaces lined by a layer of endothelial cells (arrow) and separated by fibrous stroma. HE, x 200

Fig. 7. Heart; dog. Neoplastic tissue consisting of narrow vascular spaces lined by a layer of endothelial cells (arrow) and separated by fibrous stroma. MT, x 100

Fig. 8. Lung; dog. Lung parenchyma displaying changes in histoarchitecture due to the proliferation of atypical cells forming pseudovascular structures containing neoplastic cells and blood cells. Evident pleomorphism, anisokaryosis and nuclear hyperchromatism. HE, x 400

Fig. 9. Liver; dog. Neoplastic tissue consisting of narrow vascular spaces lined by a layer of neoplastic endothelial cells and separated by loose fibrous stroma. HE, x 100

Fig. 10. Spleen; dog. Clusters of blood vessels formed by neoplastic cells; lumen containing blood cells and endothelial neoplastic cells, surrounded by lymphocytes and plasma cells. HE, x 200

Fig. 11. Angiosarcoma: solid arrangement of CD 31 positive atypical endothelial cells within vessel (arrow). Immunohistochemically labeled for CD 31. x 200

Fig. 12. Heart; dog. Angiosarcoma: F VIII RAg positive atypical endothelial cells lining vascular structures (arrow). Immunohistochemically labeled for F VIII RAg. x 200
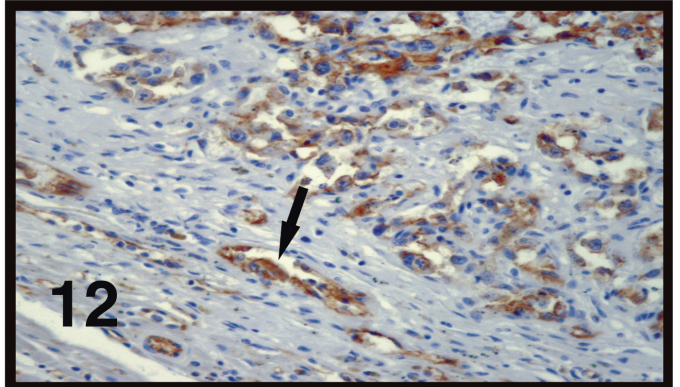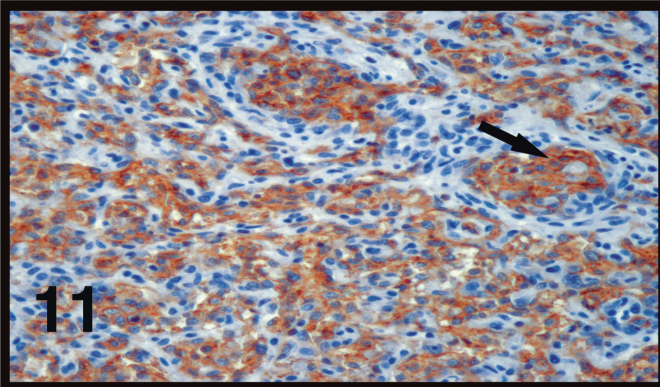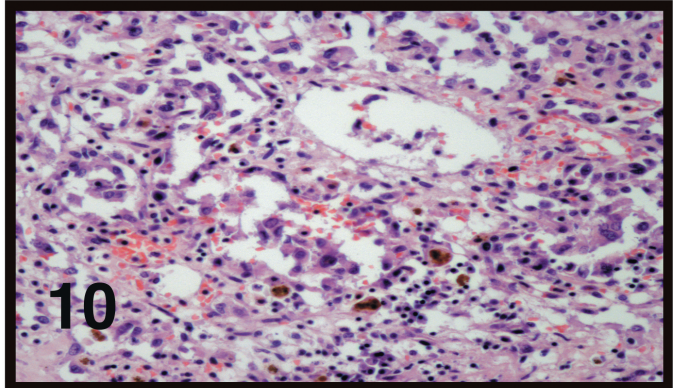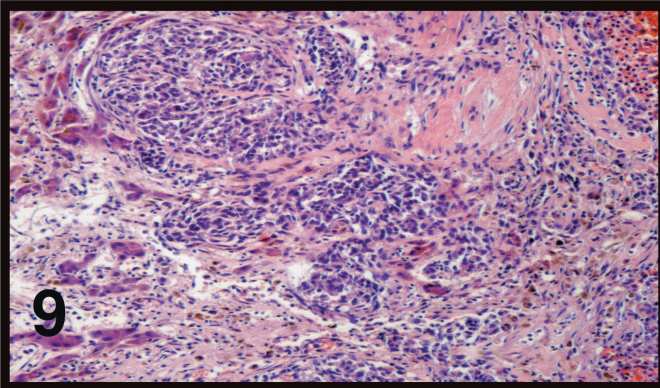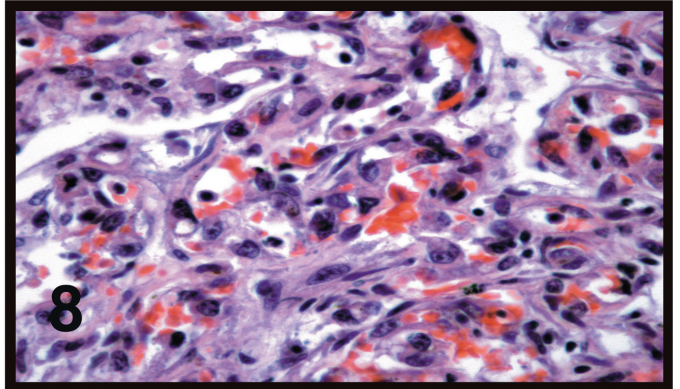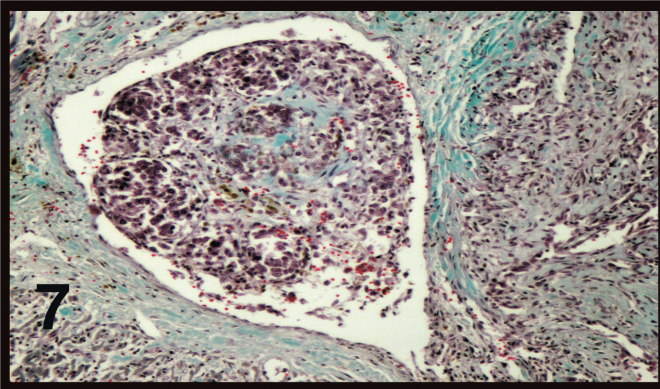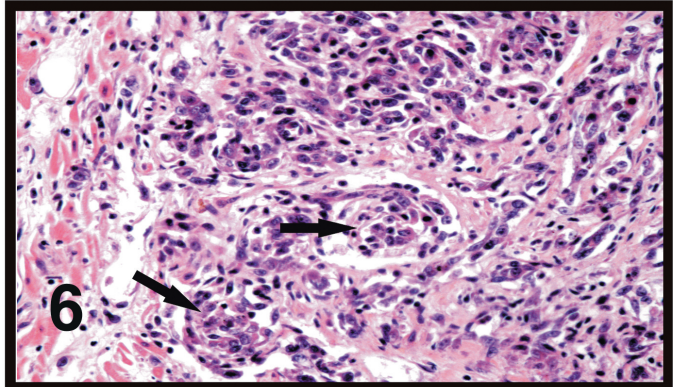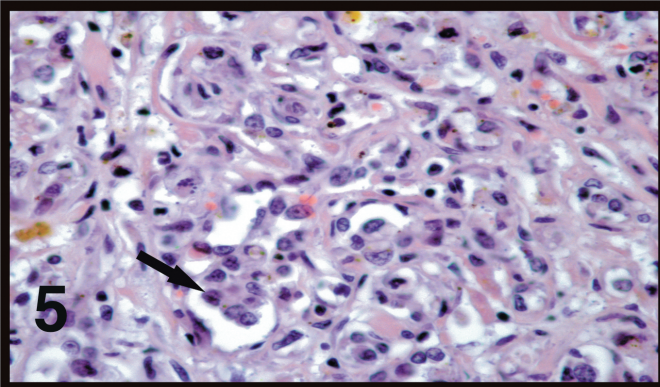
Spindle-shaped and polygonal cells were also visible, forming neoplastic cell nests, again similar to those recorded in myocardium. Vascular structures were separated by fibroconnective neoplastic stroma, although in the absence of capsule formation there was no break between the neoplastic area and normal lung parenchyma.

Neoplastic areas in liver parenchyma comprised heterotypical vascular structures forming both capillaries and lined vascular spaces (Fig. 9), filled with degenerated blood cells, necrotic debris and both polygonal and spindle-shaped neoplastic cells.

Spleen parenchyma displayed extensive areas of necrosis and bleeding, as well as a focal infiltrate, of polygonal and spindle-shaped neoplastic cells, forming vascular structures or clusters of cells showing clear signs of anaplasia and atypia (Fig. 10).

Immunohistochemistry

In the myocardium, lung, liver and spleen all epithelioid components of the tumors were negative for CK 18, the monoclonal antibody specific for keratinizing cells.

The neoplasm in the ventricular myocardium stained strongly positive with factor CD31 (Fig. 11) and moderately positive with Factor VIII Rag (Fig. 12) antibodies. Positive staining was also observed in neoplastic foci of lung, liver and spleen.

Discussion

This paper reports on an epithelioid variant of AS located in the myocardium, spleen, liver and lung of a female Cocker Spaniel. Canine AS is a malignant tumor of vascular endothelial origin that accounts for around 5% of all non-cutaneous primary malignant tumors and 12% to 21% of all mesenchymal neoplasms in this species (Smith, 2003; Fosmire et al., 2004). AS presents in two topographic distribution patterns: visceral and cutaneous (Ward et al., 1994). For visceral AS, the spleen is the most common primary site, but it can occur as a primary tumor in many other organs, including lung, liver, heart and prostate (Goldschimidt and Hendrick 2002). Typically, visceral ASs are very aggressive, with rapid and widespread metastasis through the hematogenous route (Lattanzi et al., 2001; Robinson and Maxie, 2007).

Clinical signs, blood count and blood biochemistry results were similar to those reported by other authors (Lattanzi et al., 2001; Shor et al., 2009). Similar hematological changes observed in the current case can be seen in dogs (Hargis and Feldman, 1991; Hammer et al., 1991) and cows (Stock et al., 2011) with angiosarcoma. Common clinicopathologic findings include anemia due to intracavitary hemorrhage, and spontaneous bleeding prompted by thrombocytopenia secondary to microangiopathic hemolysis (Brown et al., 1985; Hammer et al., 1991; Lattanzi et al., 2001; Hammond and Pesillo-Crosby, 2008; Stock et al., 2011).

This is a case report of a epithelioid angiosarcoma located in the ventricular myocardium, particularly the left ventricle; other authors have previously reported this tumor, either primary or metastatic, in the left ventricle (Keene et al., 1990), especially in the lateral wall of the right atrium (Hayden et al., 1992; Warman et al., 2006; Dennler et al., 2007). Vascular neoplasms in the heart lead to ventricular dysfunction and cardiac arrhythmia; moreover, tumor ingrowth into the atrial or ventricular cavity can prompt obstruction both of the ventricles and of the atrioventricular vestibule, giving rise to congestive heart failure (Von Beust et al., 1988; Villar de et al., 1995; McGrotty, 2001; Warman et al., 2006). These patho-physiological changes were not observed here, since there was no ingrowth into cardiac cavities.

Neoplastic lesions were present in heart, spleen, lung and liver. However, the primary site of the neoplasia can not be identified in case of widespread tumors, and the neoplasm could possibly be multicentric in origin. The skin has been reported as a common primary site for disseminated angiosarcoma (Hargis et al., 1992; Galeotti et al., 2004; Shiu et al., 2011; Stock et al., 2011). In the current case, no neoplastic skin lesions were detected. It is essential to verify the origin of AS cells, and especially to distinguish whether these cells arise from mature endothelial-lining cells or from hemangioblasts; i.e. bone marrow-derived hematopoietic precursors with capacity for endothelial differentiation. It has been reported that AS originates from bone marrow precursors that arrest their differentiation at the hemangioblast to angioblast stage (Schatteman and Awad, 2004). Neoplastic vascular spaces and newly-formed capillaries comprised endothelial cells displaying marked anaplasia and atypia. These cells were surrounded by a variable amount of eosinophilic cytoplasm with relatively distinct borders. Most cells contained 1-2 large vacuoles. Histologically, therefore, they shared certain features with hemangioblasts (Fosmire et al., 2004; Lamerato-Kozicki et al., 2006).

Epithelioid AS is a variant of the histologically conventional AS that has little or no morphological evidence of a vascular origin and has been reported rarely in domestic animals. Distinctive findings in the case reported here included the presence of epithelial-like cells, also reported by other authors in dogs (Machida et al., 1998; Shor et al., 2009), as well as in horses and cows (Machida et al., 1998; Warren and Summers, 2007; Arenas-Gamboa and Mansell 2011). In this case, the epithelial nature of the tumor has been ruled out by the negative immunoreaction against the cytokeratins, coinciding with that previously reported (Bertazzolo et al., 2005). Immunohistochemical staining revealed CD 31 and Factor VIII Rag positivity, while cytokeratin was negative, confirming the diagnosis of epitheloid angiosarcoma. Endothelial vascular tumors in humans are divided into three groups: hemangiomas, hemangioendotheliomas and hemangiosarcomas (Weiss and Enzinger, 1982; Kempson et al., 2001). Hemangiomas are the most benign, and angiosarcomas the most malignant. The present case was identified as a

epithelioid angiosarcoma, since the distinctions between hemangiosarcoma and lymphangiosarcoma cannot be based on histology, and both tumors express CD 31 and Factor VIII Rag, as previously reported (Galeotti et al., 2004).

The etiology of angiosarcoma remains incompletely understood. Its common occurrence in dogs suggests predisposing factors favor its development in this species. These factors could represent a constellation of heritable characteristics that promote transformation events and/or facilitate the establishment of a microenvironment that is conducive for survival of malignant blood vessel-forming cells (Tamburini et al., 2010). AS cells may release proteins that stimulate the growth of new blood vessels. The presence of numerous blood vessels helps supply growing tumors with nutrients and oxygen and may serve as a highway for the cancer cells to metastasize to distant parts of the body (Clifford et al., 2001).

Histopathological findings in lung and liver were similar to those reported by other authors (Brown et al., 1985; Warren and Summers, 2007). Microscopic patterns of the proliferative endothelial cells varied from more conventional capillary-like to cavernous vessels, which blended into areas of branching tubular and gland-like structures. The neoplastic endothelial cells of the newly-formed capillaries were spindled, whereas in epithelioid areas, cells were larger and predominantly polygonal (Warren and Summers, 2007). A number of authors argue that neoplasms in the liver and lung are metastases from primary tumors in the spleen or some other unknown origin (Brown et al., 1985; Latanzi et al., 2001; Warren and Summer, 2007).

The spleen neoplasm prompted a total loss of spleen structure and morphology. The whole surface of the spleen was covered by neoplastic vascular spaces of varying sizes, together with focal neoplastic epithelioid structures. This combination of vascular spaces and epitheloid areas has been seen by some authors as evidence that the spleen is the primary tumor site, neoplasms elsewhere being metastatic (Lattanzi et al., 2001; Shor et al., 2009). Although it was not possible to locate the primary tumor in the current case, neoplastic cell thrombi were found only in peripheral veins in the heart neoplasm, coinciding with what was previous report in humans (Ge et al., 2011) and dogs (Chandler et al. 2009; Wenzlow et al., 2009; Shiu et al., 2011).

In the present case, the skin was unaffected. Primary canine cutaneous angiosarcomas are relatively uncommon in humans (Sundaram et al., 2011) and dog (Ward et al., 1994; Wilkerson et al., 2002; Gross et al., 2005), and in cases of disseminated disease it is difficult to differentiate de novo from metastatic dermal angiosarcomas (Warren and Summers, 2007; Sabattini and Bettini, 2009)

Factor VIII-related antigen (F VIII RAg) is one of several components of the factor VIII glycoprotein complex, which is present in plasma and plays an essential role in blood coagulation and platelet function. Synthesis of F VIII Rag by endothelial cells has been proven in studies on endothelial cell cultures (Jeanneau and Sultan, 1982).

An early study of 83 canine endothelial neoplasms suggested that F VIII RAg is a reliable endothelial cell marker, not only in normal and reactive canine endothelial cells, but also in neoplasms of endothelial origin (Von Beust et al., 1988). The ontogeny of AS cells can be confirmed by the expression of selected endothelial markers such as F VIII RAg and CD31 (Fosmire et al., 2004; Sabattini and Bettini, 2009). Use of the monoclonal antibody CD31 for the immunostaining of neoplastic endothelial cells on fixed material from canine neoplasms is reported to provide better results than F VIII Rag (Quezada et al., 1993; Ferrer et al., 1995); this was the case in the present study. The case reported here was diagnosed as epithelioid angiosarcoma located in ventricular myocardium, spleen, liver and lung. Further IHC studies of anaplastic cells combining endothelial markers, such as F VIII RAg and CD31, with lymphatic vascular markers, such as Lymphatic Vessel Endothelial receptor - 1, to confirm hemangiosarcoma or lymphangiosarcoma are required to determine the histogenesis of epithelial AS cells.

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