Cutaneous leiomyosarcoma with osteoid metaplasia in a budgerigar (*Melopsittacus undulatus*): a case report

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**ABSTRACT:** Cutaneous leiomyosarcoma with osteoid metaplasia was diagnosed at the base of the right wing in a five-year-old female budgerigar. Grossly, the tumour mass was well circumscribed and solitary, and had a nodular appearance on section. On histological examination, the mass was composed of randomly arranged bundles of spindle-shaped cells that included mitotic, multi-nucleated and bizarre cells. In addition, within and between tumour areas, there were osteoid metaplasia foci. The bony trabeculae were usually localised in the areas around the haemorrhage, and exhibited focal mineralisation. Tumour cells were stained red using Masson's trichrome staining method. In addition, tumour cells were intensively positive for smooth muscle actin and focally positive for desmin and vimentin, but were negative for CD68 and S100 by immunohistochemical staining. The tumour reported here was defined as a cutaneous leiomyosarcoma with osteoid metaplasia by histopathological and immunohistochemical findings. Our findings may indicate that osteoid metaplasia should be considered in leiomyosarcoma in budgerigars.

**Keywords:** desmin; CD68; smooth muscle actin; spindle-shaped cells; S100

Leiomyosarcomas are malignant tumours which develop from smooth muscle tissue. Although, malignant smooth muscle tumours constitute only about 10% of total smooth muscle tumours in domestic animals (Hulland 1990), malignant muscle neoplasms in avian species have been reported more than twice as frequently as benign tumours (Latimer 1997). Leiomyosarcomas, known as smooth muscle sarcomas, have most commonly been reported in spleen, and at different anatomical locations of the alimentary and genital tracts in pet birds including budgerigars (Blackmore 1966; Latimer 1997; Zamani-Ahmadmahmudi et al. 2015). Well-differentiated leiomyosarcomas have a classic histological pattern of interrupted cellular fascicles consisting of spindle-shaped tumour cells with abundant eosinophilic cytoplasm and elongated nuclei (Hulland 1990; Grabellus et al. 2006). However, poorly differentiated leiomyosarcomas may exhibit various uncommon histopathological changes such as myxoid and epitheloid changes or osteoid differentiation, which is a rare finding for leiomyosarcomas (Grabellus et al. 2006; Chen et al. 2011). Smooth muscle tumours with metaplastic bone formation have been reported in humans (Lidang et al. 1998; Bush et al. 2003; Grabellus et al. 2006) and in a cat (Finnie et al. 1995). To our knowledge, there has been no report of leiomyosarcoma with osteoid metaplasia in domestic animals and avian species. We describe a case of cutaneous leiomyosarcoma with osteoid metaplasia at the base of the right wing in a budgerigar.

**Case description**

A dead five-year-old, female budgerigar, weighing 50 g with a neoplastic mass on the right wing was referred to the Department of Pathology, Faculty of Veterinary Medicine, University of Firat, for necropsy. This revealed the presence of a well-circumscribed nodule located on the dorsal surface of the right wing. Tissue samples collected from the tumour mass and visceral organs were fixed in 10% neutral buffered formalin, routinely processed, paraffin-embedded, sectioned at 5 µm thickness, and stained with H-E, and Masson’s trichrome for his-
Immunohistochemical staining of selected sections was performed using the avidin-biotin-complex (ABC; DAKO, Carpinteria, CA, USA) method with monoclonal antibodies specific for smooth muscle actin (MS-113-P0, Lab Vision Cooperation, Fremont, USA, diluted 1 : 800), desmin (MS-376-S0, diluted 1 : 100), vimentin (MS-129-P0, diluted 1 : 1000), S100 (MA-20119, Genemed Synthesis, diluted 1 : 200) and CD68 (168M-98, Cell Marque, diluted 1 : 100). After visualisation of the positive antigen-antibody reaction by incubating the slides with diamino-benzidine, the slides were slightly counterstained with Mayer haematoxylin.

The tumour was located on the dorsal surface of right wing near the metacarpal bone and it was in the subcutaneous area (Figure 1). After excision, the tumour mass was 2.5 × 2.5 × 3.5 cm in size and weighed 15 g. The appearance of the tumour was pasty and solid. The cut surfaces of the tumour mass were greyish-white and brownish-red, and nodular in appearance (Figure 2). These nodules were of varying size and had a cystic appearance. No gross lesions were observed in the other organs of the budgerigar. Histological evaluation demonstrated that the tumour was encapsulated by a thin connective tissue and was non-invasive. The tumour was densely cellular and composed of randomly arranged bundles of spindle-shaped cells surrounded by an irregularly oriented scant fibrous stroma (Figure 3). Spindle-shaped tumour cells were extremely pleomorphic varying from oval to fusiform or elongated nuclei. The tumour contained multi-nucleated and bizarre giant cells and numerous cells with enormously enlarged nuclei. The tumour displayed a moderate mitotic rate (> 4 per high-power fields). In addition, there were numerous bone formations within the tumour, consistent with osteoid metaplasia (Figure 4). The bone formation with trabeculae was well-differentiated with evident proliferation of osteocytes embedded in bone matrix (Figure 4). These osteoid foci were usually localised in the areas around the haemorrhage, and exhibited focal mineralisation. Along with large areas of necrosis and haemorrhage, haemopoietic cells and lymphoplasmacytic infiltration were also seen in the tumour. Ectatic vascular channels filled with red blood cells were also found. The spindle-shaped tumour cells stained as bright red, and collagen bundles and osteoid metaplasia as blue using Masson’s trichrome staining method (Figure 5). Immunohistochemically, tumour cells showed intense cytoplasmic positivity for smooth

Figure 1. The tumour mass located on the dorsal surface of the right wing of the budgerigar

Figure 2. The cut surface of the tumour was greyish-white and brownish-red in colour, and nodular in appearance
muscle actin (Figure 6), focal positivity for desmin and vimentin, but were negative for S100 and CD68.

**DISCUSSION AND CONCLUSIONS**

Osseous metaplasia describes bone formation in a non-osseous tissue and is caused by differentiation of local cells into osteogenic cells as a response to pathological events such as tumourigenesis, metabolic disorders or physical injury (Lynch and Scagliotti 2007; Park et al. 2010). The histogenesis of osseous metaplasia in neoplasia is poorly understood. In animals, there are some published reports on osseous metaplasia associated with neoplasms, including a horse with adenocarcinoma (Rottman et al. 1991), dog with fibroma (Park et al. 2010), cat with multiple piloleiomyomas (Finnie et al. 1995) and a cow with cholangiocarcinoma (Ohfuji 2012). Although, there are some published reports of leiomyosarcomas in animals (Blackmore 1966; Hulland 1990; Reece 1992; Latimer 1997), leiomyosarcomas with osseous metaplasia have not been reported in domestic animals and avian species previously. Mitchell (1982) reported a case of mesenteric leiomyosarcoma and perirenal osseous metaplasia in a deer, but suggested that there was no connection between bone metaplasia and the tumour because of the distinct localisation of the tumour and the bone. In humans, some cases of leiomyosarcomas with osseous metaplasia have been reported (Lidang et al. 1998; Bush et al. 2003; Grabellus et al. 2006; Chen et al. 2011), and it has been observed that leiomyosarcomas can exhibit osseous differentiation (Lidang et al. 1998; Grabellus et al. 2006). It has been speculated that the osteoid...
metaplasia may develop as reactive non-neoplastic ossification or dystrophic calcification in smooth muscle tumours (Bush et al. 2003). Reya et al. (2001) stated that mesenchymal stem cells have the most extensive potential for differentiation. In our case, bone formation, consistent with osteoid metaplasia was seen in the tumour and these foci were usually localised around the haemorrhagic areas. This finding indicates that osteoid metaplasia, as found in the budgerigar in this report, should be considered in the pathological and radiographical diagnosis of smooth muscle tumours in animals (Bush et al. 2003).

Leiomyosarcomas are generally local-invasive, and metastasis is a rare event, but metastasis to the liver, spleen, bone marrow, and thoracic cavity has been reported (Blackmore 1966; Latimer 1997). In the present case, the tumor was encapsulated by a thin connective tissue and was non-invasive, similar to a previous report from a budgerigar (Zamani-Ahmedmahmudi et al. 2015). Poorly differentiated leiomyosarcomas must be distinguished from fibrosarcomas, rhabdomyosarcomas, malignant peripheral nerve sheath tumours, histiocytic tumours and haemangiopericytomas (Hulland 1990). The microscopic appearance along with the Masson’s trichrome and immunohistochemical staining help in the diagnosis of poorly differentiated mesenchymal tumours. In the present study, the tumour cells were negative for S100 and CD68, which are useful markers for nerve sheet tumours (Stasik and Tawfik 2006) and histiocytes and histiocytic tumours (Binder et al. 1992), respectively. However, the neoplastic cells stained as bright red with Masson’s trichrome, and the diagnosis was also confirmed by positive staining for smooth muscle actin by immunohistochemistry. Smooth muscle actin is a specific marker for smooth muscle tumours (Grabellus et al. 2006; Chen et al. 2011). In conclusion, the tumour reported at the base of the wing of a budgerigar was diagnosed as a cutaneous leiomyosarcoma with osteoid metaplasia on the basis of histopathological and immunohistocemical findings. Our findings may indicate that osteoid metaplasia should be considered in smooth muscle tumours in avian species, including budgerigars.

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