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Contribution Regarding the Use of Immunohistochimical Examination in Hemangiosarcoma

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Abstract*. A post-mortem examination of a 5-year-old female Labrador sex female was performed to find out the cause of death. The macroscopic examination revealed a large amount of blood in the abdominal cavity (hemoperitoneum) and pericardial (hemopericard) and also the rupture of the right atrium. The spleen was taken for histopathological examination.*

Keywords*: hemangiosarcomas, immunohistochemical, haemorrhagic*

1.Introduction

This test revealed undifferentiated tumor cells. Hemangiosarcoma is suspected but can not be confirmed because the definitive vascular model is missing. Immunohistochemical examination was performed to confirm the diagnosis.

Hemangiosarcoma (HSA) is a malignant tumor with a high degree of aggression found in the vascular endothelium or endothelial precursor cells. The origin of the tumor is frequently in the spleen in percentage (30-50%), in the right atrium and auricle in percentage (5-50%) and less often in subcutaneous tissues (10-15%). It can also be found in other organs such as: liver, lung, bladder, prostate, peritoneum, kidneys, pulmonary artery, aorta, muscles, bone, oral cavity, tongue, vertebral body and central nerve System. [5, 7, 8, 10, 13, 14, 16].

Metastasis is first established by haematogenous systemic and transabdominal circulation by implantation after disruption of the vessels and the discharge of blood into the cavity. Approximately 80% of dogs can have metastases until the diagnosis. Ware and Hopper emphasize that HSA has the highest incidence at heart level. HSA accounts for 69% of all encountered cardiac tumors.

From the etiopathogenetic point of view, adult dogs (8-13 years old) and the German Shepherd Dog are the most frequently affected. HSA does not cause pain and evolves asymptomatically. Because the vessels have a sinuous tract and malformation, the blood tends to clot and causes the death of the tumor cells. which leads to the production of small ruptures in the tumor, allowing the blood to spill into the abdomen. Hemangiosarcoma tends to spread aggressively and produces remote metastases. Dog death with HSA results from tumor rupture resulting in severe haemorrhage, collapse, and hypovolemic shock [1, 2, 3, 11, 12].

2. Materials and methods.

The necropsy exam was performed on a 5-year-old Labrador female femel after death. To perform the laboratory examination (histopathological), portions were taken from the affected areas of the spleen.

The samples were processed: fixing the alcoholic solution at the usual room temperature (preventing tissue change due to the activity of enzymes; keep the tissuetexture; improves optical differentiation), then dehydration carried out in alcoholic solutions in concentrations increased from 70-100%, in five stages, each lasting two hours, cleaning with benzene,then embedded in paraffin at 56°C. Sectioning of the samples is done at (6 μm), and staining is done by the hematoxylin.

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Staining followed steps: deparaffinization for which benzene was used, then rehydration for which alcohol was used, removal of excess alcohol by distilled water, staining with hematoxylin, alcohol, staining with eosin and staining with methyl blue, removal of water using an increase of alcohol concentration, mounting on slides

In the cytoplasm of infected cells using the immunohistochemical test, both viral antigens and nucleocapside are highlighted. For this, immunoperoxidase is used. Then, under the microscope, the antigen-antibody complexes are highlighted. Small brown formations will be observed in the infected cells, at the level of the cytoplasm. The kit used was purchased from BIO-X Diagnostic.

Also very important was the detection of coagulation Factor 8, for which the above-mentioned kit was used.

The standard protocol provides for three stages of work.

To perform it, it was necessary to take samples from the spleen, from the portions with changes observed macroscopically.

Stage I.

Fixation of the samples, for which paraformaldehyde was used in a concentration of 4%. the fixing time is 24 h. Then followed by rinsing with water, and introduction into alcohol of increased concentrations (50% -70% -95%) for one hour for each, then introduction into toluene for one hour.

Then followed the inclusion in two paraffin baths, at a temperature of 60% (2 h first bath, 1 h second bath).

For stages II and III the standard protocol of the NPDS system (Novolink Polymer Detection System) was used.

This involves performing the section at the microtome, deparaffining in two successive baths of toluene, then rehydration for which ethanol solution was used in decreasing concentrations. This is followed by washing with water, then endogenous peroxedase is neutralized with Peroxidase (10 min), washing in two successive baths of TBS solution, incubation using Protein Block (10 minutes) and again washing in two successive baths of TBS (5 min).

The primary antibody is added to the slide containing sections at a dilution of 1: 100. Then the slides previously stored in the refrigerator for 24 h are washed in successive TBS baths, incubated with Post Primary for 30 min, again washed in two successive baths of TBS solution, then DAB (diaminobenzidine) is added for 5 min and then freshly washed with water. Then add hematoxylin for 40 seconds. Then wash the reeds in two successive water baths (5 min). Finally, the slides are washed with UNYHOL, UNYHOL PLUS and BIOCLEAR. And then after drying and fixing it is examined under a microscope.

3.Results and discussions

At necropsy there was a large amount of blood in the pericardial cavity (hemopericard). A rupture of the right atrium was seen in the examination of the heart. When examining the spleen, a round, protruding surface was observed. Specimens from the spleen were taken from histopathological examination where undifferentiated tumor cells were observed that could not confirm the diagnosis of hemangiosarcoma in the absence of the characteristic vascular design.

Continued immunohistochemical investigations were required as a result of which viral antigens were highlighted by Factor VIII of the cuagle, thus confirming the diagnosis. Specialty literature mentions that HTA represents about 2% of all tumors observed in dogs. The concomitant occurrence of lesions in the spleen and the right cord represents 25% of the cases of HAS.

Data from the literature highlights that several splenic lesions (eg, HSA, hematoma, hemangioma) may have a similar ultrasonographic appearance, but this does not necessarily indicate malignancy.

Immunohistochemistry, highlighting antibodies to vascular endothelial markers is useful for the diagnosis of HAS.

In humans immunohistochemistry was demonstrated to be a useful diagnostic tool, but not useful as a prognostic one. In dogs) it was shown that the r/h F VIII RAg antibody was a positive marker of both

normal and neoplastic endothelial cells. As the HSAs are specificaly by a minimal or absent expression of endothelial markers the specific identification of the HSA is difficult.[1, 4, 5, 7].

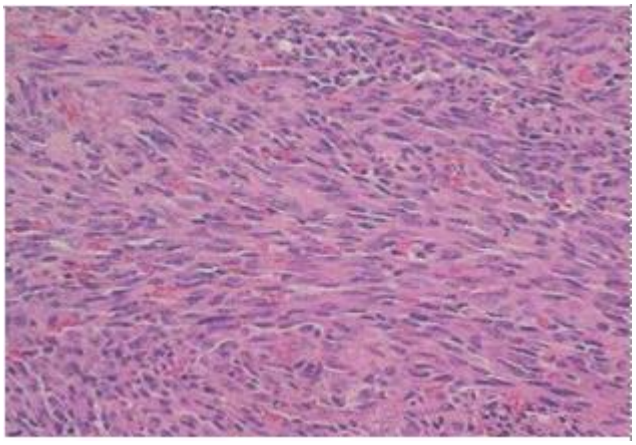

Figure 1. Spleen dog.
Histopathological aspect.
Undifferentiated tumor cells

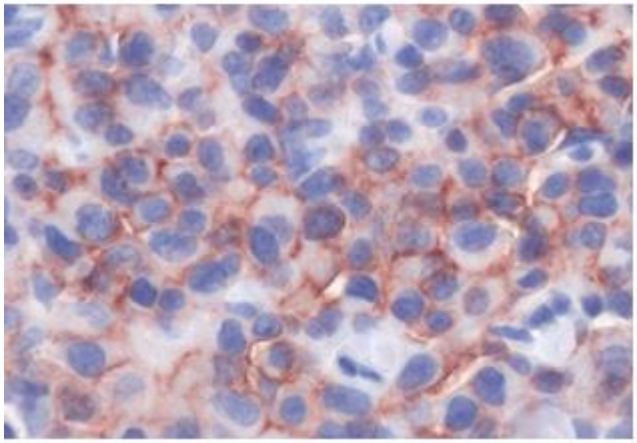

Figure 2. Dog Spleen.
Immunohistochemical exam.
The presence of viral antigens
having a brown color

4.Conclusions

Splenic hemangiosarcoma usually develops asymptomatically until splenic rupture occurs, at which time an overactive abdominal haemorrhage, hypovolemia, shock and ultimately death occur.

Histopathological examination at which undifferentiated tumor cells were observed could not confirm the diagnosis of hemangiosarcoma in the absence of the characteristic vascular design.

It was necessary to continue the investigations by immunohistochemical examination, as a result of which the viral antigens were highlighted with the help of Factor VIII of the cuagle confirming the diagnosis of hemangiosarcoma.

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