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CASE REPORT

Late prostatic metastasis of an uveal melanoma in a miniature Schnauzer dog

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Key Clinical Message

This manuscript describes a previously unreported clinical case of canine uveal melanoma in a miniature Schnauzer dog with an unusual location of metastasis (prostate) and delayed occurrence (3 years after primary tumor diagnosis and enucleation). Immunohistochemical labeling of both tumors with Melan A, Ki-67, and c-kit added some valuable information.

Keywords

Canine, immunohistochemistry, prostatic metastasis, uveal melanoma.

Introduction

Tumors of melanocytic origin are the most common primary ocular neoplasm in dogs [1, 2]. In this species, 47% of the ocular and adnexal neoplasms affect the globe itself and 53% affect the adnexia [3]. In the tumors of the globe, 64.6% are melanocytic tumors.

Although both benign and malignant forms of canine ocular melanocytic tumors are frequent, evidence of metastatic disease in primary ocular melanocytic neoplasia is rarely reported, even in tumors that are malignant by cellular criteria [1, 4, 5].

There are several types and locations of canine ocular melanocytic tumors, with different incidence and biological behavior. Melanocytic lesions may occur as benign melanocytoma or malignant lesions. Lid margin are usually melanocytomas and benign, while the majority of canine conjunctival melanomas are malignant [6]. Limbal melanocytomas are generally benign, but it is important to distinguish between limbal melanocytoma and extraocular extension of uveal or conjunctival melanomas [6].

Most of canine uveal melanocytic neoplasms are benign melanocytomas. From these, anterior uveal melanocytomas are more common (94%) and only 6% are located in the choroid [6]. The majority of canine uveal malignant melanomas are also in the anterior uvea. These are characterized by histopathological features of malignancy yet few metastasize. Immunohistochemistry for melanocyte markers such as Melan-A [7], and for membrane receptors involved in melanogenesis, such as c-kit [8], may be useful in diagnosing amelanotic tumors. In canine ocular melanoma, the mitotic index has been considered a highly important behavior predictor [2, 9]. The use of cell cycle markers such as Ki67 is, therefore, justified, being a more accurate indicator of the proliferation index [10]. These markers are putative prognostic indicators not yet validated for canine ocular melanomas. Although ocular melanoma in dogs rarely metastasize, prognosis being only slightly guarded, metastases of uveal melanoma have been reported in lungs, regional lymph nodes, and liver [1, 5, 6].

In cats, melanocytic neoplasms presented as diffuse iris melanoma and shows considerable variation in cellular

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morphology. In humans, uveal malignant melanoma is the most common primary intraocular tumor of adults, occurring predominantly in the choroid (85%), with about 10% in the ciliary body and 5% in the iris [6]. Except in the iris, human uveal malignant melanoma is a highly aggressive tumor, and about half of patients die with hepatic metastatic disease 10–15 years of diagnosis [6].

Morphological aspects such as tumor size, location, and extension within the globe, together with histological features such as cellular atypia and mitotic index, have been used as predictors of long-term biologic behavior of these neoplasms validated with published results of series [1, 9, 11]. At present, adding molecular and genetic characteristics to the information provided by morphology is useful in defining prognostic indicators.

In the present case, metastases were identified initially in the prostate, an unusual location, followed by dissemination leading toward a fatal outcome. To the best of our knowledge, this is the first report of a prostatic metastatic disease secondary to an uveal malignant melanoma in a dog.

Case Report

A 9-year-old male intact miniature Schnauzer dog presented to the Ophthalmology consultation at the Teaching Hospital of the Faculty of Veterinary Medicine of the University of Lisbon for evaluation of changes in the right eye. A complete ophthalmic examination was performed. The menace response, the dazzle reflex, the pupillary light reflexes, and the blink reflex were normal bilaterally. Schirmer tear test-1 (STT-1, Dina strips Schirmer-Plus, Luneau SAS, Chartres, France) readings were 19 mm/min OU. Intraocular pressure, measured using applanation tonometry (Tono-Pen XL, Medtronic Solan) following instillation of a topical anesthetic (Oxibuprocaine, Anestocil®, Laboratórios Edol, Lisbon, Portugal), was 17 and 16 mmHg for the right and left eye, respectively. Slit-lamp biomicroscopy (SL14 Kowa Company, Tokyo, Japan) revealed that there were no lesions affecting the anterior chamber or the lens of the left eye and there was diffuse iris thickening and melanosis of the right eye. A drop of 1% tropicamide (Tropicil TopTM, Laboratórios Edol, Lisbon, Portugal) was applied onto each eye to facilitate funduscopic evaluation, which revealed no lesions. No additional problems were identified on physical examination. At that time ocular ultrasonography was advised for further investigation, but the owner did not return until 6 months later.

The right eye presented intense corneal neovascularization, neopigmentation, and granulation tissue secondary to an intraocular mass that occupied the anterior chamber, making it impossible to identify any intraocular structure. On complete ophthalmic examination, the menace response and the dazzle reflex were present in the left eye and absent in the right eye. The pupillary light reflexes were impossible to evaluate in the right eye and normal in the left eye. The blink and the corneal reflexes were normal bilaterally. STT-1 (Dina strips Schirmer-Plus, Luneau SAS, Chartres, France) readings were 17 mm/min OU. Intraocular pressure, measured using applanation tonometry (Tono-Pen XL, Medtronic Solan) following instillation of a topical anesthetic (Oxibuprocaine, Anestocil®, Laboratórios Edol, Lisbon, Portugal), was 47 and 17 mmHg for the right and left eye, respectively. Slit-lamp biomicroscopy (SL14 Kowa Company, Tokyo, Japan) revealed intense corneal neovascularization, neopigmentation, and granulation tissue as well as the presence of an intraocular mass that occupied the anterior chamber, making it impossible to identify any intraocular structure (Fig. 1). The left eye presented no abnormalities on biomicroscopy or funduscopy.

A medical history was obtained and a complete physical examination was performed, since schnauzers may be predisposed to melanocytic lesions and melanomas in other areas. Differential diagnosis included cystic primary neoplasia such as pigmented adenoma or adenocarcinoma of the ciliary body, metastatic neoplasia, or ocular melanocytic tumors and prompted further investigation.

Since one of the hypotheses was an intraocular tumor, evaluation of the extension of the disease was achieved with complementary examinations. Three-view thoracic X-ray and abdominal ultrasonography were performed, and did not reveal any nodular formations compatible with metastatic disease. Complete blood count and serum

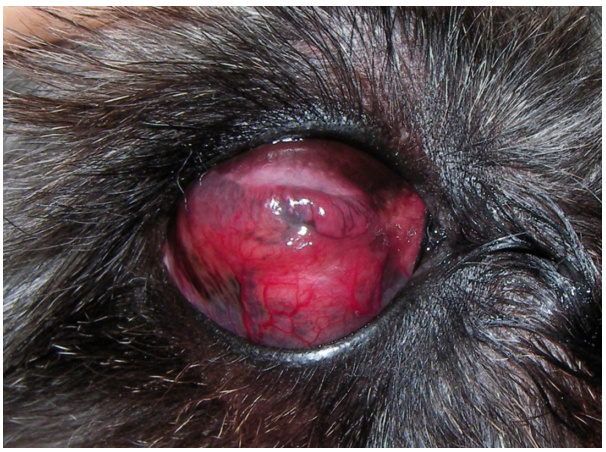

Figure 1. Severe corneal neovascularization, neopigmentation, and granulation tissue secondary to an extensive intraocular mass that occupied the anterior chamber, making it impossible to identify any intraocular structure.

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biochemistry analysis were within normal limits. Clinical reevaluation of the regional lymph nodes did not reveal any changes. Considering that it was a blind, painful eye, highly suspicious of having an intraocular tumor, urgent exenteration of the right eye was accomplished, trying to obtain tumor-free margins (Fig. 2). Recovery was uneventful.

Histopathology of the primary tumor

The intraocular tumor was a solid and deeply pigmented mass that occupied the posterior and the anterior chambers and the vitreous. The tumor invaded the sclera, but did not show extrascleral extension. Histologically, the tumor was composed of compact round to spindle-shaped melanocytic cells with variable amounts of melanin, measuring about 26 $\times$5 μm (Fig. 3A). It showed various criteria of malignancy such as atypical nuclei, including anisokaryosis, karyomegaly, and large nucleoli. The mitotic index was estimated as corresponding to four mitosis per 10 high-power fields ($\times$400). The diagnosis was of invasive malignant uveal melanoma, most probably deriving from the anterior uvea.

Follow-up

The dog had clinical rechecks every 6 months that included complete physical and ophthalmic examination. The left eye remained normal and no local recurrence at the surgical site in the right orbit was observed. For 3 years, the patient remained asymptomatic, after which it presented to the Teaching Hospital of the Faculty of Veterinary Medicine with urinary tract complaints. Abdominal ultrasonography showed prostate enlargement, asymmetry, and a nodular lesion with mixed echogenicity and calcifications in the right lobe, compatible with neoplastic disease. The other abdominal organs showed no changes. Three-view thoracic X-ray and blood analysis were normal. Orchiectomy plus an exploratory laparotomy were performed. During the procedure it was possible to identify and biopsy the nodule detected by ultrasonography, which was darkly colored. The biopsy was triangular prism shaped and measured approximately 5 $\times$ 5 $\times$ 5 mm. Differential diagnosis of the prostatic lesion included prostatitis, prostatic cysts, benign prostatic hyperplasia, and neoplasia. Recovery was uneventful.

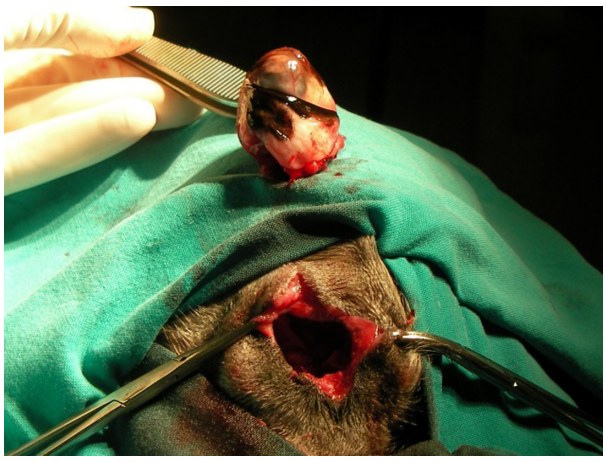

Figure 2. During the exenteration procedure, right buphthalmos secondary to the extensive intraocular tumor.

Histopathology of the prostatic tumor

Histopathology of the prostate biopsy revealed replacement of the glandular tissue by spindle cells, some of which were pigmented (Fig. 4A). These cells displayed features of malignancy, including nuclear atypia (anisokaryosis) and frequent mitotic figures. A presumptive diagnosis of metastasis of the uveal melanoma was issued. The testicles showed no histological changes.

Immunohistochemistry

To characterize the tumor cells in all analyzed tissues the following three primary antibodies were used: Melan A (reference M 7196; Dako, Queluz de Baixo, Lisbon, Portugal), c-Kit (reference A 4502; Dako), and Ki67 (reference NCL-L-Ki67-MM1; NK, Newcastle, UK). Antigen retrieval for the first two markers was achieved with Ethylenediamine tetraacetic acid (EDTA) pH 9.0 in microwave oven, and for Ki-67 with citrate buffer pH 6.0 in pressure cooker. For detection of the primary antibodies the Novolink Polymer Detection System was used (reference 7140; Leica, Carnaxide, Portugal) in which the chromogen is diaminobenzidine. As the presence of pigment could interfere with the interpretation of the technique, the removal of melanin was performed with 3% hydrogen peroxide. Counterstaining was obtained with Mayer's hematoxylin.

The Ki-67 proliferation index was determined by assessing the percentage of positively staining tumor cell nuclei in 500 tumor cells, using Image J open source software (version 1.46r).

Immunohistochemical labeling with Melan A showed strong positive staining of the cytoplasm of both uveal tumor cells and of the cells of the prostatic nodule (Figs. 3B & 4B). In view of the results obtained with Melan A, the diagnosis of metastasis of the uveal tumor in the prostate was issued. The equivalent staining intensity for Melan A in the eye tumor and in the prostate

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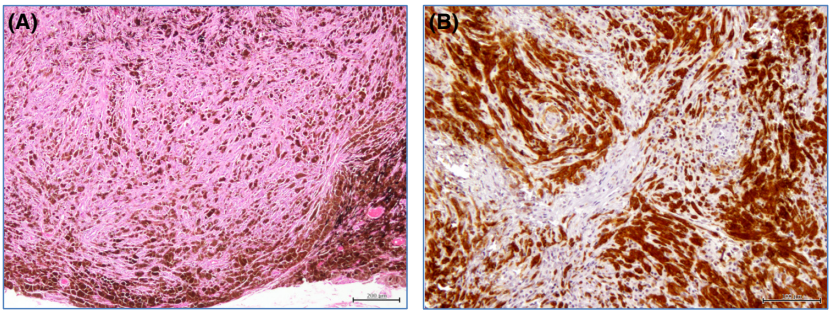

Figure 3. Uveal melanoma. (A) The melanocytic cells are arranged in compact lobules, with variable amounts of melanin (H&E). (B) Melan A labeling positive for 80% of the cells present in the tumor. Intensity was considered strong (Novolink Polymer Detection System, Mayer's hematoxylin). The brown color is due to the used chromogen and not to melanin.

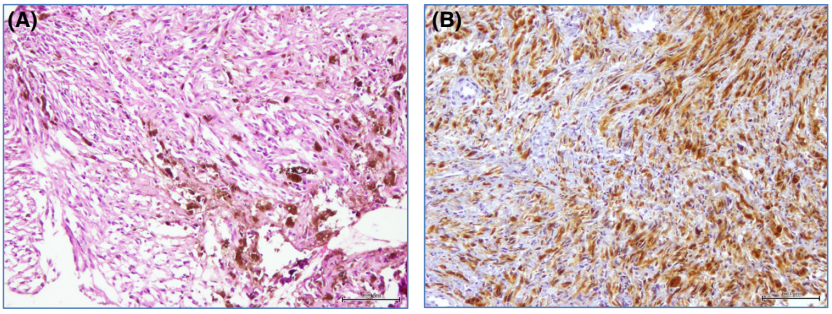

Figure 4. Prostate metastasis of the uveal melanoma. (A) The prostate glandular tissue was replaced by spindle cells, part of which were pigmented (H&E). (B) Melan A labeling positive for 80% of the cells present in the tumor. Intensity was considered moderate to strong (Novolink Polymer Detection System, Mayer's hematoxylin). The chromogen used for labeling is brown.

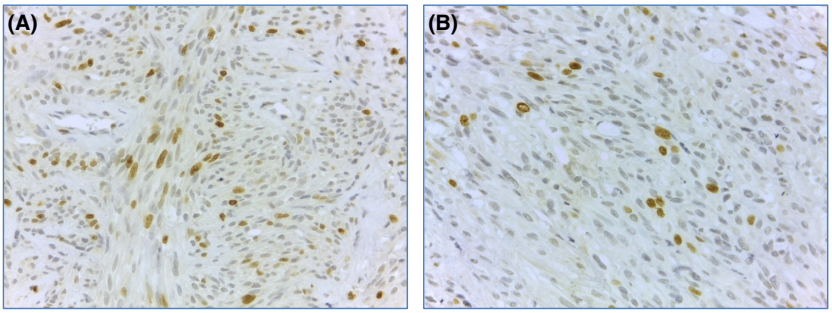

Figure 5. Cell proliferation estimated by Ki-67 labeling. (A) Nuclear labeled positive cells in the uveal melanoma corresponds to a proliferation index of 18.9. (B) The number of positive cells in the prostate metastasis with this immunomarker corresponds to a proliferation index of 7.2 (Novolink Detection System and Mayer's hematoxylin counterstain, ×400).

showed that there was no loss of differentiation in the metastasis process.

Ki-67 yielded a high score of nuclear labeled positive cells in the uveal melanoma (proliferation index of 18.9) and a medium score of positive cells in the prostatic metastatic melanoma (proliferation index of 7.2) (Figs. 5A and B). Labeling for c-kit was negative in the uveal melanoma and in the prostate metastasis.

Outcome

Radical prostatectomy or chemotherapy was declined by the owner. Six months later, due to worsening of the clinical condition, the animal was submitted to tomographic examination and diagnosed with metastatic disease in the mesenteric lymph nodes, right kidney, and lungs, and was euthanized.

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Discussion

Canine primary intraocular melanocytic neoplasia remains an important disease entity. The majority of these tumors are found in middle to older aged dogs and have an anterior uveal tract distribution [1], which correlates well with the case presented.

The precise origin of the tumor was considered to be the anterior uvea, due to the initial clinical examination and histopathological examination. In canine ocular neoplasms, only uveal tumors are reported as having malignant potential similar to what was observed in this case. In fact, 15–20% of all canine anterior uveal tumors of melanocytic origin are histologically malignant [2, 11], and approximately one third of these have been confirmed to be behaviorally malignant by virtue of extraocular metastases [11]. Uveal melanomas considered malignant showed the same histological criteria of malignancy that were reported in the present case, such as invasive and destructive growth, anisokaryosis, and a mitotic index of 4. The most reliable behavior predictor in these cases was thought to be the mitotic index [9]. In retrospective studies, a mitotic index in excess of 3 in 10 high-power fields has been associated with a moderate risk of metastasis, with most metastasized tumors having an index equal or higher than four mitosis per 10 high-power fields [9, 12], the same mitotic index of the ocular tumor hereby described.

The most common metastases of uveal malignant melanoma have been described in the lungs, lymph nodes, and liver [13]. Contrary to the prevailing idea that ocular melanoma in dogs rarely metastasize, prognosis being therefore only slightly guarded [6], in the present case the tumor metastasized to an unusual location, the prostate, the metastatic disease being the reason for euthanasia.

Regardless of the tumor classification, recurrence of ocular melanomas at the surgical site following enucleation appears to be minimal [1].

Concerning the delayed occurrence of the metastasis, in the few metastasizing uveal melanomas in dogs reports in the literature, metastasis occurred 3 [4] and 18 months after enucleation [5]. In humans, uveal melanoma typically can lead to death caused by delayed metastasis several decades after the primary tumor was definitively treated. Where the metastatic cells reside in apparent dormancy and which events delay clinical metastasis are still unanswered questions [13, 14].

Our dog is a miniature Schnauzer, and there is breed predisposition to nonocular melanocytic lesions and melanomas [15].

The choice for the immunohistochemical markers used was based on the results obtained in melanomas in dogs and in humans. Both tumors tissues, eye and prostate, showed positive staining for Melan-A, which helps confirming the melanocytic origin of the prostatic metastasis. Melan A is a melanocyte differentiation marker to ascertain the melanocytic origin of a non or little pigmented tumor and it has been validated in dogs [16, 17]. Labeling for Ki-67 revealed the primary uveal tumor to have a high proliferation index of 18.9. Ki-67 index has been extensively used to associate proliferative markers with prognosis, but so far it has not been validated for ocular melanomas [18].

Smedley et al. [18] published a study on prognostic factors for melanocytic tumors of the skin, digits, lips, and oral cavity of dogs. Unfortunately, ocular melanocytic neoplasms were not included owing to insufficient material. Labeling for Ki-67 revealed the primary uveal tumor to have a proliferation index of 18.9 which was markedly decreased in the metastasis. In a study by Bergin and colleagues (2011) of oral melanomas, a proliferation index higher than 19.5 was considered predictive of death or euthanasia within a year after the initial diagnosis of melanoma [10].

Recently C-kit expression has also been considered a prognostic marker as its expression may reveal mutations on tyrosine kinase receptor protein, although there is no consensus in the literature [8]. According to Gomes and colleagues, immunohistochemical labeling for c-kit is stronger in cutaneous melanocytic benign tumors, its expression being reduced or lost in malignant tumors [8]. The negative labeling of the uveal melanoma for this marker may be an additional indicator of malignancy.

Immunohistochemistry was, therefore, useful for further characterization of the uveal melanocytic tumor and its metastasis. As more cases will be studied in the future, Ki-67 proliferation index may also prove to be an interesting prognostic indicator in ocular melanomas, and is the case of other melanomas [18].

In humans, uveal melanoma typically can lead to death caused by delayed hepatic metastasis several decades after the primary tumor was definitively treated. Where the metastatic cells reside in apparent dormancy and which events delay clinical metastasis are still unanswered questions [13, 14]. There are few, but some reports on metastasizing uveal melanomas in dogs, namely one case of pulmonary metastasis [4] and another of vertebral metastasis [5].

In conclusion, our finding of a late distant prostatic metastasis of an uveal melanoma in a dog is in contrast to the expectation that these tumors are of little clinical significance beyond their effects on the eye. It is important to remember that some canine uveal melanocytic tumors can give rise to distant metastases.

Recently, a study evaluated 14 genes that discriminate metastasizing human uveal melanomas for their potential

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utility as gene expression markers for predicting canine uveal melanoma metastasis, which may be another tool to use in the future [19].

Metastasis may occur in an unusual location as the prostate and may be delayed. Until now we do not have predictive features that help distinguishing cases that will and that will not metastasize, but immunohistochemistry panels are useful tools for accurate diagnosis and may help establishing a prognosis.

Conflict of Interest

None declared.

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