

Hemangiosarcoma of the Third Eyelid in an American Pit Bull Terrier

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ABSTRACT

Background: Hemangiosarcoma (HSA) is a malignant neoplasm arising from the endothelial cells of blood vessels. It has fast growth, and severe local infiltration and metastasis power, in addition to risk of hemorrhage due to the fragility of its vessels. HSA develops in dogs aged 8 to 13 years but can affect younger animals too. Ocular involvement in HSA is rare, but when identified, the third eyelid and bulbar conjunctiva close to the limbus are the most affected sites by this neoplasm. This study aimed to report the clinicopathological aspects of a case of HSA in the third eyelid of an American Pit Bull Terrier breed.

Case: A 10-year-old male American Pit Bull Terrier with a history of a red hemorrhagic mass on the third eyelid of the left eye was examined at a veterinary clinic. On physical examination, the animal showed signs of ocular discomfort and bleeding. On ophthalmologic examination, a raised red mass, approximately 2 cm in diameter, was identified on the anterior surface of the third eyelid. The mass was surgically excised. The excised tissue fragment was fixed in 10% buffered formalin solution for 24 h and sent for histopathological examination. Macroscopically, the fragment was irregular, soft, and brownish and measured 2.0 × 1.0 × 0.5 cm. Histologically, proliferation of non-delimited and non-encapsulated atypical endothelial cells, which were organized in vascular arrangements forming small lakes filled with red blood cells, was observed. The cells exhibited elongated and basophilic cytoplasm, oval nuclei with coarse chromatin, and evident nucleoli. Moderate anisocytosis and anisocariosis were observed, with no mitotic figures. Epithelial hyperplasia with mild mixed inflammatory infiltrate was noted. Surgical margins were compromised. Sections of neoplastic tissue were processed for immunohistochemical evaluation with anti-CD31, anti-factor VIII, and anti-Ki-67 antibodies. Neoplastic cells exhibited marked immunostaining for CD31 and factor VIII, and only 8% of these cells were immunostained for Ki-67.

Discussion: The diagnosis of HSA in the third eyelid was based on histological features and positive immunostaining for CD31 and factor VIII. The Ki 67 protein is a marker of cell proliferation, being highly expressed in malignant cells, and has been applied as a prognostic marker for different types of neoplasms. Hemangiosarcoma of the third eyelid is a rare malignant neoplasm in small animals. Dogs are the species most affected by this tumor, with the incidence age varying from 8 to 13 years; however, it can also affect younger animals. Animals with thin, light hair and glabrous regions, especially on the face and periocular region, may be more predisposed to this neoplasm. Surgical excision with a wide margin of safety is the recommended treatment for HSA. In addition, chemotherapy may be indicated as a complement to the surgical procedure, especially if the margins are compromised. The main chemotherapy protocols used for this neoplasm include VAC I and VAC II, which are associated with the drugs, doxorubicin, vincristine, and cyclophosphamide. Another alternative to conventional protocols is the use of metronomic chemotherapy, which involves intensifying an anti-tumor immune response and decreasing tumor vascular density. Differential diagnoses for hemangiosarcoma (HSA) of the third eyelid in dogs include other neoplasms with ocular-conjunctival involvement, such as third eyelid gland adenocarcinoma, conjunctival melanoma, mastocytoma, and squamous cell carcinoma.

Keywords: angiosarcoma, HSA, malignant neoplasm, immunohistochemistry, eye, ocular, dog.

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INTRODUCTION

Hemangiosarcoma (HSA) is a malignant neoplasm arising from the endothelial cells of blood vessels [2]. This tumor has fast growth, severe local infiltration power, and capacity for metastases, in addition to a high risk of hemorrhage due to the fragility of its vessels [18]. This neoplasm can cause disseminated intravascular coagulation due to its ability to modify the physiological process of the coagulation cascade [10].

In dogs, HSAs develops in animals ranging from 8 to 13 years of age but can affect younger animals as well. Large dog breeds, especially German Shepherds, Golden Retrievers, and Labradors, appear to be more predisposed to the development of this neoplasm [17].

Canine primary HSA can develop in any vascularized tissue but is mainly observed in the spleen, liver, and heart, with the right atrium being the most affected site. Clinical signs are nonspecific and vary according to the tumor location [3,7].

Ocular involvement by HSAs is rare, but when identified, the third eyelid and bulbar conjunctiva close to the limbus are the most affected sites by this neoplasm [13]. This study aimed to describe the clinical and pathological aspects of a case of HSA in the third eyelid of an American Pit Bull Terrier.

CASE

A 10-year-old male American Pit Bull Terrier with a history of a red hemorrhagic mass on the third eyelid of the left eye was examined in a veterinary clinic. On physical examination, the animal showed signs of ocular discomfort and bleeding. On ophthalmologic examination, a raised red mass, approximately 2 cm in diameter, was identified on the anterior surface of the third eyelid (Figure 1). No changes in blood count and serum biochemistry were observed. The mass was surgically excised. The excised mass was fixed in 10% buffered formalin solution for 24 h and sent for histopathological examination.

Macroscopically, the fragment was irregular, soft, and brownish and measured 2.0 × 1.0 × 0.5 cm. Histologically, proliferation of non-delimited and non-encapsulated atypical endothelial cells was observed, which were organized in vascular arrangements forming small lakes filled with red blood cells. The cells exhibited elongated and basophilic cytoplasm, oval nuclei with coarse chromatin, and evident nucleoli. There were moderate anisocytosis and anisocariosis, and no mitotic

figures were observed (Figure 2A and B). Epithelial hyperplasia with mild mixed inflammatory infiltrate was also noted. Surgical margins were compromised.

Sections of neoplastic tissue were processed for immunohistochemical evaluation using anti-CD31¹, anti-factor VIII¹, and anti-Ki-67¹ antibodies. Neoplastic cells exhibited marked immunostaining for CD31 and factor VIII (Figure 2C and D), and only 8% of these cells were immunostained for Ki-67.

DISCUSSION

The diagnosis of HSA in the third eyelid was based on histological features and positive immunostaining for CD31 and factor VIII. These immunohistochemical markers are widely used for the diagnosis of HSA, as factor VIII is a glycoprotein synthesized in endothelial cells and megakaryocytes, and the CD31 antigen is a glycoprotein expressed only by endothelial cells, platelets, and macrophages [4]. In addition to these immunohistochemical markers, claudin-5, CD117, and VEGF can be used in the diagnosis of HSA in dogs [6]. The Ki-67 protein is a marker of cell proliferation, being highly expressed in malignant cells, and has been used as a prognostic marker for different types of neoplasms [15]. In this case, immunostaining for Ki-67 was observed in 8% of tumor cells. Tumors with positive immunostaining, between 10% and 14%, for Ki-67 have a worse prognosis [20].

Hemangiosarcoma of the third eyelid is a rare malignant neoplasm in small animals. Dogs are the species most affected by this tumor [11]. HSA has been described to originate from vascular endothelial cells; however, recent studies indicate that HSA originates from bone marrow progenitor cells that reside in the endothelium and subsequently undergo neoplastic transformation [6]. Some studies suggest that environmental factors, such as high sun exposure, may be involved in its development [2,12]. Animals with thin, light hair, and glabrous regions, especially on the face and periocular region, may be more predisposed to this neoplasm [16,19].

In this study, the affected animal was a male dog of the Pitbull breed. Male and large breed dogs, such as German Shepherds, Golden Retrievers, Labradors, Boxers, and Pit Bulls, have been reported to be more frequently affected by HSAs [8,10,16].

The neoplastic behavior of dermal HSA differs from the visceral behavior, especially in the absence

of subcutaneous involvement, and the nodule remains limited to a small region. A higher metastasis rate has been observed in visceral HSA [6].



Figure 1. Hemangiosarcoma of the third eyelid in an American Pit Bull Terrier. A red mass of approximately 2 cm in diameter was observed on the anterior surface of the third eyelid.

Surgical excision with a wide margin of safety is the recommended treatment for HSA [3,14]. Excision is usually curative, and the prognosis is favorable if there are no signs of metastasis. However, relapses can occur, primarily if the safety margin is compromised [8]. In addition, chemotherapy may be indicated as a complement to the surgical procedure, especially if the margins are compromised [9].

The main chemotherapy protocols used for cancer include VAC I and VAC II, which are associated with the drugs, doxorubicin, vincristine, and cyclophosphamide [9]. Another alternative to conventional protocols is the use of metronomic chemotherapy, which involves intensifying an anti-tumor immune response and decreasing tumor vascular density. The protocol aims at continuous administration of low

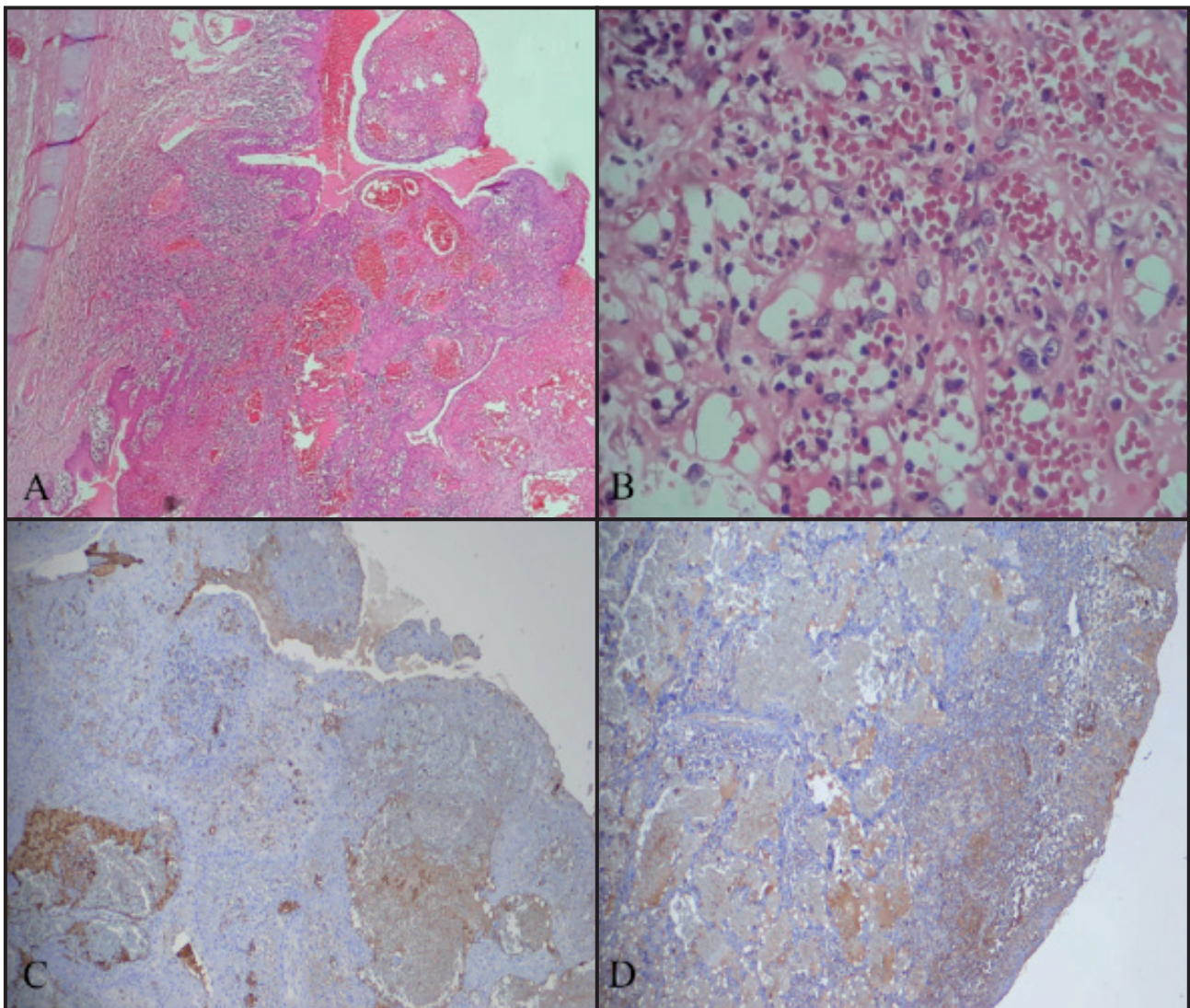


Figure 2. Hemangiosarcoma of the third eyelid in an American Pit Bull Terrier. A- Proliferation of non-encapsulated atypical non-delimited endothelial cells organized in vascular arrangements forming small lakes full of red blood cells [HE; 10x]. B- Neoplastic cells exhibit elongated cytoplasm, oval nucleus with coarse chromatin, and evident nucleolus. There is moderate anisocytosis and anisocariosis [HE; 40x]. C- Positive immunostaining is observed for CD 31 [IHC-DAB; 20x]. D- Positive immunostaining is observed for factor VIII [IHC-DAB; 20x].

doses of chemotherapy without interruption for long periods to reduce the likelihood of drug resistance and genetic mutations, since endothelial cells are more sensitive to this type of exposure [5].

Differential diagnosis of HAS of the third eyelid in dogs includes other neoplasms with ocular-conjunctival involvement, such as third eyelid gland adenocarcinoma, conjunctival melanoma, mastocytoma, and squamous cell carcinoma [1].

MANUFACTURER

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REFERENCES

- 1 **Conceição L.F., Ribeiro A.P., Piso D.Y.T. & Laus J.L. 2010.** Considerations about ocular neoplasia of dogs and cats. *Ciência Rural.* 40(10): 2235-2242.
- 2 **Dantas A.V.E., Carneiro S.C.M.C., Brito E.S.A., Costa A.P.A., Moura V.M.B.D., Carvalho R.O.A. & Lima A.M.V. 2018.** Hemangiossarcoma de terceira pálpebra em uma cadela da raça boxer – relato de caso. *Arquivos de Ciências Veterinárias e Zoologia da UNIPAR.* 21(1): 43-46.
- 3 **Ferraz J.R.S., Roza M.R., Caetano Jr. J. & Costa A.C. 2008.** Hemangiossarcoma canino: revisão de literatura. *Jornal Brasileiro de Ciência Animal.* 1(1): 35-48.
- 4 **Ferrer L., Fondevila D., Rabanal R.M. & Vilafranca M. 1995.** Immunohistochemical Detection of CD31 Antigen in Normal and Neoplastic Canine Endothelial Cells. *Journal of Comparative Pathology.* 112:319-326.
- 5 **Freitas J., Yi L.C. & Forlani C.S. 2019.** Hemangiossarcoma canino: revisão. *Pubvet Medicina Veterinária e Zootecnia.* 13(8): 1-9.
- 6 **Griffin M.A., Culp W.T.N. & Hebhun R.B. 2021.** Canine and feline haemangiosarcoma. *Veterinary Record.* e585: 1-13.
- 7 **Laus J.L., Ortiz J.P.D., Brito F.L.C., Lisboa C.B.S., Silva Jr. V.A. & Maia F.C.L. 2008.** Hemangiosarcoma of the nictitant membrane in a brazilian fila dog: case report. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia.* 60(6): 1413-1417.
- 8 **Liapis I.K. & Genovese L. 2004.** Hemangiosarcoma of the third eyelid in a dog. *Veterinary Ophthalmology.* 7(4): 279-282.
- 9 **Martins K.P., Almeida C.B. & Gomes D.E. 2019.** Hemangiossarcoma canino. *Revista Científica Unilago.* 1(1): 1-12.
- 10 **Moroz L.R. & Schweigert A. 2007.** Hemangiossarcoma em cão. *Campo Digital.* 2(1): 55-55.
- 11 **Multari D., Vascellari M. & Mutinelli F. 2002.** Hemangiosarcoma of the third eyelid in a cat. *Veterinary Ophthalmology.* 5(4): 273-276.
- 12 **Pigatto J.A.T., Albuquerque L., Voll J. & Driemeier D. 2015.** Hemangiosarcoma of the third eyelid in a dog. *Acta Scientiae Veterinariae.* 43(Suppl 1): 91. 4p.
- 13 **Pirie C.G., Knollinger A.M., Thomas C.B. & Dubielzig R.R. 2006.** Canine conjunctival hemangioma and hemangiosarcoma: a retrospective evaluation of 108 cases (1989-2004). *Veterinary Ophthalmology.* 9(4): 215-226.
- 14 **Redaelli R., Alburquerque L., Cavalcante R.L., Rodarte A.C., Gomes C., Franzen A.A., Driemeier D. & Pigatto J.A.T. 2007.** Hemangiossarcoma de terceira pálpebra em um cão. *Acta Scientiae Veterinariae.* 35(Suppl 2): s642-s643.
- 15 **Rozolen J.M., Teodoro T.G.W., Sobral R.A. Sueiro F.A.R., Laufer-Amorim R., Elias F. & Fonseca-Alves C.E. 2021.** Investigation of prognostic value of claudin-5, PSMA, and Ki67 expression in canine splenic hemangiosarcoma. *Animals.* 11(8): 11-14.
- 16 **Schultheiss P.C. 2004.** A retrospective study of visceral and nonvisceral hemangiosarcoma and hemangiomas in domestic animals. *Journal of Veterinary Diagnostic Investigation.* 16(6): 522-526.
- 17 **Smith A.N. 2003.** Hemangiosarcoma in dogs and cats. *Veterinary Clinics: Small Animal Practice.* 33(3): 533-552.

- 18 Soares N.P., Medeiros A.A., Szabó M.P.J., Guimarães E.C., Fernandes L.G. & Santos T.R. 2017.** Hemangiomas e hemangiossarcomas em cães: estudo retrospectivo de 192 casos (2002-2014). *Ciência Animal Brasileira*. 18: 1-10.
- 19 Szivek A., Burns R.E., Gericota B., Affolter V.K., Kent M.S., Rodriguez Jr. C.O. & Skorupski K.A. 2008.** Clinical outcome in 94 cases of dermal haemangiosarcoma in dogs treated with surgical excision: 1993-2007. *Veterinary and Comparative Oncology*. 10(1): 65-73.
- 20 Yang C., Zhang J., Ding M., Xu K., Li L., Mao L. & Zheng J. 2018.** Ki67 targeted strategies for cancer therapy. *Clinical and Translational Oncology*. 20(5): 570-575.