



## Case Report

# Bilateral canine seminoma with ocular metastasis: histochemical and immunohistochemical characterization

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## Abstract

A 10-year old male dog was examined due to a buphthalmia in the left eye and a nodule in the two testicles. Due to the limited resources of the owner and loss of visual acuity of the patient, the enucleation and castration were chosen as treatment. Microscopic analysis of the testicular tissue revealed neoplastic germ cells. Morphologically, neoplastic cells were characterized by distinct cell borders, scarce and eosinophilic cytoplasm, large round nucleus, with thick chromatin and a prominent nucleolus. Binucleated and multinucleated neoplastic cells were also frequently observed. In 10 high power fields (400x), 62 typical and atypical mitosis were counted. Similar neoplastic cells were identified within the vessels of the retina, sclera and in the sub-epithelial conjunctive tissue of the eyelid. The neoplastic cells observed in the testicle and in the eye were positive for PAS. By immunochemistry technique was identified an intense immunostaining of the neoplastic cells for Vimentin and Ki-67 in both testicular and ocular tissue. While, discrete immunoreactivity was identified to c-KIT from the neoplastic cells in both organs. Based on morphological, histochemical and immunohistochemical analysis, it was possible to characterize the ocular lesion as seminoma metastasis.

**Key words:** C-KIT, Ki-67, inhibin- $\alpha$ , immunohistochemistry, neoplasia, dog.

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## Introduction

Testicular neoplasms (TNs) are more frequent in dogs than in other species, including the human (4, 2). Among the TNs, canine seminoma is one of the most frequent, being observed mainly in aged dogs (4). The histogenesis of the tumor is possibly found in germinative tissue as is the case in teratoma and in embryonic carcinoma. Given that dogs and humans are often exposed to the same environmental conditions and present a similar genome, it has been suggested that the canine species can serve as an animal model for the study of human testicular neoplasms (2). Canine seminoma generally presents

benign behavior, despite the observation of histological characteristics of malignancy. However, in cases of aggressive behavior, metastasis was identified in several organs (3, 8). Previously, two cases were reported with metastasis of seminoma in the eyeball, but the immunohistochemical characterization of the tumor or of the metastasis was not included (8). The purpose of this report is to describe the macroscopic, histopathological, histochemical and immunohistochemical findings observed in a canine seminoma with ocular metastasis.

## Case report

A 10-year-old, mixed breed, male dog was reported at the Veterinary Hospital of College of Veterinary Medicine and Animal Science (HV-FMVZ) at UNESP-Botucatu, Brazil, due to a buphthalmia in the left eye. Clinically, it was reported that the owner noted, on the same day of the Hospital visit, ocular secretion and irritation in the affected eye. The owner also noted a nodule in the two testicles a month ago.

Due to the limited resources of the owner and loss of visual acuity of the patient (diagnosed after an ophthalmologic examination), the enucleation of the affected eye and castration were chosen as treatment. Before the castration, a cytological exam of the testicles and regional lymph nodes was performed. Round neoplastic cells were identified in the testicles (Fig. 1A) but not in the lymph nodes.

After surgery, the eye and both testicles and were fixed in 10% buffered formaline and sent for analysis to

the Veterinary Pathology Service of FMVZ-UNESP. At gross examination, the testicles were firm and presented an asymmetrical enlargement. A section from the right testicle revealed a 2 cm in diameter, well circumscribed yellow nodule divided into fine trabeculae of conjunctive tissue measuring. A similar lesion of 3.3 cm diameter was observed in the left testicle. In the eyeball and adnexa it was not possible to identify any macroscopic lesion.

Samples were routinely processed for histopathological evaluation. Immunohistochemistry (IHC) was carried out with the Envision Dual link System-HRP (Dako, CA, USA). The primary antibodies used were vimentin (V9), Ki-67 (MIB-1), E-cadherin (NHC-38), inhibin- $\alpha$  (R1), c-KIT (CD117) and calretinin (DAK Calret 1), the dilution and incubation for each antibody is present in Table 1. The chromogen was 3-3'-diaminobenzidine (DAB, Substrate System, Dako), and Harris's hematoxylin was used to Counterstain. Sections of a normal canine testicle were employed as positive control.

**Table 1.** Immunohistochemistry protocol and primary antibodies used.

Antibody (Clone)	Dilution	Antigen-retrieval method	Incubation period
Vimentin (V9)	1:300	Citrate pH 6.0/Pascal Pressure Cooker (PPC)	18 h/ 4 °C
Ki-67 (MIB-1)	1:50	Citrate pH 6.0/PPC	18 h/ 4 °C
c-KIT (CD117)	1:300	Citrate pH 6.0/PPC	18 h/ 4 °C
E-cadherin (NHC-38)	1:300	Citrate pH 6.0/PPC	18 h/ 4 °C
Inhibin- $\alpha$ (R1)	1:50	Citrate pH 6.0/PPC	18 h/ 4 °C
Calretinin (DAK Calret 1)	1:50	Citrate pH 6.0/PPC	18 h/ 4 °C

Histologically, the samples of both testicles presented a diffuse non-encapsulated proliferation of germinative neoplastic epithelial cells, reaching the stroma and the tunic albuginea (Fig. 1B). The cells were characterized morphologically by a cytoplasm that was well-delimited, scarce and eosinophilic, as well as by a large nucleus, round and centrally located, with thick chromatin and an evident nucleolus. Anisocytosis, anisokaryosis, accentuated pleomorphism and frequent karyomegalia were noted. Binucleated and multinucleated neoplastic cells were commonly observed. In 10 high power fields (400x), 62 typical and atypical mitosis were counted.

In the eyeball and adnexa, the most affected area was the vascular tunic layer, in which small aggregates of neoplastic cells, morphologically similar to those mentioned in the testicles, were observed close to the blood vessels. Neoplastic cells were also observed within the vessels in the external plexiform layer and in the layer of retinal photoreceptors, in the sclera, and in the sub-epithelial conjunctive tissue of the eyelid (Fig. 1C). Some neoplastic cells in the testicles and in the eye were positive for PAS staining (Fig. 1D).

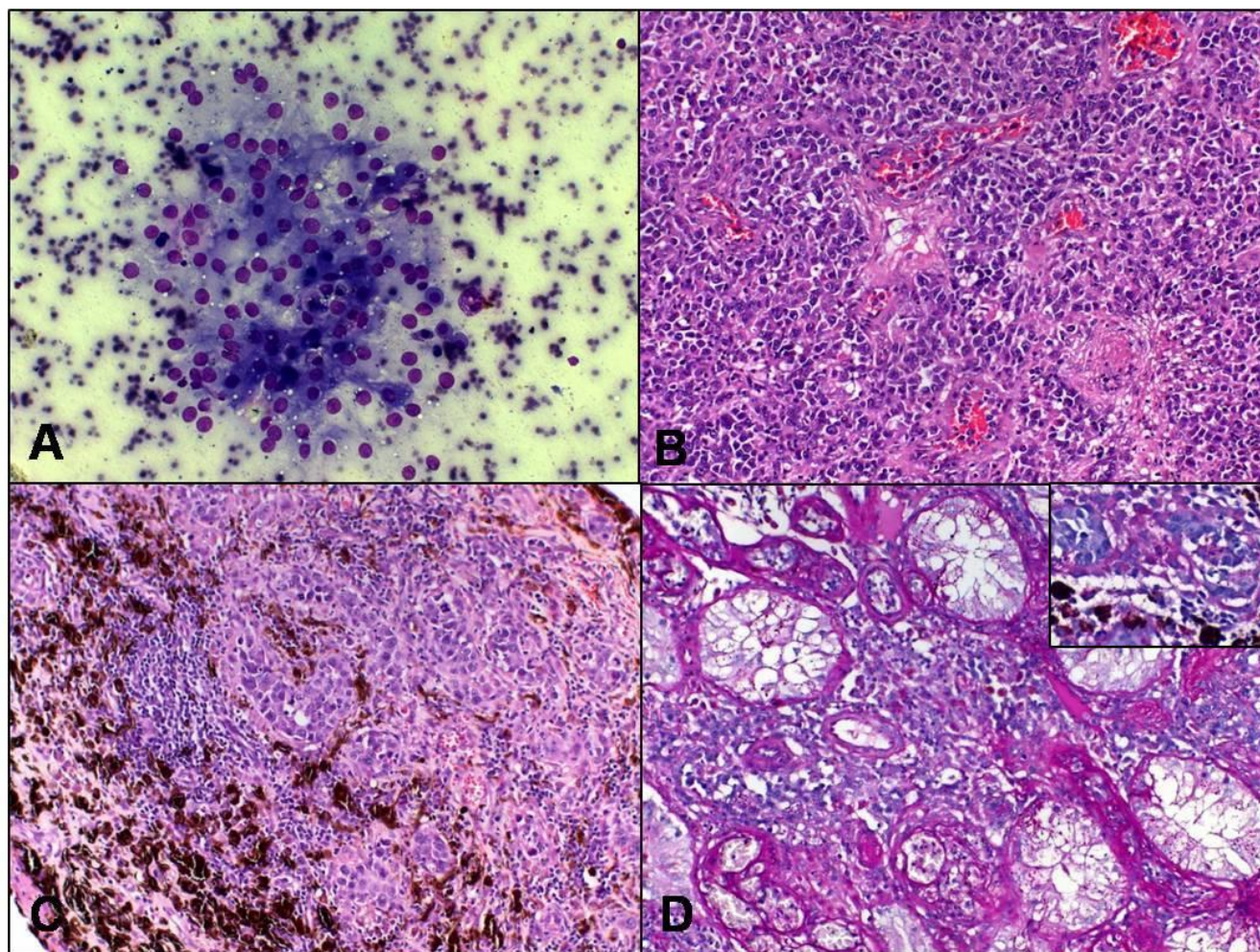
Immunohistochemistry (IHC) reaction revealed intense vimentin and Ki-67 staining in neoplastic cells in both testicular and ocular tissue (Fig. 2A and B). Likewise,

discrete immunoreactivity was identified to c-KIT from the neoplastic cells in both organs (Fig. 2C and D).

On the other hand, the neoplastic epithelial cells were negative for E-cadherin, inhibin- $\alpha$  and calretinin (Table 2). According to the histological, histochemical and immunohistochemical findings, it was possible to diagnose diffuse canine seminoma with metastasis in the eyeball and its adnexa.

## Discussion

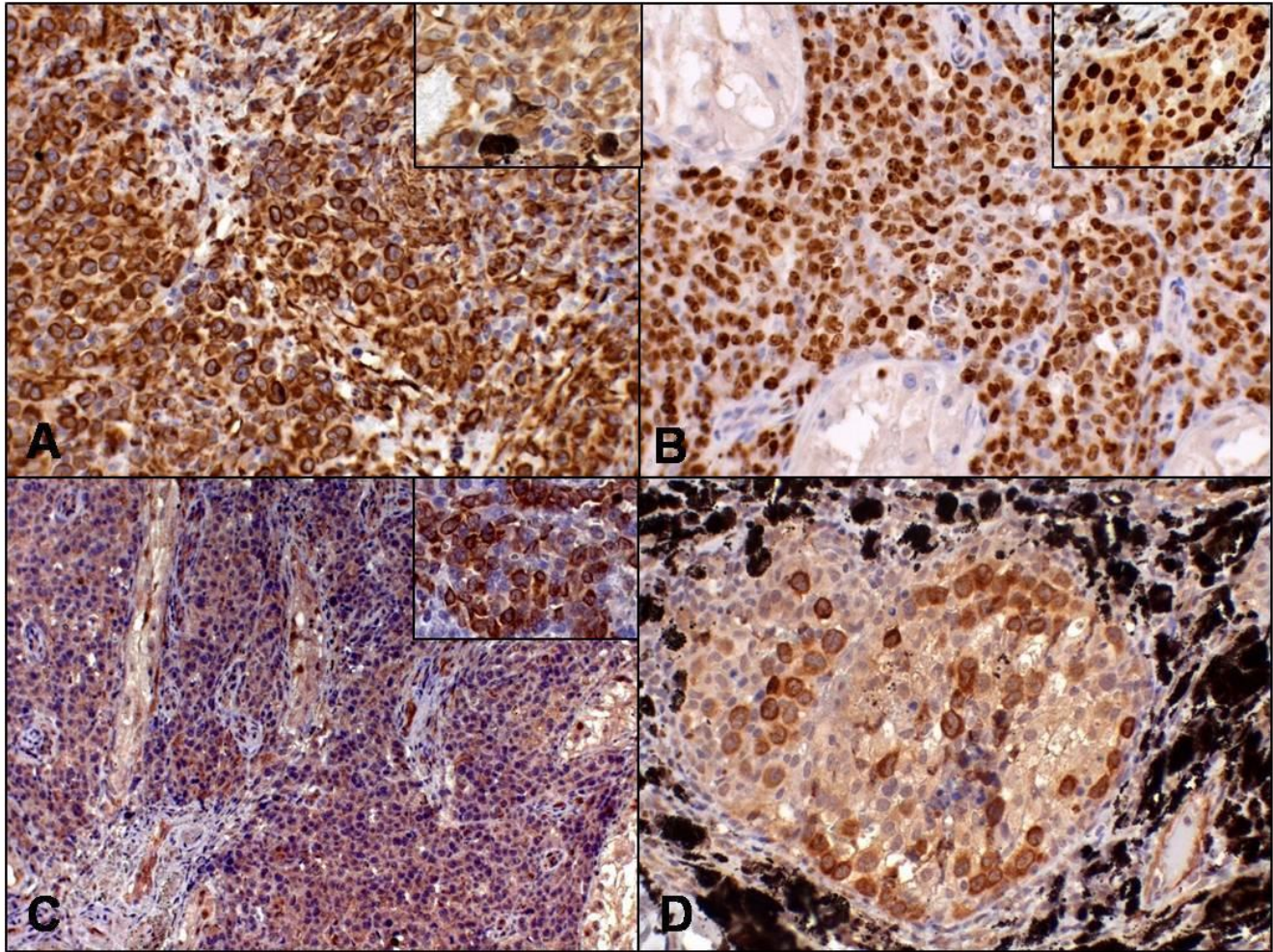
Some papers describing the immunohistochemical analysis of proteins expression in human and canine seminomas are present in literature (2, 10). We selected some of these proteins to immunocharacterize the primary seminoma and its ocular metastasis. CIAPUTA et al. (2014) identified 75% of canine seminomas as being negative for inhibin- $\alpha$ , while 50% and 30% of neoplasias did not express E-cadherin or calretinin, respectively. TANIYAMA et al. (2001) also observed that all the canine seminomas studied were negative to inhibin- $\alpha$ . These works demonstrate that canine seminomas can be negative for these three proteins, as occurred in the present case. Furthermore, we observed staining of the internal positive control, demonstrating the correct functioning of the immunohistochemical test in these proteins.



**Figure 1.** Cytological, histological and histochemical features in the canine seminoma. **A.** The cytological features of the seminoma showed grouped neoplastic cells with variable amounts of bluish cytoplasm, large and round nuclei with reticular chromatin and one or more nucleoli. Panoptic stain, 20x. **B.** Neoplastic cells within and around seminiferous tubules showed strong variation in size (anisocytosis), with distinct cell borders and eosinophilic cytoplasm, nuclei are large, central and round. Hematoxylin & Eosin (HE), 20x. **C.** Metastatic foci mixed with lymphocytes in the chorioidea, left eye. (20x). **D.** Some neoplastic cells positive for Periodic acid-Schiff stain (PAS) in the testicle (20x). Inset, upper right: the neoplastic cells positive for PAS in the chorioidea (40x).

The staining of c-KIT receptor was reported in canine primary and metastatic seminoma (1,5,10). In the present study, more than 75% of neoplastic cells presented cytoplasmic staining for c-KIT with weak intensity, although a strong staining intensity was observed in neoplastic cells found inside blood vessels (Fig. 2D). Similar results were demonstrated in a work by HOHŠTETER et al. (2014) in which half of the diffuse canine seminomas presented 90% of neoplastic cells reactive to c-KIT. Furthermore, Thorvaldsen et al. (2012) identified that 32 out of 44 seminomas evaluated were positive for the c-KIT marker.

In addition, the marker for cellular proliferation Ki-67 and vimentin were positive in the majority of neoplastic germ cells and may be important for characterizing testicular tumors (2, 7). In the present case, some neoplastic cells presented granular PAS staining. One description of similar staining was also reported in other studies, although these works also demonstrated that positivity for this staining might be very low in canine seminomas (6, 10).



**Figure 2.** Immunohistochemical features in the canine seminoma and metastasis. **A.** Immunohistochemistry (IHC) for Vimentin in seminoma, testicle, 20x. Inset, upper right: metastatic foci positive for Vimentin in the chroidea (40x). **B.** IHC for Ki-67 in seminoma, testicle (20x). Inset, upper right: nuclear labeling for Ki-67 antibody in the metastatic foci (chroidea) (40x). **C.** IHC for c-KIT in primary tumor. Inset, upper right: c-KIT showed higher expression in the neoplastic cells (20x) inside the blood vessels than in neoplastic cells in the testicular parenchyma (40x). **D.** IHC for c-KIT in the metastatic foci (chroidea), some neoplastic are strongly positive for the marker (40x).

**Table 2.** Results of immunohistochemical staining in the seminoma, ocular metastasis and normal testicle.\*

Antibody (Clone)	Seminoma			Ocular metastasis Cells	Normal Testicle		
	Tumors Cells	Sertoli Cells	Leydig Cells		Germ Cells	Sertoli Cells	Leydig Cells
Vimentin (V9)	+++	+++	+++	+++	++	+++	+++
Ki-67 (MIB-1)	+++	+	-	++	++	-	-
c-KIT (CD117)	+	-	-	++	+	+	+
E-cadherin (NHC-38)	-	-	-	- (#)	-	-	-
Inhibin- $\alpha$ (R1)	-	-	-	-	-	-	++
Calretinin (DAK Calret 1)	-	-	-	- (&)	-	-	-

\* - it means without staining; intensity of staining+ weak, ++ moderate, +++ strong. # it means positive corneal epithelium (control), & it means positive retinal external nuclear layer (control).

## Conclusion

Based on morphological, histochemical and immunohistochemical analysis, it was possible to characterize the ocular lesion as seminoma metastases.

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