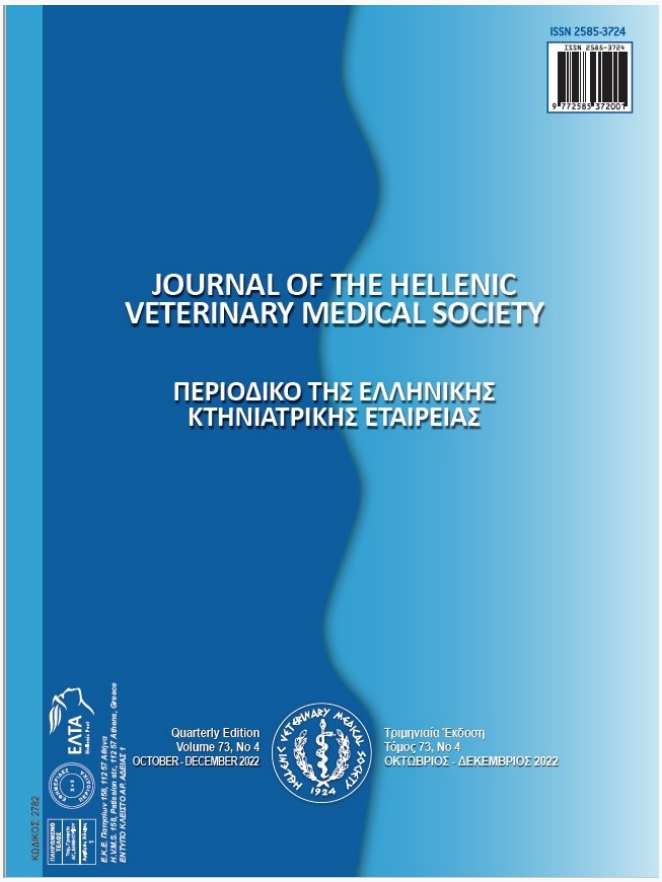
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Case report
Κλινικό περιστατικό

A case of giant cell osteosarcoma in a Scottish fold cat

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ABSTRACT: This study describes a case of giant cell osteosarcoma (GCO) located on the right scapulae in a six-year-old neutered male Scottish Fold cat. GCO is a rare tumor in domestic animals.This tumor is also known as giant cell-rich osteosarcoma. Multinucleated giant cells and osteoid islands are observed in GCO.The mass was 4.6x4.4x4.3 cm in size and very firm. Microscopically, fusiform-shaped cells and multinucleated giant cells were observed. There were 4-8 mitotic figures in three random high-power fields (400x). Osteoid islands and necrosis were detected in several areas. In these areas, atypia was observed in the multinucleated giant cells and fusiform-shaped cells. Immunohistochemically, vimentin expression was observed in neoplastic cells.No immunoreactivity against actin and cytokeratin was observed in neoplastic cells. Additionally, vimentin expression was detected in the periphery of blood vessels and actin expression in blood vessel smooth muscle cells. As a result ofhistopathological and immunohistochemical findings, the mass was determined to be GCO.

Keywords:Giant cell osteosarcoma, Scottish fold cat, scapulae, multinucleated giant cell

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CASE HISTORY

Osteosarcoma is a malignant tumor of bone tissue (Thompson and Dittmer, 2017). This tumoris the most common primary bone tumor in cats (Heldmann et al., 1995). According to the World Health Organization (WHO) classification, there are different osteosarcoma types (Craig et al., 2016).Giant cell osteosarcoma (GCO), considered one of these tumors' types,is rarely observed in humans(Fu et al., 2011) or domestic animals (Thompson and Dittmer, 2017). These tumors are also known as giant cell-rich osteosarcoma (Craig et al., 2016; Thompson and Dittmer, 2017).

In the histopathological evaluation of GCO, especially multinucleated giant cells are numerous and distinct. On the other hand, osteoid islands are observed along with neoplastic cells (Craig et al., 2016; Thompson and Dittmer, 2017).In addition, GCO is defined as a tumor consisting of many osteoclast-like giant cells admixed with malignant bone-forming cells. (Verma et al., 2011).The prognosis of GCO is evaluated according to clinical examinationand histopathological findings (Dimopoulou et al., 2008) and the definitive diagnosis is obtained through histopathological evaluation (Ehrhart et al., 2013).

According to the literature, only seven cases have been reported in animals so far (Table 1), and only four of them were observed in cats. To the bestof our knowledge, this study is the first case of GCO described on the scapulae in a Scottish Fold cat.

This case describes a six-year-old neutered male Scottish fold catwho was taken to a private veterinary clinic with a history of tumor on theright scapulae about 1.5 months before the biopsy was taken.In the anamnesis, it was stated that there was no history of vaccination at the tumor site. It was reported that there was no change in hematology and serum biochemistry, and also no radiological examination was performed. It was reported that the mass had grown rapidly in the previous two weeks, and an excisional biopsy was performed with the patient'sowner'sconsent with suspicion of neoplasia.

Table 1. 7 cases of giant cell osteosarcoma previously reported

Type-Breed	Age-Sex	Location	Macroscopic Findings	Microscopic Findings	References
Cat-Shorthair	13-female	Calvarium	1.5x2 cm, white, firm, expansive, multilobate mass	H&E: Multinucleated giant cells, polygonal-shaped pleomorphic cell bundles, multifocal islands of osteoid IHC: Vimentin (+)	Negrin et al., 2006
Cat-American Shorthair	7-male	L1 vertebral arch	Unknown	H&E: Multinucleated giant cells, multifocal islands mineralized foci of osteoid	Nakata et al., 2017
Cat-Persian	4-male	Humerus-liver+spleen metastasis	3.5x2.5x2 cm, firm mass	H&E: Multinucleated giant cells,polygonal cells, multifocal osteoid matrix, mitotic figures IHC: Vimentin (+)	Farjanikishet al., 2018
Cat-Unknown	4-male	Pelvic limb	5 cm, ulcerated mass	H&E: Fusiform shaped pleomorphic neoplastic cells, multinucleated giant cells, osteoid matrix	Jaretta et al., 2020
Dog-German Shepherd	7-male	Humerus	7.5x5 cm, white, firm, mass	H&E: Osteoclast-like multinucleated giant cells, polygonal-fusiform shaped stromal cells, rare osteoid matrix IHC: Vimentin (+)	Fattahian et al., 2008
Dog-Scottish Deerhound	6.5-female	Tibia	Unknown	H&E: Multinucleated giant cells, hemorrhage, pleomorphic cell bundles, atypical neoplastic cells, mitotic figures IHC: Vimentin (+)	Oryan et al., 2015
Ranch mink-*Mustela vision*	2-female	Lumbosacral vertebrae	4x3x1.5 cm, white, firm, multilobate mass	H&E: Pleomorphic polygonal and fusiform shaped osteoblast-like cells, osteoclast-like multinucleated giant cells, focal osteoid islands	Mikaelian et al., 1998

Tissue samples were fixed in a 10% formalin solution. After routine processing,samples were embedded in paraffin. Paraffin blocks were cut at 4µm in thickness and stained with hematoxylin &eosin (H&E) to be evaluated with light microscopy (Nikon Eclipse 80i). Also,immunohistochemical (IHC) stainings were performed using a commercial staining kit (UltraVision ONE Detection System, HRP polymer & DAB Plus chromogen, TL-015-HDJ, Thermo Fisher Scientific, Waltham, MA, United States) according to the manufacturer's instructions.Briefly, sections were deparaffinized in xylene, rehydrated in series of ethanol, washed in distilled water and phosphate-buffredsaline (PBS). Afterwards sections were subjected to citrate buffer (pH 6.0) (AP-9003, Thermo Fisher Scientific, Waltham, MA, United States) in a microwave at 600 W for 2x5 minutes for antigen retrieval. Sections were incubated with hydrogen peroxide for 15 minutesfor endogenous peroxidase activity.Slides were then incubated with primary antibodies against cytokeratin (1:200, mouse monoclonal, Sc-57004, Santa Cruz BiotechnologyInc., Dallas, TX, USA), vimentin (1:500, mouse monoclonal, M0725, Dako/ Agilent Technologies, Santa Clara, CA, USA), and actin (1:500, mouse monoclonal, Sc-8432, Santa Cruz Biotechnology Inc., Dallas, TX, USA).Slides were then rinsed in PBS and incubated with secondary antibody provided in the IHC staining kit for 30 minutes at room temperature.3,3-diaminobenzidine (DAB) (40 µl DAB Plus chromogen solution, 2 ml DAB Plus substrate solution) was used as the chromogen for 2 minutes.Subsequently, the slides were counterstained with Harris'Hematoxylin (HHS 16, SigmaAldrich, St Louis, MO, USA) and the sections were evaluated by Olympus Stream Image Analysis.

The tumor taken on the right scapulae was 4.6x4.4x4.3 cm in sizeon the gross examination. It was a very firm, solid, and circumscribed mass. The cut surface of the mass was white and lytic with cystic structures and hemorrhages.Onthe microscopic examination, in some areas, an eosinophilic homogeneous osteoid matrix among the neoplastic cells was detected (Figure 1 A). Extensively multinucleated giant cells were observed in the stroma (Figure 1 B).The nuclei of giant cells were round to ovoid, and their cytoplasm was eosinophilic. The number of nuclei of multinucleated giant cells ranged from 6-30.Throughout the tumor, fusiform-shaped cells, and areas of necrosis were detected.The fusiform-shaped cells contained ovoid nuclei. Significant atypia was observed in the neoplastic cells.These cells differed in size and shape.Mitotic figures were examined in three random high-power fields (400x) (Jaretta et al., 2020).There were 4-8 mitotic figures in three random high-power fields.In some areas, hemorrhages, and hyperemia were observed.It was determined that the neoplastic cells showed a strong positive reaction against vimentin antibody on immunohistochemical examination (Figure 2). However, these cells did not express actin and cytokeratin.Also, smooth muscle cells of blood vessels expressed the actin protein.According to the WHO classification (Craig et al., 2016),the presented case was defined as GCO as a result of routine histopathological methods and immunohistochemical staining.

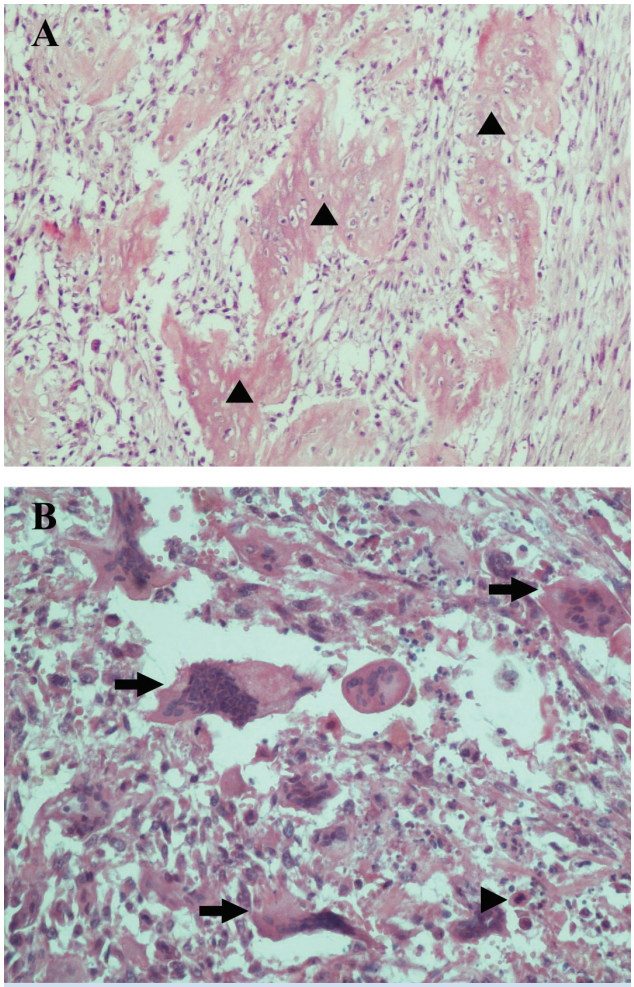


Figure 1. (A):Multifocal islands of osteoid among the neoplastic cells (arrowheads), (B):Mitotic figure (arrowhead), and multinucleated giant cells surrounded by fusiform-shaped cells (arrows) (H & E, x200)

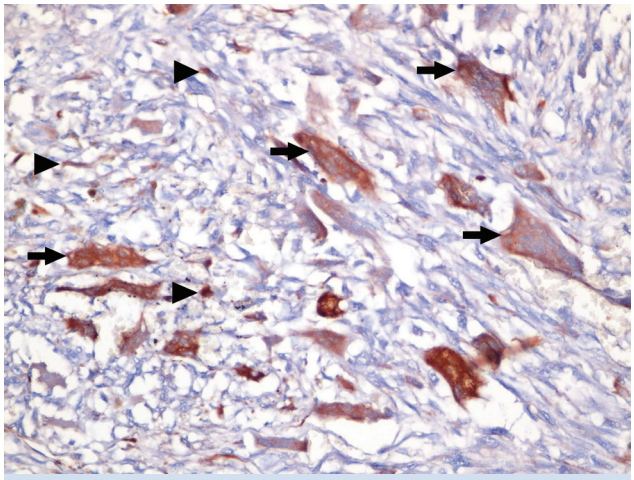


Figure 2. Multinucleated giant cells (arrows),and fusiform-shaped cells (arrowheads) expressing vimentin (Streptavidin-biotin-peroxidase, x400)

DISCUSSION

Bone tumors are uncommon in cats. Osteosarcomas are the most common malignant tumors that constitute 70-80% of primary bone tumors in cats (Quıegley and Leedale, 1983; Craig et al., 2016; Thompson and Dittmer, 2017). They are usually seen in middle-aged or older cats (Craig et al., 2016).

GCO is basically characterised by osteoid production and the presence of giant cells formed by osteoclast-like cells (Negrin et al., 2006; Gambarotti et al., 2011; Thompson and Dittmer, 2017).GCOs are difficult to differentiate histologically from giant cell tumors of bone.In differential diagnosis, neoplastic cells in GCOs show more anaplasia and atypia. In addition, they can be characterised by the production of osteoid matrix (Bertoni et al., 2003; Craig et al., 2016; Thompson and Dittmer, 2017).However, GCO is more aggressive than giant cell tumors of bone (Craig et al., 2016; Thompson and Dittmer, 2017).Osteosarcomas are also malignanttumors that tend to metastasize. It has been reported that GCO metastasized to the liver and spleen in a Persian cat (Farjanikish et al., 2018).

Osteosarcomas are classified as vimentin positive tumors. (Fain et al., 1993). In studies on GCO, it has been reported that vimentin is expressed by multinucleated giant cells, fusiform-shaped cells, and osteoclasts (Fattahian et al., 2008; Oryan et al., 2015; Farjanikish et al., 2018). In this study, vimentin expression of the fusiform-shaped cells and multinucleated giant cells indicated that the tumor originated from the mesenchymal cells. In contrast, it did not express cytokeratin indicated that this tumor was not of epithelial origin.Besides, the positive reaction with actin was only seen in smooth muscle cells of blood vessels. The absence of actin expression in neoplastic cells and multinucleated giant cells indicated that thetumor didnot have a smooth muscle origin.In some studies, it has been stated that tartrate-resistant acid phosphatase (TRAP) staining was used to show osteoclasts (Mikaelian et al., 1998; Negrin et al., 2006). However, we did not perform this staining in our study.Also,in some studies,S100 (Negrin et al., 2006; Farjanikish et al., 2018),CD20-CD3-CD68 (Farjanikish et al., 2018), desmin (Fattahian et al., 2008; Farjanikish et al., 2018), and CD34 (Fattahian et al., 2008) primary antibodies were examined in addition to vimentin, cytokeratin, and actin primary antibodies for immunohistochemical staining.However, these antibodies were not present in our immune panel.

In conclusion, histopathological changes such as the observation of osteoid islands, fusiform-shaped cells, andmultinucleated giant cells in this study were consistent with the previously reported GCO in humans and animals.In addition to the routine histopathological methods we used, immunohistochemistry was also helpful in confirming the diagnosis of the GCO.However, we could not comment on the patient's current status in this study because we did not have any data on metastasis, and the owner did not want treatment for GCO.

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CONFLICT OF INTEREST

None declared by the authors.

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