Hematoxylin - eosin-methylene Blue Staining in a Dog Hemangiosarcoma Case

ADRIAN STANCU[1]*, ROMEO CRISTINA[1], MIRELA AHMADI[1], LILIANA CARPINISAN[1], ALINA GHISE[1], MARIUS PENTEA[1], DELIA MIRA BERCEANU VADUVA[2]

[1] Faculty of Veterinary Medicine Timisoara, 119 Calea Aradului, 300645, Timisoara, Romania

[2] "Victor Babes" University of Medicine and Pharmacy Timisoara, 2 Eftimie Murgu Sq., 300041, Timişoara, Romania

A post-mortem examination of an 8 years old German Shepherd dog was performed in order to find out the death cause. The macroscopically examination revealed a large amount of blood in the abdominal (hemoperitoneum) and pericardial cavity (hemopericardium) and also the rupture of the right atrium. The spleen was sampled for histopathological examination. Hematoxylin-eosin–methylene blue staining was performed in order to prepare the samples for microscopical examination. Based on the specific histopathological changes and taking into account all the pathological data, the diagnosis was hemangiosarcoma.

Key words: hemangiosarcoma, German Shepherd dog, hematoxylin-eosin–methylene blue staining.

Hemangiosarcoma (HSA) is an aggressive, malignant tumor that arises from either the vascular endothelium or endothelial precursor cells [1]. The origin of tumour is frequently in the spleen (28–50%), right atrium and auricle (3–50%) and subcutaneous tissues (13%) [3-6]. HAS can also primarily occur in liver, kidney, bladder, prostate, peritoneum, lung, pulmonary artery, aorta, muscle, bone, oral cavity, tongue, vertebral body, and central nervous system [6,7].

Metastasis occurs early in the disease, via hematogenous routes, because of the access to the systemic circulation, but also through transabdominal implantation following rupture. Around 80% of the dogs can have metastasis by the time of diagnosis. Up to 63% of dogs with right atrial HSA have concurrent metastatic disease [8-10]. The overall prevalence of HSA is reported to be 0.3–2.0% of all tumors in dogs, and represent about 5-7% of all malignancies in dogs [5,7,11].

Ware and Hopper emphasize that HSA is the most common cardiac tumor identified in dogs, with an overall incidence of 0.19%. HSA represent 69% of all cardiac tumors in dogs and is 10-fold higher than the second most common cardiac tumor in dogs, the aortic body tumor [12].

From ethiopathogenetic point of view, adult dogs (range 8-13 years old) and German Shepherd breed are most frequently affected [4,7,13].

HSA does not cause pain and there are no clinical signs to emphasize a life threatening disease. Because the vessels are tortuous and malformed, the blood tends to clot and does not reach the tumoral cells, causing them to die. Death of the cells lead to small ruptures in the tumor, that allow the blood to escape into the abdomen, heart sac, chest, or subcutaneous space. If the hemmorhage is mild, affected dogs may show transient non-specific signs (lethargy and weakness). Unfortunately, the clinical signs are recurrent, and they are going unnoticed for some time. Hemangiosarcoma tends to spread aggressively and the distant metastasis already occurred once the disease is finally diagnosed. The death of patients with HSA often follows the rupture of a large or rapidly growing tumor, which results in acute, severe hemorrhage, collapse and shock [11].

We consider to present this case data because of the few scientific papers to emphasize HAS in Romania [14,15].

Experimental part

Materials and methods

It was performed an 8 years old German Shepherd dog post-mortem examination, following a sudden death. There were taken spleen samples for histopathological examination.

The samples preparation was carried out as follows: 24 h alcohol fixation at room temperature (prevent the tissue alteration due to the enzymes activity; preserve the tissue texture; improves the optical differentiation), alcohol dehydration (five steps: 70, 80, 90, 100% and 100% alcohol, each step for two hours), clearing with benzene, paraffin wax at 56°C, embedding tissues into paraffin blocks, trimming of paraffin blocks (6 μm), sections mounting on the glass slides (using Meyer albumin), hematoxylin - eosin - methylene blue staining [16,17].

Staining was performed as follows: deparaffination of tissue sections in benzene, rehydration using decreasing concentrations of alcohol, rinsing in distilled water, hematoxylin staining, alcohol, eosin staining and methylene blue staining, water removal using increasing concentrations of alcohol, cover slide mounting [17]. Hematoxylin will stain the nuclei in blue and the mucins in light blue. Eosin will stain the cytoplasm in pink, collagen in pale pink, red blood cells in bright red, and colloid in red. Methylene blue improves the blue colour of the nuclei, making them more observable [17].

The microscopical examination is useful as differentiating diagnosis method only if chemical preparation of samples is applied [18].

Results and discussions

The gross examination of the German Shepherd dog cadaver revealed no pathological signs. Following necropsy, a large amount of blood was observed in the abdominal cavity (hemoperitoneum) (fig. 1).

Figure 1. Blood collection in the abdominal cavity (hemoperitoneum).

The pericardial cavity opening emphasizes local blood accumulation (hemopericardium) (fig. 2). On heart gross examination it was observed a rupture in the right atrium and the heart sectioning revealed a dark-red homogenous tissue.

* *email: astancu2002@yahoo.com*

It was assumed that the death occurred following a super acute hemorrhage.

The spleen gross examination revealed the presence of two round formations, nonencapsulated, of different sizes, localized on the spleen head; respectively tail (fig. 3). Following the spleen sectioning, a homogeneous dark red tissue was observed (fig. 4).

The complete examination of the cadaver does not show other pathological lesions.

Based on the gross examination findings the presumptive diagnosis was HSA.

The cadaver age (8 years) fits in the normal range specific for HSA. The scientific data reveal the adult age (8-13 years) as being specific for HSA occurrence [4,7,9,11,13]

The diagnosis is also supported by the unicity of the case during the last 5 years of necropsies. The specific literature emphasize that the frequency of HSA is generally rare 0.3–2.0% [5] or 5-7% [11] of all tumors seen in dogs.

Applying the "double two thirds rule" according to which approximately 2/3 of dogs with splenic masses will have malignant tumors and approximately 2/3 of those malignant tumors will be diagnosed as HAS (43%) [19,20], and taking into account that 63 to 70% of dogs presenting splenic masses with nontraumatic hemoabdomen were HAS [21] we assumed that the found out formation was HSA.

The concomitant occurrence of the lesions in the spleen and right heart in 25% of HAS cases [5,7,10] supports our presumption, even if some authors suggest that this percentage at initial presentation is significantly lower (5% or less) [22].

Also, on notice that the cardiac or pericardial tumors are responsible for approximately 60% of cases of pericardial effusion in dogs [23].

Specific literature data emphasize that several splenic mass lesions (e.g., HSA, hematoma, hemangioma) can have a similar gross and ultrasonographic appearance, and large masses do not necessarily denote malignancy [24]. According to the mentioned affirmation, we consider to perform the histopathological examination of spleen samples for a definitive diagnosis.

Following the hematoxylin-eosin-methylene blue staining, the microscopically examination of the spleen samples emphasizes immature, endothelial cells forming vascular spaces containing variable amounts of blood and/or thrombi (fig. 5), according to the specific literature data [25].

We know that if histopathological features are minimal but HSA is suspected, immunohistochemistry for von Willebrand's factor (factor VIII–related antigen) or CD31/platelet endothelial cell-adhesion molecule (PECAM) can be used to demonstrate endothelial derivation and support the diagnosis of HAS [25,26,27], but our findings were sufficient to confirme the diagnosis.

We could not specify which was the primary site of tumor occurrence, the right atrium or the spleen, but according scientific papers, the origin of the tumors is equal noticed (50 %) [3,5,6].

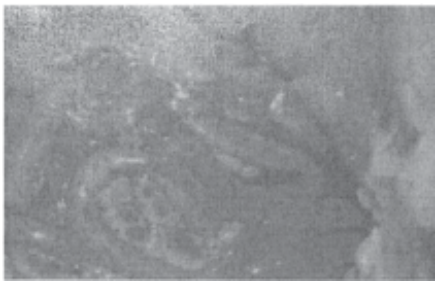

Fig. 1. Blood collection in the abdominal cavity (hemoperitoneum)

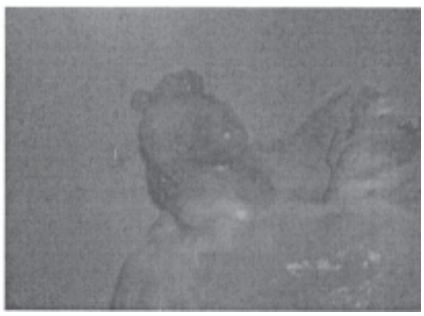

Fig. 2. Heart - rupture of the right atrium

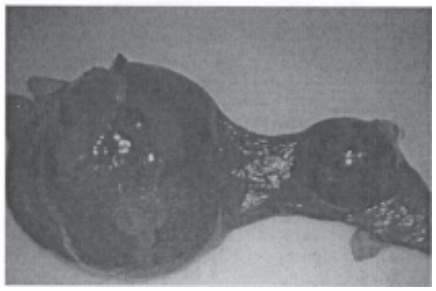

Fig. 3. Spleen – round reddish-black formations.

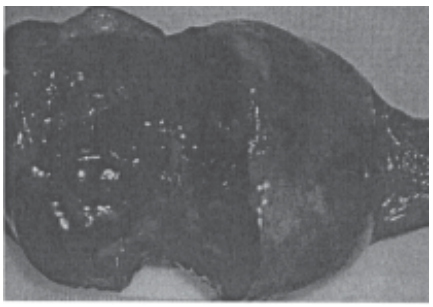

Fig. 4. Spleen - hemorrhagic aspect.

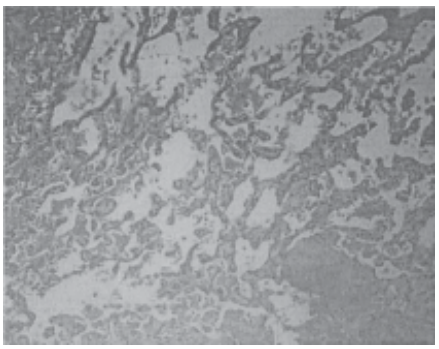

Fig. 5. Spleen - abnormal blood vessels and blood-filled channels and spaces. Hematoxylin-eosin-methylene blue staining, 20x

Conclusions

The German Shepherd dog post mortem gross examination revealed hemoperitoneum, hemopericardium and right atrium rupture that, based on the specific literature data, lead to the presumption of hemangiosarcoma.

The confirmation of hemangiosarcoma diagnosis was made taking into account the histopathological examination of the spleen samples, following hematoxylin-eosin-methylene blue staining. The presence of the specific histopathological aspects (blood-filled channels or spaces) confirmed the HSA diagnosis, without demanding immunohistochemistry tests.

The rare founded pathological aspects (once in 5 years), show that HSA has quite low frequency in dogs.

References

1. LAMERATO-KOZICKI A.R., HELM K.M., JUBALA C.M., CUTTER G.C., MODIANO J.F., Exp Hematol. 34, no.7, 2006, p. 870.
2. OGILVIE G.K., MOORE A.S., Managing the Veterinary Cancer Patient; A Practice Manual, Veterinary Learning Systems; Trenton, NJ, 1995, p. 367–376 in CRAIG A.C., ANDREW J.M., HENRY C.J., Vet. Intern Med., no. 14, 2000, p. 479.

3. BROWN N.O., PATNAIK A.K., MACEWEN E.G., J, Am Vet Med Assoc. 1; no.186, 1985, p. 56.
4. ARONSOHN M.J., Am Vet Med Assoc., Nov 1; 187(9), 1985, p. 922.
5. HOSGOOD G. Compend Cont Educ, Pract Vet;13 1991, p. 1065 In: PASTOR J., Canine Hemangiosarcoma Clinical Update, WSAVA Congress Proceedings, 2002;
6. WARD H., FOX L.E., CALDERWOOD-MAYS M.B., ET AL., J Vet Intern Med, 8, 1994, p. 345–348 in CRAIG A.C., ANDREW J.M., HENRY C.J., Vet. Intern Med., no. 14, 2000, p. 479.
7. HAMMER A.S., COUTO C.G., Vet Med,;87, 1992, p. 188 In: CRAIG A.C., ANDREW J.M., HENRY C.J., Vet. Intern Med., no. 14, 2000, p. 479.
8. THAMM D. M., Miscellaneous tumors. . *In*: Small Animal Clinical Oncology, 4th ed. Withrow, S. J. and Vail, D. M. eds., Saunders, St. Louis, 2007, p. 785–795.
9. PASTOR J., Canine Hemangiosarcoma Clinical Update, WSAVA Congress Proceedings, 2002;
10. WATERS D, CAYWOOD D, HAYDEN D, et al, J Small Anim Pract, 29, 1988, p. 805–814, In THAMM D. M., Hemangiosarcoma; In Withrow and MacEwen's Small Animal Clinical Oncology; WITHROW S.J., VAIL D.M., PAGE R.; Elsevier Health Sciences, 2013, p.679.
11. MODIANO J.F., RITT M.G., BREEN M., BREEN T., Discoveries, 24, American Kennel Club, Canine Health Foundation, 2007.
12. WARE W.A., HOPPER D.L., J Vet Intern Med., 13, no 2, p. 95.
13. ETTINGER S.J., FELDMAN E.C., Textbook of Veterinary Internal Medicine, 6th edition, Elsevier Science Health Science Division, 2004. p. 758
14. CĂTOI C., MOUSSA R., BOLFA P., TAULESCU M., NEGREA O., Lucrări Stiinţifice Medicină Veterinară Vol. XLI, , Timisoara, 2008, p. 100.
15. IVANA S., IVANA F.I., Rev. Rom. Med. Vet, 7, no. 4, 1997.
16. ŞINCAI M., Tehnici de citohistologie normală şi patologică. Ghid practic, Ed. Mirton, Timişoara, 2000. p.6
17. ALEXANDRU N., Investigaţia histologică în diagnostic, Ed. I, uz intern, Laboratorul Sanitar Veterinar de Diagnostic, Bucureşti, 1995
18. COTOI O.S., SUCIU M., PACURAR MARIANA, ORMENISAN ALINA – Rev. Chim. (Bucharest), 66(2), 2015, 219-222.
19. SPANGLER W.L., CULBERTSON M.R., J Am Vet Med Assoc, 200, 1992, p. 829, In THAMM D. M., Hemangiosarcoma; In Withrow and MacEwen's Small Animal Clinical Oncology; WITHROW S.J., VAIL D.M., PAGE R.; Elsevier Health Sciences, 2013, p.679.
20. GAMLEM H, NORDSTOGA K, ARNESEN K, APMIS Suppl, 2008, p. 41, In THAMM D. M., Hemangiosarcoma; In Withrow and MacEwen's Small Animal Clinical Oncology; WITHROW S.J., VAIL D.M., PAGE R.; Elsevier Health Sciences, 2013 p.679.
21. PINTAR J., BREITSCHWERDT E.B., HARDIE E.M., ET AL, J Am Anim Hosp Assoc 39, 2003, p. 518, In THAMM D. M., Hemangiosarcoma; In Withrow and MacEwen's Small Animal Clinical Oncology; WITHROW S.J., VAIL D.M., PAGE R.; Elsevier Health Sciences, 2013, p.679.
22. THAMM D. M., Hemangiosarcoma; In Withrow and MacEwen's Small Animal Clinical Oncology; WITHROW S.J., VAIL D.M., PAGE R.; Elsevier Health Sciences, 2013, p.679.
23. BERG R.J., WINGFIELD W., J Am Anim Hosp Assoc 20, 1984, p. 131, In THAMM D. M., Hemangiosarcoma; In Withrow and MacEwen's Small Animal Clinical Oncology; WITHROW S.J., VAIL D.M., PAGE R.; Elsevier Health Sciences, 2013, p.679.
24. MALLINCKRODT M.J., GOTTFRIED S.D., J Am Vet Med Assoc 239, 2011, p. 1325 In THAMM D. M., Hemangiosarcoma; In Withrow and MacEwen's Small Animal Clinical Oncology; WITHROW S.J., VAIL D.M., PAGE R.; Elsevier Health Sciences, 2013 p.679.
25. GAMLEM H, NORDSTOGA K, ARNESEN K, APMIS Suppl, 2008, p. 19, In THAMM D. M., Hemangiosarcoma; In Withrow and MacEwen's Small Animal Clinical Oncology; WITHROW S.J., VAIL D.M., PAGE R.; Elsevier Health Sciences, 2013, p.679.
26. von BEUST B.R., SUTER M.M., Summers B.A., Vet Pathol 25, 1988, p. 251 In THAMM D. M., Hemangiosarcoma; In Withrow and MacEwen's Small Animal Clinical Oncology; WITHROW S.J., VAIL D.M., PAGE R.; Elsevier Health Sciences, 2013, p.679.
27. FERRER L, FONDEVILA D, RABANAL RM, et al, J Comp Pathol 112, 1995, p. 319, In THAMM D. M., Hemangiosarcoma; In Withrow and MacEwen's Small Animal Clinical Oncology; WITHROW S.J., VAIL D.M., PAGE R.; Elsevier Health Sciences, 2013, p.679.

Intrat in redactie: 30.03.2015