Cytology from a dermal mass in a pet gold fish (*Carassius auratus*)

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Specimen: Fine-needle aspirate cytology of a dermal mass

Signalment: 6-year, unknown sex, gold fish (*Carassius auratus*)

History:

An approx. 6-year-old gold fish was presented for investigation of a large dermal mass on the left side. The fish was owned for two years after it was bought from fish nursery and a small dermal mass was noted shortly after purchase but did not seem to affect the fish. Initially the owner had treated this with a tea tree-based treatment into water. The mass had since gradually increased in size and recently a mark had appeared on the mass and additional masses had developed. He was reported anorexic on the day of presentation.

Clinical findings

The fish was bright, alert and responsive on clinical exam and was swimming appropriately. A 50x50mm pedunculated, soft (jelly-like), lobulated, pink-orange mass on left dorsal-lateral flank. Multiple 5x5mm lobulated, pink-orange nodules located around the body and base of the tail. An exophthalmos of the right eye was also noted.

Fig. 1: Dermal mass on the left flank of a gold fish.

Fine-needle aspirates (FNA) of the largest mass were taken with and without negative pressure. The mass was subsequently surgically removed, and both samples were submitted to the diagnostic services of the RVC.

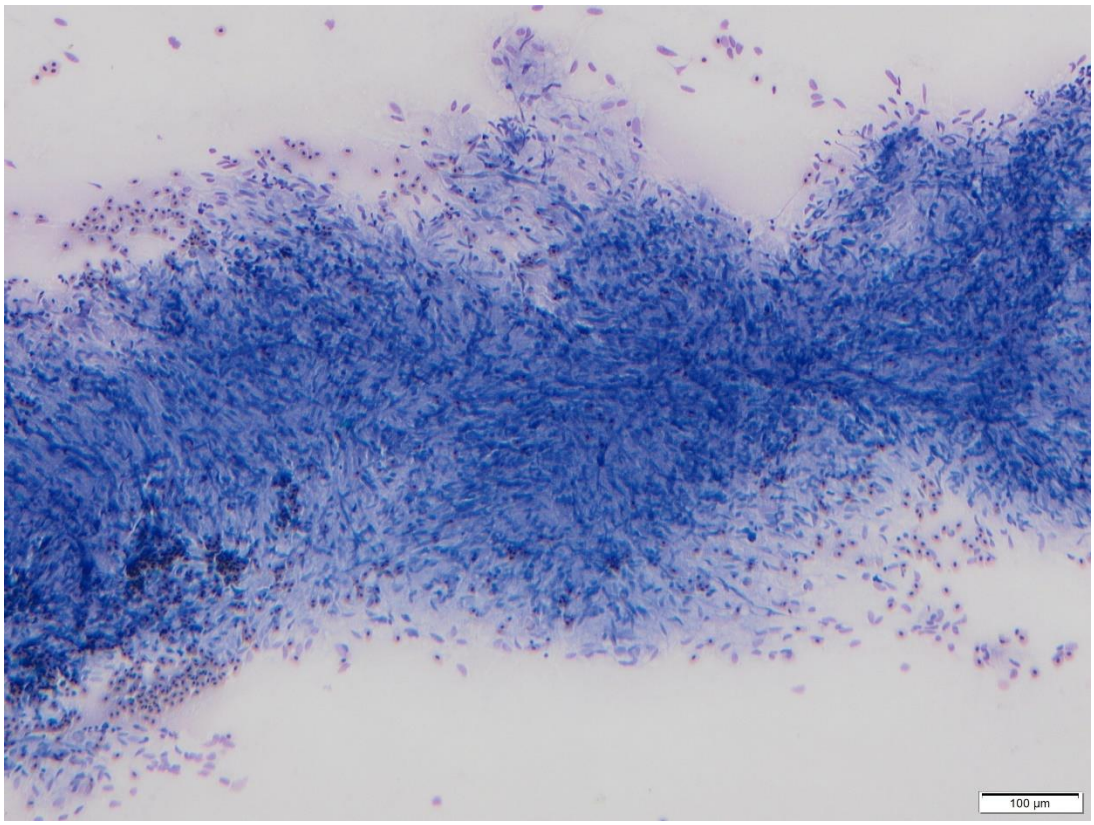

Fig. 2 FNA flank mass, modified Wrights stain (x100)

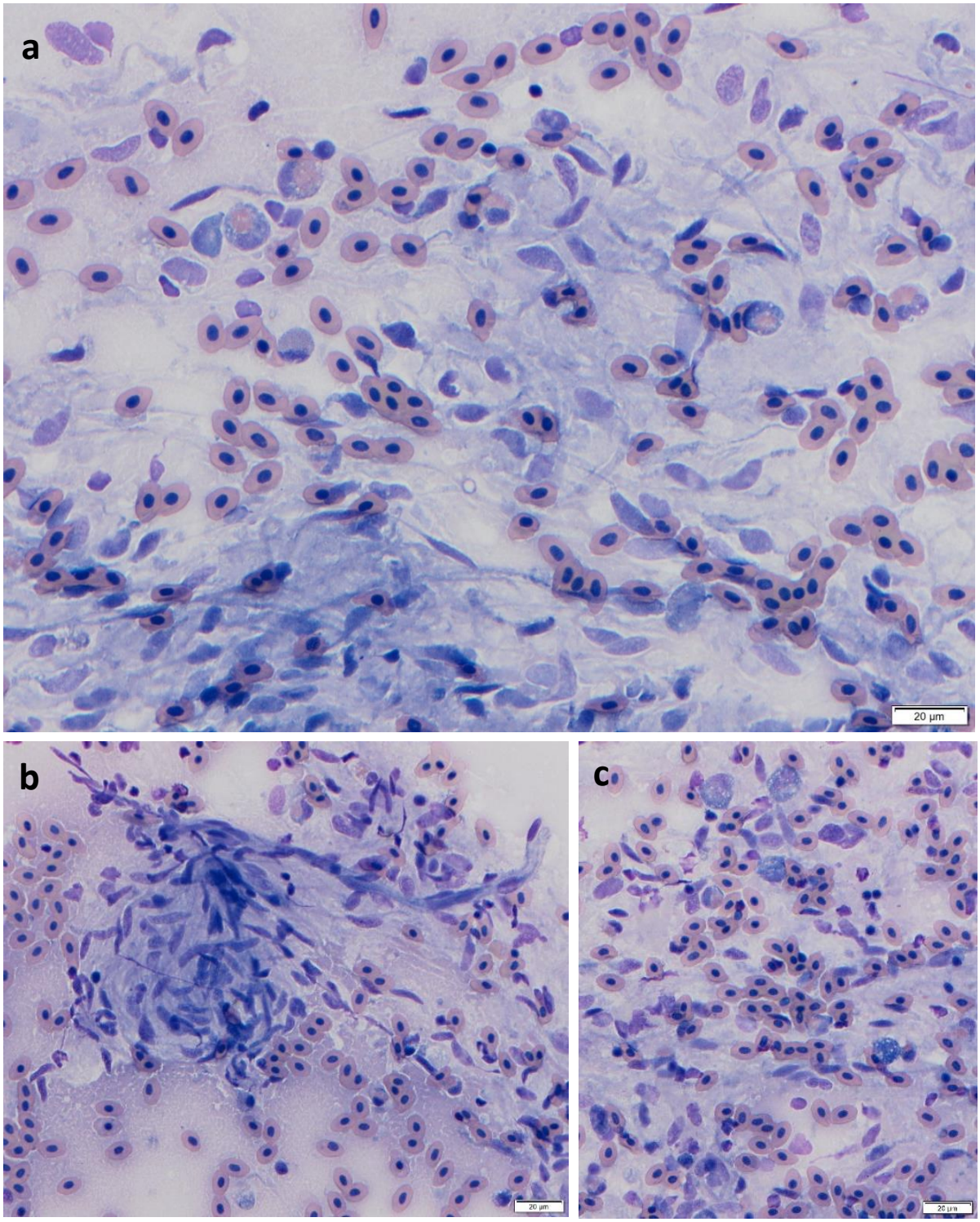

Fig. 3 a-c FNA flank mass, modified Wrights stain (x400)

Questions

1. Describe the cytological findings?
2. What is the most common skin neoplasia in fish?
3. What is your top differential for this case?

Interpretation/Diagnosis

Mesenchymal proliferation, suspect spindle cell tumour

Additional information

Three slides were submitted for cytological examination which were highly cellular with moderate to good cell morphology preservation and moderate blood content on a pale pink background containing many lysed cells. A population of mostly slender spindle cells were present, both individualised and in small aggregates with a fusiform arrangement and rarely whirl-like formation (Fig.3). The cells had indistinct cell borders and light blue cytoplasm. Nuclei (10-15µm) were mostly oval to elongated with finely to coarsely stippled chromatin with indistinct nucleoli. A low number of heterophils, macrophages and small lymphocytes were scattered throughout. Based on the predominance of a spindle cell population with only low numbers of inflammatory cells a spindle cell neoplasia was thought most likely, although a reactive (e.g. granulation) proliferation could not be excluded.

Due to the cytology report, surgical excision of the largest mass and histopathological exam was agreed with the owner. The fish received 0.2mg/kg meloxicam and 0.4mg/kg butorphanol intramuscular and was induced with MS222 100mg/L a few hours later. General anaesthesia was maintained with 50mg/L MS222 through buccal cavity and over gills. The mass was removed with scalpel blade perpendicular to the body wall. Mild haemorrhage as a result was stopped with compression. Iodine applied to surface followed by orahesive (repeated twice) and a protective layer was formed.

Representative sections of the flank mass (4.2 x 4.1 x 2.5cm) were processed. One section composed of the epidermis present with three to multiple layers with secretory cells and the dermis separated from the epidermis by a basal membrane is examined. Expanding the dermis, raising the contour of the overlying epithelium was an unencapsulated, poorly demarcated, infiltrative mesenchymal cell neoplasm. Neoplastic mesenchymal cells were arranged in streams, bundles and interlacing fascicles supported by dense fibrovascular stroma. Neoplastic cells were spindloid, contained a moderate amount of eosinophilic fibrillar cytoplasm and an oval to elongate nucleus with hyperchromatic chromatin and indistinct nucleoli. There was moderate anisocytosis and anisokaryosis, 16 mitotic figures in ten high power fields (2.37mm2) and frequent bizarre mitoses. Infiltrating throughout the neoplasm there were moderate numbers of lymphocytes and plasma cells.
The histopathologic diagnosis confirmed presence of a spindle cell neoplasm. Immunohistochemistry of the mass was performed with anti-S-100, anti-PNL-2 and anti–smooth-muscle antibody (ASMA). The neoplastic cells uniformly did not express the aforementioned immunohistochemical markers. An additional section of the mass was stained with Masson’s trichrome histochemical stain.

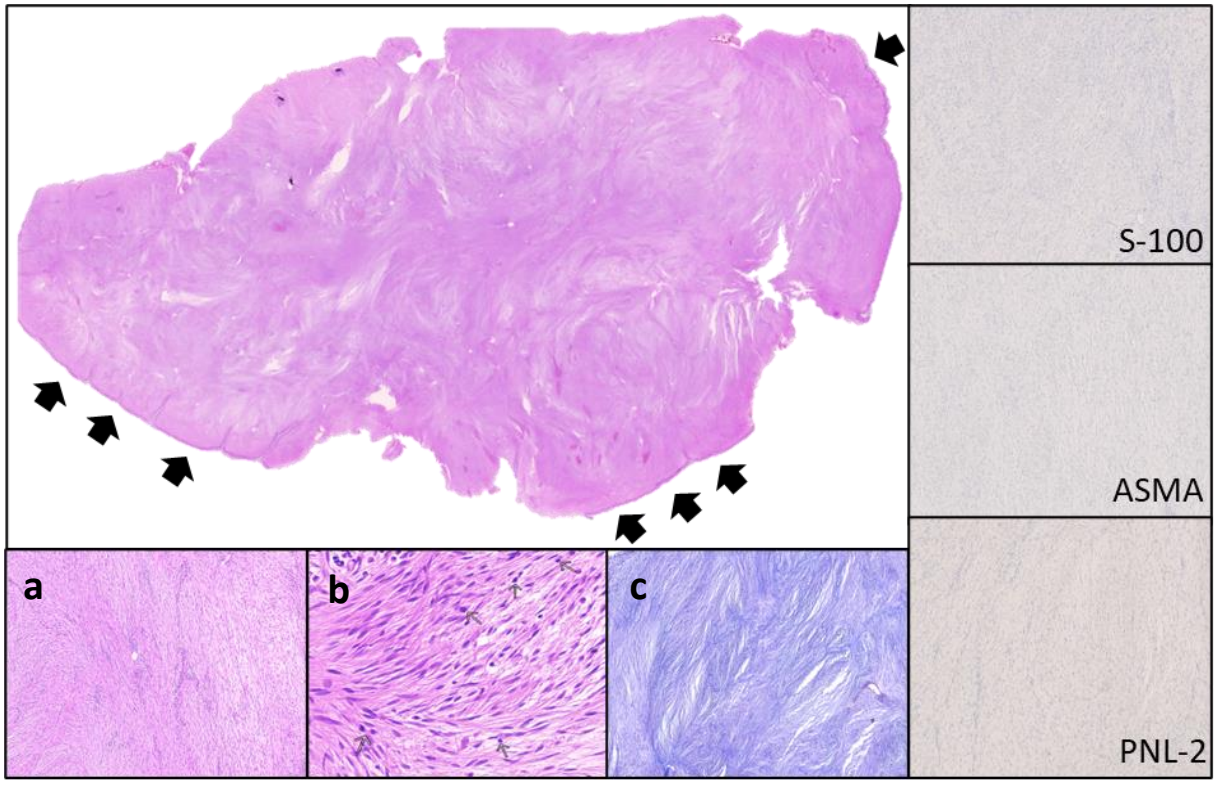

Fig. 4 Left flank mass (large black arrows point to the epidermis) composed of neoplastic mesenchymal cells arranged in streams, bundles and interlacing fascicles (a) with increased mitotic activity (b, small arrows) and supported by dense fibrovascular stroma that stains positive for Masson's trichrome histochemical stain (c). The mass was negative for anti-S-100, anti–smooth-muscle antibodies (ASMA) and anti-PNL-2 immunohistochemical markers.

Follow up and clinical outcome

The fish recovered well from the general anaesthesia but acutely deteriorated the following morning and was found dead. Post-mortem examination was not performed.

Discussion

Whilst fine-needle aspirates and cytologic examination of dermal masses are a common diagnostic process in domestic animals, cytologic preparations from fish are uncommonly seen by most clinical pathologists. Papillomas, chromatophoromas and spindle cell tumours rank amongst the most common skin tumours in many fish species including the gold fish (*C. auratus*) and the latter include benign (fibromas, schwannomas, neurofibromas) and malignant (fibrosarcomas, schwannomas, neurofibrosarcomas) mesenchymal neoplasms (Vergneu-Grosset et al 2017, Reaville et al 2007). Macroscopically, spindle cell tumours are generally tan to red, raised, firm nodules that are occasionally pedunculated, and may be locally infiltrative and recurrent (Reaville et al 2007). Surgical removal is usually the therapy of choice but reports of intralesional chemotherapy have been published (Reaville et al 2007, Love et al. 1997).

Although general descriptions can be found in text books and publications (Campbell 2015, Reaville et al. 2007, Vergneau-Grosset et al. 2017) only few publications describe detailed cytological findings of spindle cell tumours in gold fish:

A case report of a schwannoma in a gold fish described the cytologic features of the FNA as a highly cellular sample consisting of oval to elongate, medium-sized spindle cells either individually or in aggregates with a storiform pattern arrangement, and a moderate amount of extracellular faintly eosinophilic material. Cells had indistinct cell borders and wispy cytoplasmic extensions, a scant to moderate amount of lightly basophilic and occasionally microvacuolated cytoplasm and oval to cigar-shaped nuclei with finely stippled to lacy chromatin and 1–3 variably sized prominent nucleoli. Anisocytosis and anisokaryosis were described as moderate (Sirri et al. 2015).
A recent publication looked at neurofibromas in six gold fish with a similar cytological description, however described small, magenta intracytoplasmic and extracellular granules and occ. multinucleated cells (Armando et al. 2021). In addition to the spindle cell population, all three publications describe a variable number of erythrocytes, macrophages, and either endothelial cells resembling capillaries, or an additional epithelial cell population (Clyde et al. 1995, Sirri et al. 2015, Armando et al. 2021).

No magenta inclusions were noted but otherwise the descriptions match our cytological findings regarding the cellular features, arrangement and presence of capillaries and therefore led to an initial diagnosis of a spindle cell tumour.

On the basis of similar histopathological findings, this case was diagnosed as a soft tissue sarcoma with considerable mitotic activity, nuclear atypia and areas of hypercellularity. According to the histopathological features, differential diagnoses included fibrosarcoma, neurofibroma, leiomyosarcoma and undifferentiated chromatophoroma.

To further characterize the tumours, several histochemical and immunohistochemical stains were performed. Immunohistochemical examination with anti-S-100, anti-PNL-2 and anti–smooth-muscle antibody (ASMA) demonstrated that neoplastic cells uniformly did not express the immunohistochemical markers. S-100 is used for identification of tumours derived from neural crest cells, such as peripheral nerve sheath tumours and thus for the diagnosis of schwannomas or neurofibromas (Armando F et al, 2021). Similarly, both anti-PNL-2 and anti-smooth muscle actin (ASMA) were consistently negative further excluding differentials such as chromatophoromas and smooth muscle origin tumours respectively (Siniard, Wesley C., et al 2019, Vergneau-Grosset, Claire, et al, 2016).

A diagnosis of fibrosarcomas grossly resembles fibromas, although fibrosarcomas are more cellular and fibroblasts often present with a whirling or palisading arrangement with increased mitotic activity and atypia (Roberts 2001). In our case, mitoses were frequent and there was considerable amount of collagen present favouring a diagnosis of fibrosarcoma on routine diagnostic examination. To further confirm the presence of collagen we used Masson’s trichrome histochemical stain. The result demonstrated a patchy positivity of the present collagen in our slide. We postulate that this could be reflective of tumour heterogeneity and other individual characteristics of the tumour.

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