J. Comp. Path. 2022, Vol. 199, 81–85

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NEOPLASTIC DISEASE

Undifferentiated Wing Sarcoma in a Peach-Faced Lovebird (*Agapornis roseicollis*)

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Summary

A 10-year-old peach-faced lovebird (*Agapornis roseicollis*) was evaluated for an ulcerated and painful mass at the location of a fracture 2 years previously. Whole body radiographs showed a humeral fracture with a presumptive neoplastic proliferation in the distal diaphysis. Right wing amputation was elected but the animal died during recovery from surgery. Histopathological examination of the amputated wing revealed an infiltrative sarcomatous neoplastic proliferation. Immunohistochemistry (IHC) was carried out to characterize the tumour using antibodies against vimentin, desmin, smooth muscle actin (SMA), S-100, ionized calcium-binding adapter molecule-1 (IBA-1), CD18, cytokeratin and epithelial membrane antigen (EMA). The mesenchymal component of the mass was immunolabelled for vimentin and SMA and sparse epithelial cells were immunopositive for cytokeratin. Very few scattered cells were immunopositive for CD18 and IBA-1. The final diagnosis was consistent with an undifferentiated sarcoma with intralesional hyperplastic epithelium. According to the location, the history of a previous fracture and the histological pattern and IHC profile, the tumour was classified as an undifferentiated sarcoma with entrapped air sac epithelium.

Keywords: biphasic pattern; immunohistochemistry; lovebird; undifferentiated sarcoma

A 10-year-old entire female peach-faced lovebird (*Agapornis roseicollis*) presented with an ulcerated mass in the right wing. The bird had been housed alone in an indoor metal cage and fed a seed-based commercial diet. It had suffered a fracture of the right humerus due to a trauma 2 years previously. At that time, the owners declined surgical treatment and, therefore, the wing was immobilized with a figure-of-eight wing bandage combined with wing-body wrap. The fracture healed but a large bony callus formed because bone fragments were not aligned with the humeral axis.

General physical examination was unremarkable except for the mass in the right wing, which was ulcerated and involved the right humeral area. There were some cutaneous haemorrhages associated with self-mutilation, suggesting a painful process. Whole body radiographs showed a fracture at the right distal humeral diaphysis, lysis and remodelling of the proximal humeral fragment as well as severe osteolysis of the distal fragment, which extended to the proximal radial and cubital epiphysis. A round soft tissue opacity at the fracture site and surrounding the elbow was also seen (Fig. 1A).

Because of the rapid worsening of the case due to self-mutilation, right wing amputation was performed and samples were submitted for cytological and histological assessment. After removal, cytological examination of Wright's-stained impression smears of the mass revealed moderate cellularity and a population of fusiform mesenchymal cells that

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0021-9975/$ - see front matter
https://doi.org/10.1016/j.jcpa.2022.09.006

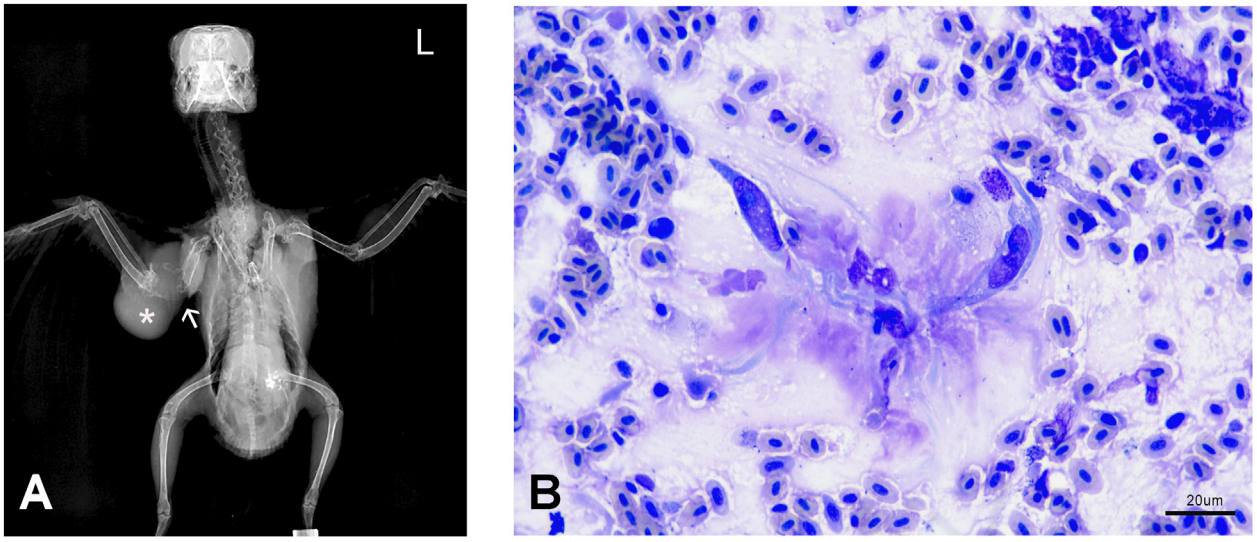

Fig. 1. Undifferentiated sarcoma, right wing, lovebird. (A) Ventrodorsal radiograph showing fracture of right distal humeral diaphysis, lysis and remodelling of proximal humeral fragment and severe osteolysis of distal fragment, extending to proximal radial and cubital epiphyses (arrow). Soft round tissue opacity associated with fracture and elbow joint (star). L, left. (B) Individual fusiform mesenchymal cells have oval nuclei with lacy chromatin and prominent nucleoli. Impression smear. Wright. Bar, 20 µm.

were arranged individually or in non-cohesive aggregates, associated with abundant extracellular pink amorphous material. Nuclei were round to oval with a finely stippled to lacy chromatin pattern, and one to two nucleoli. Cytoplasm was moderately pale blue with indistinct cell borders. Moreover, spindle-shaped cells had a moderate degree of anisocytosis and anisokaryosis. Binucleation, multinucleation and multiple nucleoli were also commonly observed (Fig. 1B). Cytological findings were consistent with a malignant mesenchymal cell neoplasm.

Post-surgical recovery was appropriate but soon after wards the bird became lethargic with wing flapping and died suddenly. Unfortunately, post-mortem examination was declined by the owners.

The right wing that had been removed during surgery was submitted for histopathology. Grossly, the distal humeral diaphysis contained a firm, brownish mass (2 cm diameter), which invaded and effaced the humeroradial joint. Multiple sagittal sections of the mass were prepared, fixed in 4% formalin, routinely processed and stained with haematoxylin and eosin (HE) for histopathological evaluation. In addition, sagittal sections of the whole joint were decalcified for 48 h in fast decalcifier with saline corrector, fixed in formalin and processed as for histopathological evaluation.

Examination of the samples from the whole joint and the associated mass revealed the same histological pattern, which consisted of a highly cellular non-encapsulated and infiltrative neoplastic proliferation that distorted the adjacent dermal, muscular and bone tissues (Fig. 2A). Neoplastic cells had a fusiform morphology and were arranged in interlacing bundles with a basophilic myxoid matrix. These cells were spindle-shaped with moderate pale eosinophilic cytoplasm and poorly defined cell borders. Nuclei were large, oval, eccentric with coarsely stippled chromatin and a single nucleolus. There were 1–3 mitotic figures in 2.37 mm^2 and occasional aberrant mitoses were observed. Marked pleomorphism was seen with moderate anisocytosis and anisokaryosis, as well as scarce multinucleated giant cells. Moreover, there was an occasional proliferation of well-differentiated epithelial cells organized in either small clusters or tubular structures lined by a simple cuboidal epithelium. These epithelial cells were large and polygonal with moderate eosinophilic cytoplasm and well-defined cytoplasmic borders (Fig. 2B). Nuclei were round and centrally located, with two evident nucleoli. Multifocally, scattered small foci of necrosis were seen.

Immunohistochemical labelling of formalin-fixed paraffin-embedded serial tissue sections was performed to determine the phenotype of the cells. The antibody panel included vimentin 3B4 (M7020; Dako, www.agilent.com), desmin (M0760; Dako), smooth muscle actin (SMA) (M0851; Dako), S100 (Z0311; Dako), ionized calcium-binding adapter molecule-1 (IBA-1) (019–19741; Wako, https://labchem-wako.fugifilm.com), CD18 (CA1.4E9; University of California, Davis, USA), cytokeratin AE1/AE3 (PA0909; Leica Biosystems, www.leicabiosystems.com) and epithelial membrane

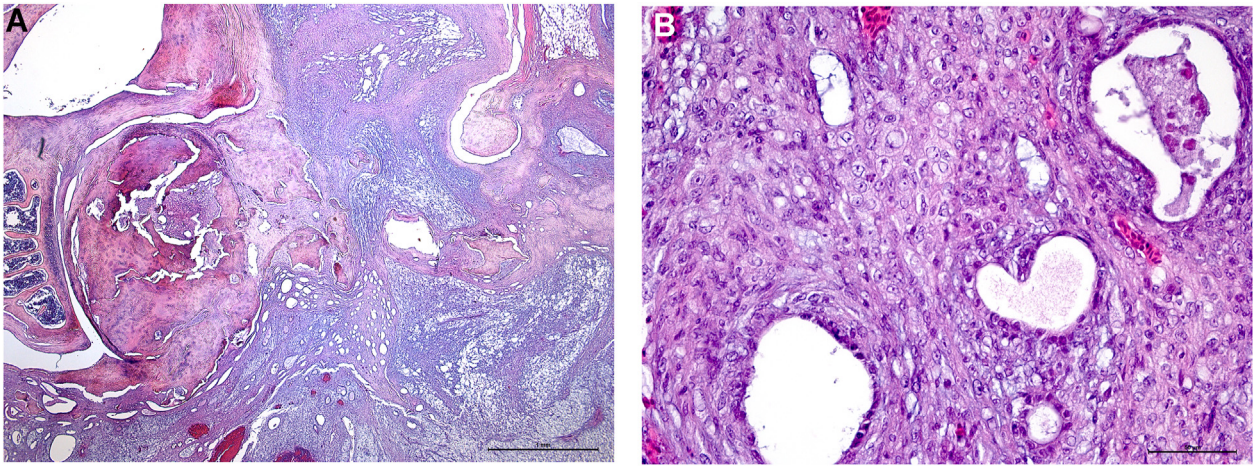

Fig. 2. Undifferentiated sarcoma, right wing, lovebird. (A) Longitudinal section of right humeral mass. Infiltrative mesenchymal neoplastic cell proliferation associated with abundant myxoid matrix and distortion of adjacent dermal, muscle and bone tissues. HE. Bar, 1 mm. (B) Higher magnification of A. Proliferation of spindloid cells and occasional epithelial cells organized in tubules. HE. Bar, 50 µm.

antigen (EMA) GP1.4 (PA0035; Leica Biosystems). Immunohistochemistry (IHC) was performed using an Autostainer Plus Immunostaining System (Dako) with 40 min of primary antibody incubation for desmin (1:40), SMA (1:500), S100 (1:500), IBA-1 (1:250) and CD18 (1:100); manually with 60 min of primary antibody incubation for vimentin (1:300); and with Leica platform with 15 min of primary antibody incubation for cytokeratin ready-to-use (RTU) and EMA RTU. Antigen retrieval was performed with high pH for desmin, SMA and vimentin and low pH for S100, IBA-1, CD18, cytokeratin and EMA. As a control, histological sections of a non-affected wing of a psittacine bird (*Amazona albifrons*) were used including skin, striated muscle, synovial membrane, cartilage and bone.

In the mesenchymal neoplastic population, most of the cells had intense immunoreactivity for vimentin and about 80% were immunopositive for SMA (Fig. 3A); occasionally, sparse histiocytic cells were weakly labelled for CD18 and IBA-1. In the epithelial cell population, the lining epithelium of the tubules and some epithelial clusters labelled for cytokeratin (Fig. 3B). No cells express positivity for EMA, desmin or S-100 (Table 1).

The neoplasm in the present report was consistent with a mesenchymal neoplastic proliferation without the presence of osteoid material and with the presence of small clusters and tubules lined by epithelial cells. Considering only the mesenchymal neoplastic population and the results of IHC, which showed vimentin positivity, we discarded histiocytic sarcoma as a diagnosis because neoplastic mesenchymal cells were CD18 negative and IBA-1 negative (only a few scattered cells, probably of inflammatory origin were immunopositive with these markers). We also excluded rhabdomyosarcoma, which is SMA negative and desmin positive, and myoepithelial or myofibroblastic tumours, which are desmin positive (Ramos-Vara and Borst, 2017). Lastly, peripheral nerve sheath tumour, which is S100 positive, and fibrosarcoma, which is S100 positive and SMA negative (Hendrick, 2017), were also discarded as possible diagnoses.

Because of the location of the tumour and the simultaneous presence of mesenchymal and epithelial polygonal cells, we could hypothesize about the diagnosis of a biphasic synovial sarcoma. In avian species, synovial cell sarcomas have been reported in a pigeon (*Columba livia*) (Liu and Moroff, 1993), a moluccan cockatoo (*Cacatua molucensis*) (Kennedy *et al*, 1994), a sulphur crested cockatoo (*Cacatua galerita*) (Van Der Horst *et al*, 1996) and a peach-faced lovebird (*Agapornis roseicolis*) (Nakano and Une, 2016). On the contrary, in dogs (Fox *et al*, 2002; Loukopoulos *et al*, 2004) and cats (Liptak *et al*, 2004; Cazzini *et al*, 2015) it usually has been described as a monophasic synovial cell sarcoma. Despite all the existing literature on synovial cell sarcoma in veterinary medicine, this tumour is currently considered not to exist in animals (Craig and Thompson, 2017). Furthermore, in our case the epithelial proliferation did not show any malignant histological features, which indicates that these epithelial clusters and follicles are not of neoplastic origin (Craig and Thompson, 2017). Therefore, biphasic synovial cell sarcoma was discarded as a differential diagnosis.

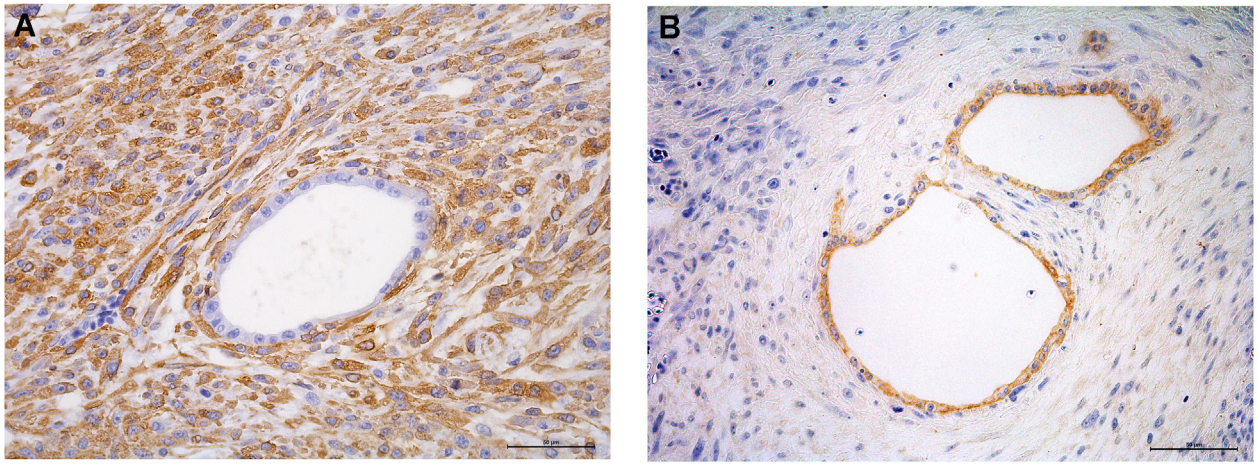

Fig. 3. Undifferentiated sarcoma, right wing, lovebird. (A) Neoplastic mesenchymal cells immunopositive for smooth muscle actin. Epithelial cells immunonegative. IHC. Bar, 50 µm. (B) Well-differentiated intratumoural epithelial cells immunopositive for cytokeratin. IHC. Bar, 50 µm.

Table 1
Immunolabelling pattern of neoplastic cells

Antigen	*Mesenchymal cells*	*Epithelial cells*
Vimentin	+ + +	–
S100	–	–
SMA	+ + +	–
Desmin	–	–
IBA-1	±	–
CD18	±	–
Cytokeratin	–	+ + +
EMA	–	–

SMA, smooth muscle actin; IBA-1, ionized calcium-binding adapter molecule1; EMA, epithelial membrane antigen; –, no positivity; ±, positivity in <5% of the cells; + +, positivity in >50% of the cells; + + +, positivity in >90% of the cells.

Interestingly, in a case report of a monophasic synovial cell sarcoma in the humerus of a pigeon (*C. livia*) (Liu and Moroff, 1993), the authors described the presence of epithelial cells that were consistent with hyperplasia of humeral air sacs and concluded that epithelial proliferation might be compatible with entrapped air sacs within the mesenchymal neoplastic growth. Therefore, to the best of the authors' knowledge, the present tumour should be classified as an undifferentiated sarcoma with the presence of intralesional epithelial proliferation.

Independently of the histological classification of this tumour, it is interesting to remark that it was associated with a previous humeral fracture at the same location without any surgical implant involved. Fracture-associated sarcomas have been reported in different mammalian species, including dogs (Isaka *et al*, 2021), cats (Sonnenschein *et al*, 2012; Baum *et al*, 2018) and goats (Steinberg and George, 1989), usually directly related with orthopaedic implants. In avian species, Nakano and Une (2016) described a synovial cell sarcoma in a peach-faced lovebird that had been treated with an intramedullary pin after a bone fracture. However, the present case is definitely related to a prior bone fracture but without any surgical implant.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Acknowledgments

The authors thank Dr. Monica Hilbe of the Institute of Veterinary Pathology of the University of Zurich for performing the vimentin 3B4 immunohistochemistry. The authors also thank Blanca Pérez and Aida Neira of Servei de Diagnòstic de Patologia Veterinària, Universitat Autònoma de Barcelona for laboratory technical assistance and Caroline Idowu for revising the English language in the manuscript.

Conflict of Interest Statement

The authors declared no potential conflicts of interest with respect to the research, authorship or publication of this article.

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[*Received, May 29th, 2022*
Accepted, September 17th, 2022]