AQUEOUS HUMOUR FROM A DOG

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Signalment:

6 year-old, female neutered, Labrador cross dog.

Clinical history:

2-month history of blepharospasm and a red left eye (figure 1).

Clinical findings:

General physical examination was unremarkable. No history of previous ocular problems.

Diagnostic procedures:

Visual responses and light reflexes including menace, dazzle and PLR's were considered within normal limits. Slit lamp biomicroscopy (Kowa SL-17) revealed episcleral congestion with distortion of the ventromedial pupil, dyscoria and increased iris pigmentation in the left eye. Aqueous flare and pigment deposition on the anterior lens capsule were detected. Intraocular pressure (tonometry) [Tono-pen Vet; Reichert] readings were recorded (12mmHg for the right eye and 13mmHg for the left eye – 10 to 25 mmHg in normal dogs). Gonioscopy revealed infiltration of the ventral and medial iridocorneal angle by an irregular, red and white, soft tissue mass. Ultrasonographical examination of the left globe confirmed thickening of a large portion of the ventral uveal tract (figure 2).

Aqueous humour was collected and submitted for cytological examination (figures 3 and 4). A cytocentrifuge preparation had moderate to high nucleated cellularity on a moderate background of red blood cells. A 500-nucleated cell differential count revealed macrophages (47%), cohesive atypical cells (27%), small lymphocytes (23%) and neutrophils (3%). Plasma cells, abnormal mitotic figures, pyknotic cells and erythrophagocytosis were rare. Moderate amounts of melanin-like

material (dark green to black to golden, round to needle-like, 1- 3 µm in diameter granules) were noted both in the background and in the cytoplasm of the macrophages and the cohesive cells. Coarsely granular, black-staining material (presumptive hemosiderin) was rarely seen in the cytoplasm of the macrophages. The cohesive, atypical cells were large (14-21 µm in diameter), polyhedral to oval in shape and arranged in clusters. These cells usually had well defined cellular borders, sometimes undulated. The cytoplasm was palely or moderately basophilic, it was present in small or moderate amounts and it sometimes contained variable numbers of 2 to 4 µm small to medium-sized, non-staining vacuoles. The nucleus measured between 7 and 14 µm in diameter and it was round, oval or slightly indented with a reticular to coarse chromatin pattern and occasional, one or two, 2 to 5 µm, round nucleoli. Anisocytosis and anisokaryosis were marked. Bi-nucleated cells were occasionally seen, multinucleated cells (up to 5 nuclei) were rare and nuclear size variation within the same cell was also noted. The NC ratio was variable. The cytological interpretation was carcinoma associated with mixed-cell, predominantly mononuclear cell inflammation and possible acute and chronic intraocular haemorrhage.

Treatment and follow-up:

The affected eye was enucleated and submitted for histopathological assessment. Macroscopically, the iris and ciliary body were focally replaced by a solid, grey-white mass (approximately 0.7x0.5x0.3cm) (figure 5). On light microscopy, the iris, ciliary body and drainage angle were focally and extensively infiltrated by a highly cellular neoplasm that infiltrated the adjacent sclera and dissected beneath the terminus of Descemet's membrane (figure 6). Neoplastic cells were polygonal to epithelioid, formed solid sheets and nests and were separated by seams of homogeneous, PAS-positive, basement membrane-like material. Neoplastic cells contained moderate amounts of palely eosinophilic cytoplasm and a single, round to ovoid, vesicular to hyperchromatic nuclei with distinct nucleoli. Rare neoplastic cells contained coarse, intracytoplasmic melanin pigment. Occasional karyomegaly associated with irregularly cleaved nuclei was evident. Cellular pleomorphism was moderate and variation in nuclear size was marked. Mitoses were frequent (38/10 HPFs) (figure 7). The main histopathological morphological diagnosis was presumptive iridociliary adenocarcinoma. Additional findings included mild lymphoplasmocytic anterior uveitis, posterior synechiae and mild vitreal haemorrhage. The neoplastic cells were immunopositive for cytokeratin (clone AE1/AE3), vimentin, S100 and NSE (figure 8) and immunonegative for GFAP, desmin, PNL-2 and Melan A (figure 9). This immunohistochemistry profile was consistent with an iridociliary adenocarcinoma. The owners declined staging at the time. 21 months post-surgery, the dog is doing well with no signs of local recurrence or metastatic spread.

Discussion

Primary ocular neoplasms are uncommon in dogs relative to neoplasia affecting other organs. Primary ocular neoplasia is more common than secondary neoplasia. Intraocular structures are most likely to be affected by the latter in comparison to the adnexal/ocular surface and the orbit.

Cytological examination of aqueous humour has been reported to have low diagnostic utility, but it may be valuable for diagnosing neoplastic uveitis, especially in cases of lymphoma. Most other intraocular tumours, either primary or secondary, do not seem to exfoliate into aqueous humour, with rare reports of mast cell tumours and large cell carcinomas. In the present case, cytological examination of aqueous humour allowed a definitive diagnosis of a carcinoma. An iridociliary adenocarcinoma was a top differential diagnosis, but metastatic neoplasia could not be ruled out as the anterior uvea is also a site for metastases of systemic carcinomas.

Iridociliary epithelial tumours are rare, but they are the second most common primary intraocular tumour in dogs after melanomas. The former arise from the pigmented and/or nonpigmented epithelial cells of the iris and/or ciliary body. Clinically, neoplasia of the ciliary body epithelium appears as nonpigmented to lightly pigmented pink masses that may protrude into the pupillary aperture, be visible in the anterior chamber and displace the iris anteriorly. Histologically, iridociliary epithelial tumours are pleomorphic and occur in either the iris and/or ciliary body. Solid, papillary or cystic tissue organisations are possible. Many of these tumours secrete thick, PAS-positive basement membrane reminiscent of the inner lining of the non-pigmented ciliary body epithelium. Secretion of hyaluronic acid is further evidence of iridociliary epithelial differentiation.

Mimicking normal neuroepithelium, iridociliary epithelial tumours are immunopositive for vimentin and NSE and variably immunoreactive for S100, desmin and GFAP. Normal iridociliary epithelium is immunonegative for pan-cytokeratin (AE1/AE3), CK7, CK20 and CK8/18. Iridociliary adenomas and carcinomas are also not immunoreactive to CK7. In contrast, non-invasive iridociliary adenomas tend to express CK20 and CKAE1/AE3 while invasive iridociliary adenomas tend to express CKAE1/AE3 and have variable expression of CK20. Iridociliary carcinomas tend to show immunoreactivity for CKAE1/AE3 and do not express CK20. The latter can be recognised by cellular features of anaplasia, increased necrosis and mitosis and aggressive infiltration of the sclera. Many iridociliary neoplasms secrete thick, PAS-positive basement membrane reminiscent of the inner lining of the non-pigmented ciliary body epithelium. Both iridociliary adenomas and adenocarcinomas are immunoreactive to telomerase reverse transcriptase (TERT), but malignant iridociliary neoplasms have increased TERT expression. The presence of pigmented epithelium, PAS-positive membranes, hyaluronic acid secretion and immunopositivity for vimentin, NSE and S100 can be useful to define iridociliary epithelial tumours in dogs and distinguish between a primary and a metastatic carcinoma.

Distant metastases of even infiltrative iridociliary carcinomas are extremely rare. The prognosis for life with ciliary body adenomas and adenocarcinomas is good, although the prognosis for the globe is poor as enucleation is required for most cases.

Conclusion:

The present case documents an occasion in which an initial interpretation of carcinoma was made possible by cytological examination of aqueous humour. This was critical in directing clinical

management. Routine cytological examination of aqueous humour may be recommended as a regular component of the ophthalmological work-up whenever neoplasia is a differential diagnosis.

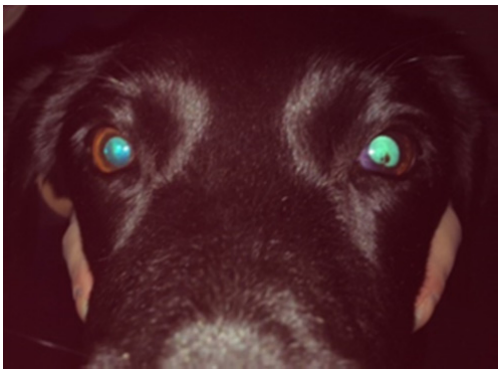

Figure 1 - Note abnormal pigmentation of the left iris.

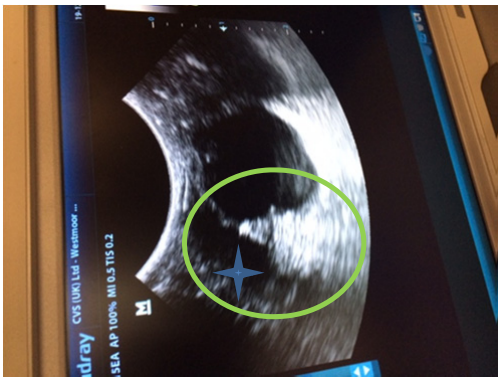

Figure 2 - Left eye ultrasonography.

Thickened ventral ciliary body and iris (within marked area)

(anterior chamber marked with a blue 4-point star)

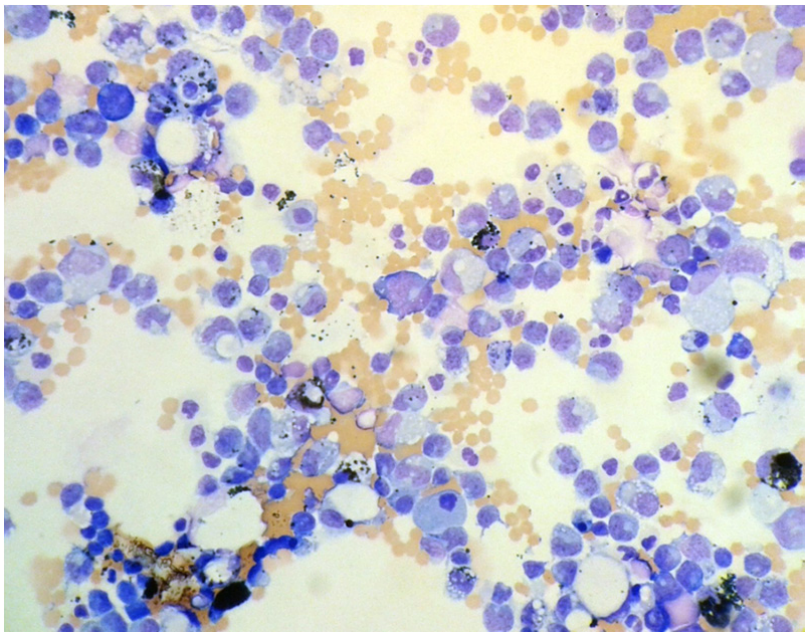

Figure 3 - Cytocentrifuge preparation of aqueous humour from a dog. Wright Giemsa, x20 objective.

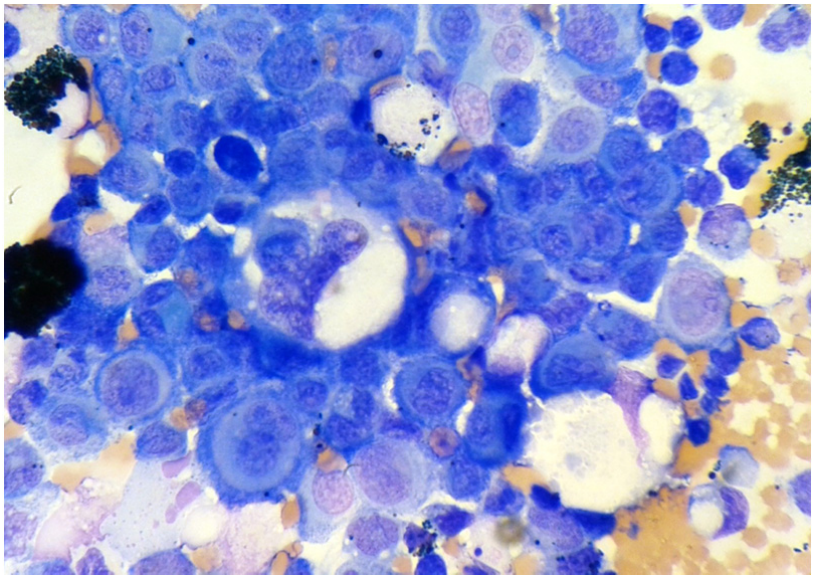

Figure 4 - Cytocentrifuge preparation of aqueous humour from a dog. Wright Giemsa, x100 objective.

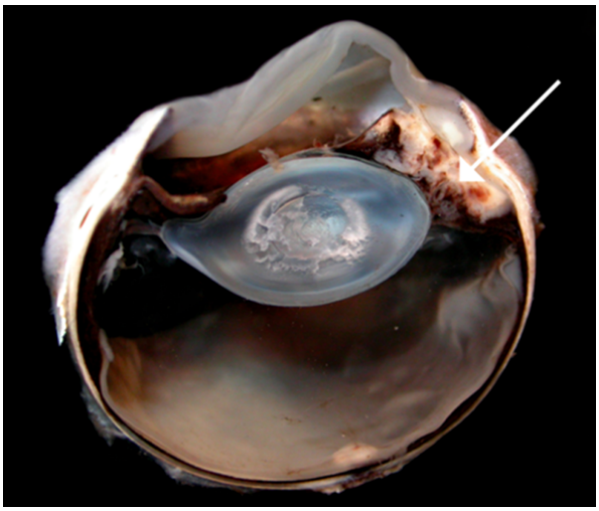

Figure 5 - Macroscopic appearance of the enucleated eye globe.

A solid, grey-white mass (white arrow) is focally replacing the iris and ciliary body.

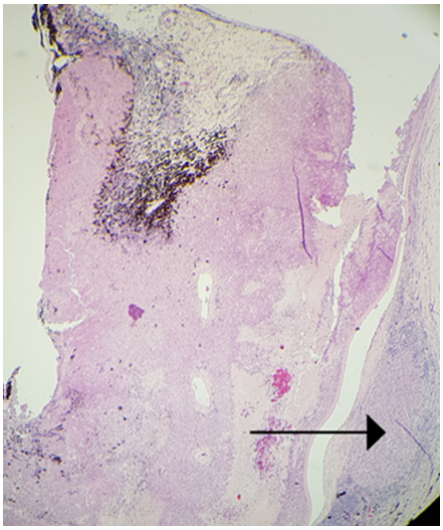

Figure 6 - Histological section of an iridociliary adenocarcinoma. H&E, x 5 objective.

The iris, ciliary body and drainage angle are focally and extensively infiltrated by a highly cellular neoplasm that infiltrates the adjacent sclera (black arrow).

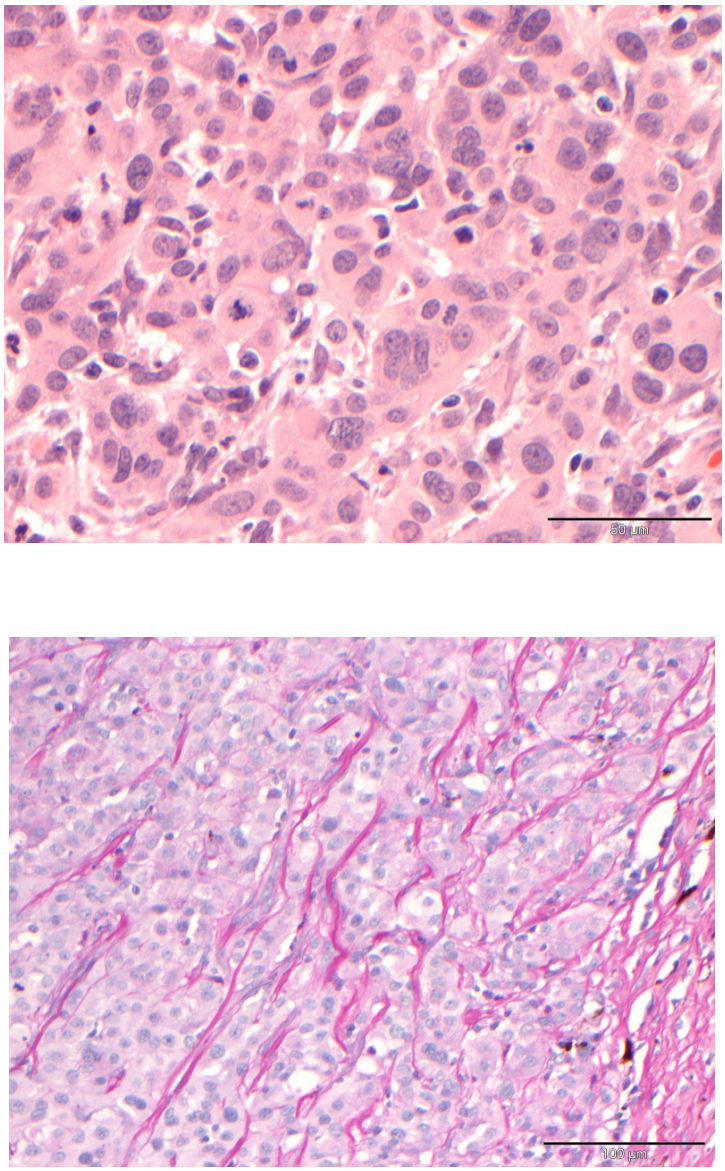

Figure 7 – Histological sections of an iridociliary adenocarcinoma.

Top: H&E, x 50 objective.

Bottom: PAS staining.

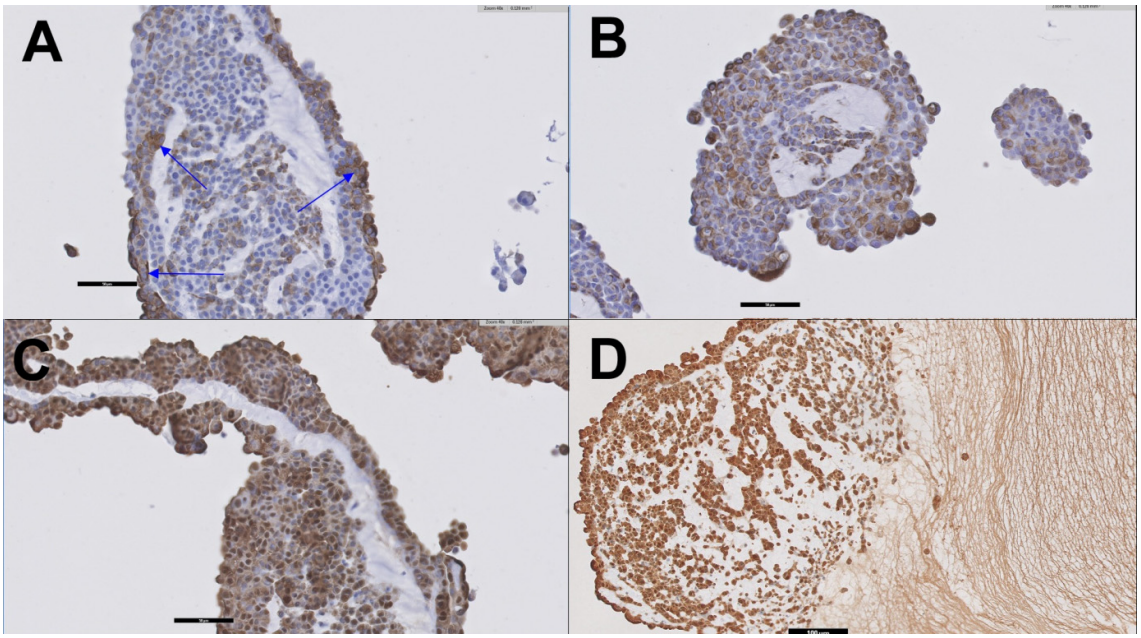

Figure 8 - Immunohistochemistry. CK (A), vimentin (B), S100 (C) and NSE (D). The neoplastic cells show immunoreactivity for these markers. X 40 objective for A, B and C, x 20 objective for D

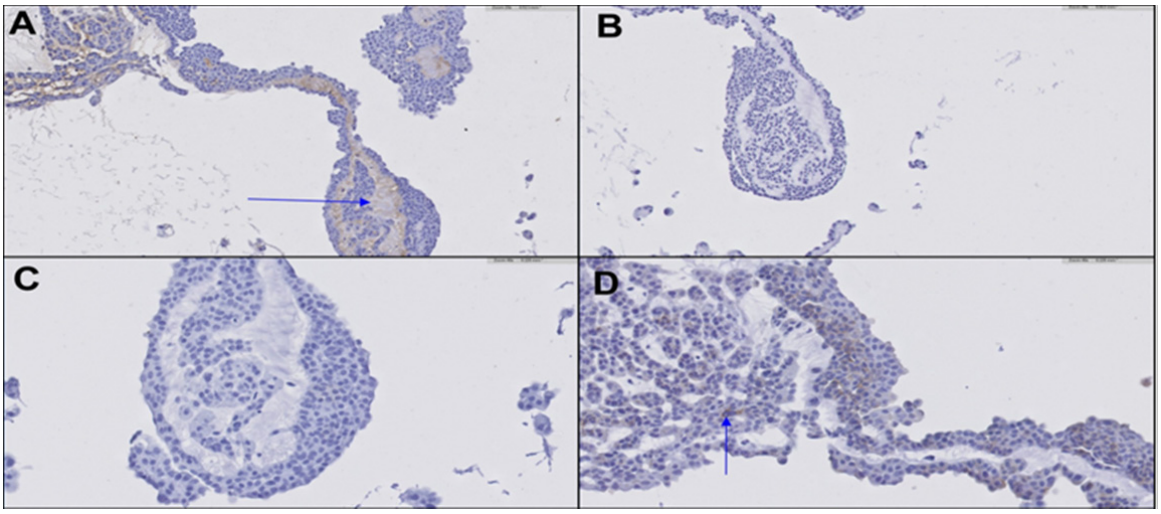

Figure 9 – Immunohistochemistry. GFAP (A – non-specific background staining indicated by arrow), desmin (B), PNL2 (C) and Melan A (D – occasional cells exhibit granular, cytoplasmic immunoreactivity).

The neoplastic cells do not show immunoreactivity for these markers.

X 20 objective for A and B and x 40 objective for C and D.

References

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