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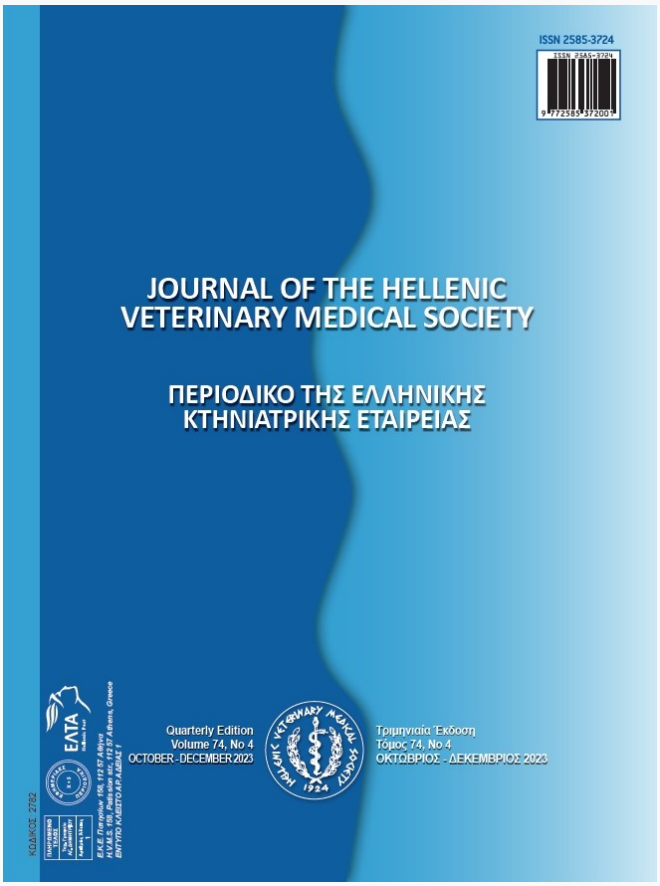

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Case report
Κλινικό περιστατικό

Presumptive injection-site sarcoma in a white tiger (*Panthera tigris tigris*): A case report

H. Moosavian[1]*, H. Esmaeilzadeh[2], M. Fazli[3], C.A. Thompson[2], A.Farajzadeh[4]

[1]*Department of Clinical Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

[2]*Department of Comparative Pathobiology, College of Veterinary Medicine, Purdue University, Indiana, USA*

[3]*Department of Biology, Faculty of Basic Science, Islamic Azad University, Tehran, Iran*

[4]*Doctor of Veterinary Medicine, Private Practice, Tehran, Iran*

ABSTRACT: A6-year-old female white tiger (*Panthera tigris tigris*) was presented with a history of a recurrent ulcerating mass on the right lumbar wall, that had been initially surgically removed one year before presentation. Cytological and histological findings were consistent with a subcutaneous injection-site sarcoma. Immunohistochemistry results revealed that the neoplastic cells were strongly positive for vimentin and weakly positive for alpha-smooth muscle actin (SMA), but negative for cytokeratin AE1/AE3, S-100 protein, and desmin. Positive staining for vimentin and SMA is consistent with myofibroblast reactivity and reflects a continuous inflammatory response observed in feline injection-site sarcomas. Feline injection-site sarcomas are the most serious adverse effect following injection of vaccines or other pharmaceutical substances and should be considered in the differential diagnosis of skin masses developing at the injection sites in the Felidae family. Insoluble adjuvants, the process of injection, wounding and trauma, with an accompanying inflammatory process, may be involved in sarcomagenesis. There is limited data published for injection-site sarcomas in tigers.This case report highlights the importance of creating vaccination recommendations for the reduction of iatrogenic fibrosarcomas occurrence, as well as a management protocol of FISS in *wild* felids.

Keywords: White tiger; Injection-site sarcoma; Histopathology; Immunohistochemistry

Corresponding Author:
Hamidreza Moosavian, Department of clinical pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
E-mail address: hrmoosavian@ut.ac.ir

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CASE HISTORY

A 6-year-old female captive white tiger (*Panthera tigris tigris*), weighing 70 kg, was presented with a recurrent subcutaneous mass on the right lumbar wall (Figure1), and history of lethargy, anorexia, and weight loss, more than 60 kg during recent months. In clinical assessment, the mass measured approximately 20 cm in length and 15 cm in width, had a firm consistency with an ulcerated irregular surface, was poorly circumscribed, and infiltrated into the surrounding tissues. The tiger was vaccinated annually against rabies, feline viral rhinotracheitis, calicivirus infection, and panleukopenia with a blowpipe, around the site of tumor development(right and left lumbar area). According to historyshe had received at least 5 vaccinations and, the subcutaneous mass was seen for the first time, 8 months after the last vaccine. For initial evaluation, several impression smears from the ulcerated mass were made on glass slides, air-dried, fixed in methanol, stained with Giemsa, and examined under a light microscope. The smears were of high cellularity and good preservation. A few RBCs were present in the background. Moderate numbers of individualized, large, irregularly shaped spindle cells were seen. The cells had moderate- to large-sized nuclei with finely stippled chromatin, one or more prominent nucleoli, and a moderate amount of moderately basophilic cytoplasm with wispy cytoplasmic tails. The nuclear-to-cytoplasmic ratio was moderate to high. Anisocytosis and anikaryosis were high .No mitotic figures or mutlinucleated cells were noted. Many degenerate neutrophils were seen. Numerous extracellular and intracellular bacteria (cocci) were found. A presumptive diagnosis of a soft tissue sarcoma; possibly vaccine-associated, similar to feline injection-site sarcoma (FISS) was considered. The tiger was anesthetized using intramuscular administration of 0.04 mg/kg medetomidine (Syva Co., Spain) and 3 mg/kg ketamine (Alfasan Inc, Holland). Surgically, the subcutaneous mass was resected with negative but 0-mm surgical margin. Medetomidine-induced sedation was reversed by intramuscular administration of 0.2 mg/kg atipamezole (Syva Co., Spain). Penicillin G 6:3:3 (1200000 IU, IM, q24h, Jaber Ebne Hayyan Pharmaceutical Co., Iran) was administered postoperatively to the tiger. The removed mass was submitted to surgical pathology. The microscopical examination was performed on the formalin-fixed and paraffin wax-embedded tissue. 5-µm thick sections were slide-mounted and stained with hematoxylin and eosin. (H & E). Histologic examination of the biopsy showed a poorly encapsulated mass with the neoplastic proliferation of polymorphic mesenchymal cells with moderate- to large-sized nuclei, one or more prominent nucleoli, and multinucleated tumor cells in a storiform pattern. Pleomorphic and bizarre tumor cells with marked atypia were seen. Moderate mitotic figures, mild necrosis, and moderate distribution of peritumoral lymphoid cells were noted (Figure 2). Focally positive surgical margins were histologically confirmed. The tumor was graded and classified based on the soft tissue sarcoma (STS) grading

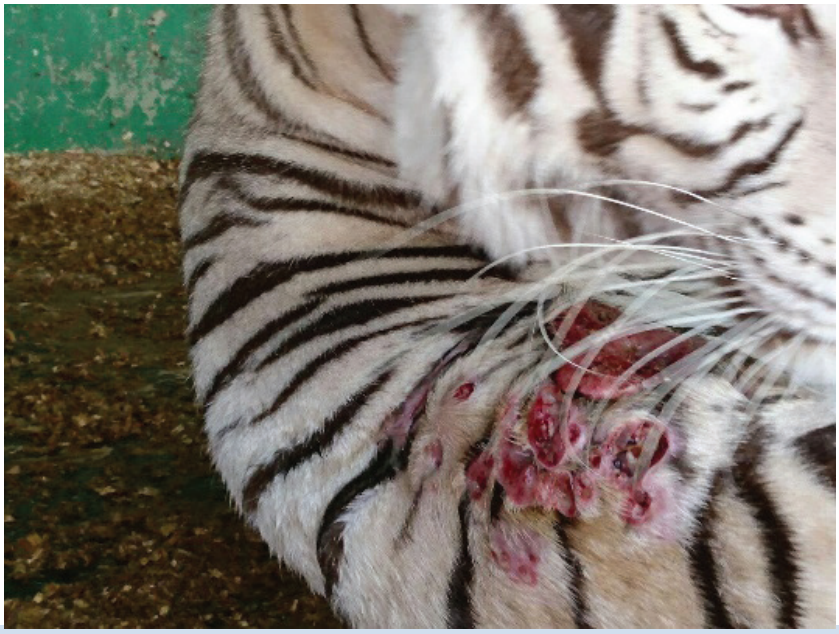

Figure 1. Clinical appearance of an injection-site sarcoma in a 6-year-old female white tiger (*Panthera tigris tigris*). A 20x15-cm lobulated ulcerative subcutaneous mass was detected on the right lumbar wall

system, previously adapted to the dog (Powers et al., 1995) and then applied to feline vaccine-associated fibrosarcomas (Couto et al., 2002; Romanelli et al., 2008), basing on cellular differentiation, presence and extension of necrosis within the neoplasm and mitotic rate. Based on the poor cellular differentiation (Tumor Cell Differentiation Score: 3/3), moderate mitotic figures (10-15 Mitoses per 10 HPF (FN22/40_ objective), Mitotic Score 2/3) and mild necrosis (Tumor Necrosis Score: 2/3), the sarcomas were designated as grade 3 (Total Grading Score: III) for overall findings. For further evaluation, the sections were incubated with a panel of antibodies specific for vimentin, alpha-smooth-muscle actin (SMA), cytokeratin AE1/AE3 (CK), S100 protein, and desmin (Table 1). Immunohistochemistry was performed using the horse-

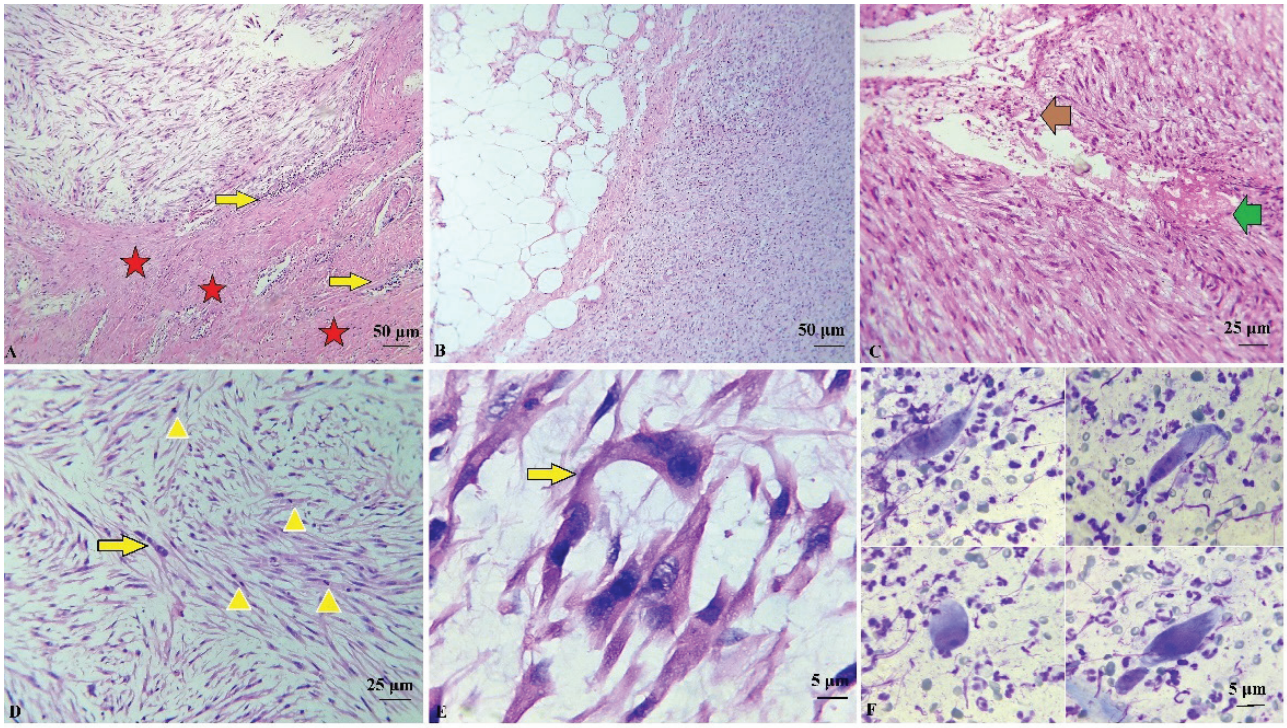

Figure 2. Photomicrographs of hematoxylin and eosin-stained subcutaneous soft-tissue mass (A-E), and tissue imprint, Giemsa staining (F), in a tiger.

A) Bordered loose fibrovascular connective tissue (red star), and lymphocytic aggregates at the periphery of the tumor; (yellow arrow).
B) The dermis was diffusely replaced by neoplastic proliferation, and the poorly circumscribed tumor invades the hypodermis and underlying tissues.
C) Scatter focal area of necrosis (green arrow) along with mild neutrophilic infiltration (brown arrow).
D) The neoplastic cells were arranged in streaming and interlacing bundles. The neoplastic cells were pleomorphic, with a scant streaming eosinophilic cytoplasm and indistinct cell borders, and pleomorphic nuclei. An increased number of multinucleated cells (yellow arrow), and mitotic figures (yellow arrowhead), were seen.
E) Multinucleated cell (yellow arrow), and marked polymorphism
F) Direct impression smear from the ulcerated surface of the mass. Individualized plump or spindle-shaped cells with wispy cytoplasmic borders, variable but mostly scant basophilic cytoplasm, moderate to high nuclear-to-cytoplasmic ratio, nuclear pleomorphism with a coarse to open chromatin pattern and with a single to multiple prominent nucleoli were seen

Table 1. Primary antibodies used for immunohistochemical evaluation of injection site sarcoma

Antigen	Antibody	Dilution	Source	Antigen retrival	Results
Vimentin	Mouse Mab*, IgG1	Ready-to-use	*Zytomed Systems*	Autoclave	Positive
Pancytokeratin (AE1 and AE3)	Mouse Mab*, IgG1	Ready-to-use	*Zytomed Systems*	Autoclave	Negative
S100	Mouse Mab*, IgG2a	Ready-to-use	Zytomed Systems	Autoclave	Negative
α-SMA*	Mouse Mab*, IgG2a-κ	1:50	Diagnostic Biosystems	Autoclave	Positive
Desmin	rabbit monoclonal antibody	1:100	Zytomed Systems	Autoclave	Negative

*Mab=monoclonal antibody, α-*SMA* =Smooth muscle alpha-actin

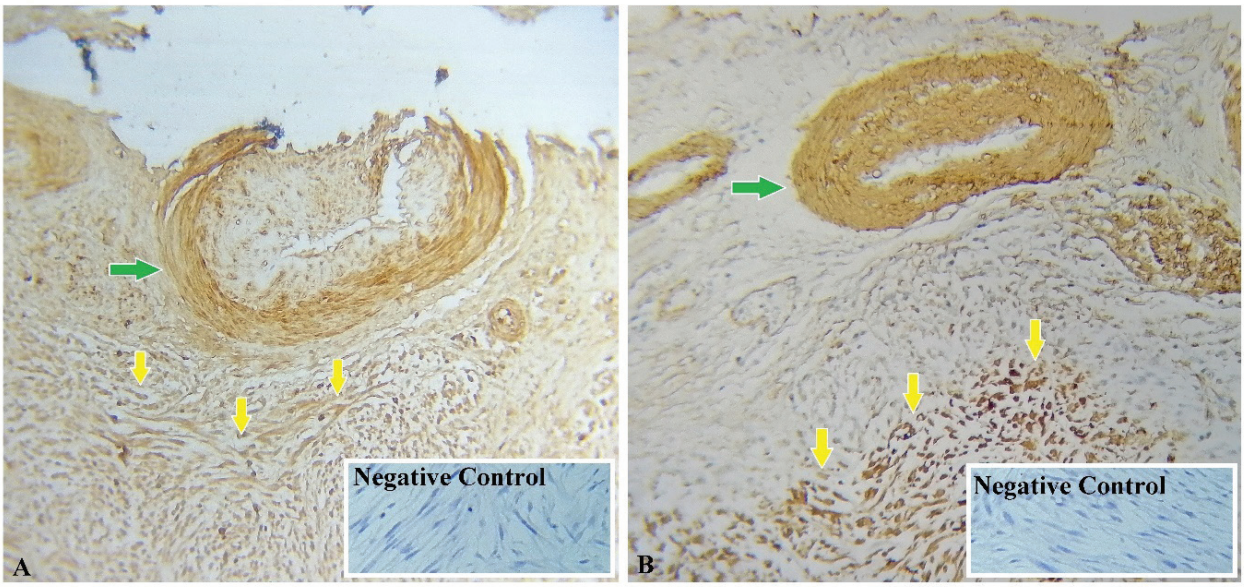

Figure 3. Photomicrographs of immunohistochemical expression of vimentin and SMA in an injection- site sarcoma in a tiger.

A) Positive immunostaining for vimentin (yellow arrow), (× 200 magnification); Smooth muscle cells from arteries were used as an internal positive control (green arrow). For negative control, additional sections were incubated with corresponding secondary antibodies and the primary antibody was omitted.
B) Positive immunostaining for SMA (yellow arrow), (× 200 magnification); Smooth muscle cells from arteries were used as an internal positive control (green arrow). For negative control, additional sections were incubated with corresponding secondary antibodies and the primary antibody was omitted.

radish peroxidase conjugated antibody and the chromogen 3.3′-diaminobenzidine-tetrahydrochloride as described (Hendrick and Brooks 1994). Positive controls for the immunostaining were found in the surrounding tissues for vimentin and SMA (smooth muscle cells from arteries), cytokeratin (epithelial cells), and desmin (endothelial cells). Feline normal adipose tissue was used as an external positive control for S100 protein. For negative control, additional sections were incubated with corresponding secondary antibodies and detection systems, and the primary antibody was omitted. The neoplastic cells were strongly positive for vimentin (more than 95% of neoplastic cells) and weakly positive for SMA (less than15% of neoplastic cells), but negative for, CK, S100 protein, and desmin (Figure 3). Based on the observed cellular pleomorphism, a diagnosis of an undifferentiated pleomorphic sarcoma was made. The mass recurred 6 months after the second surgery and appeared ulcerative. The tiger became lethargic, anorectic, and apathetic and showed severe weight loss. The owner refused to pursue further diagnostic procedures as well as chemotherapy which was recommended. Due to the recurrence of the mass and the overall poor condition of the tiger, the owner decided to pursue elective euthanasia. The tiger was humanely euthanized with pentobarbital (350 mg/kg b.w., intravenously), and owner consent for necropsy was denied.

DISCUSSION

The prevalence of feline injection-site sarcoma (FISS) in cats reported by different studies ranges from 1:1000 to 1:10 000 (Couto et al. 2002, Munday et al., 2003, Martano et al., 2011). Feline injection-site sarcomas are rare, but aggressive and locally invasive with rapid growth, while tumor necrosis and ulceration are common findings (Kang et al., 2017). Susceptibility of cats to oxidative injury, as low glutamine levels have been demonstrated in some cats with FISS, and variations in the tumor suppressor gene,which have been reported,may be risk factors for FISS formation in this species(Hendrick 1998, Couto et al. 2002, Munday, Stedman et al. 2003). The adjuvant, especially aluminum present in FeLV and rabies vaccines, has been blamed for contributing to the etiopathogenesis of FISS. Local inflammation, fibroblast stimulation, and oxidative damage to DNA following free radical formation can be induced by the adjuvant. However, in the study ofKass et al., 1993,the risk for FISS development in cats was associated with both adjuvanted (with or without aluminum) and non-adjuvanted vaccines. Different types of FISS such as rhabdomyosarcoma, myxosarcoma, chondrosarcoma, and undifferentiated sarcoma

have been described in domestic cats, with fibrosarcoma being the most common type (Chang et al., 2006). In addition to cats, FISS has previously been reported in a lion (Kinne and Tarello 2007), and ferrets (Porcellato et al., 2017). There are limited data published for injection-site sarcomas in tigers.Eikelberg et al., 2020reported two cases of recurrent subcutaneous fibrosarcomas observed in a 12-year-old, male, white tiger, and an 11-year-old, male lion. However, it is unknown if there was an injection previously in both animals (Eikelberg et al. 2020). Although it appears that injection-site sarcomas (ISS) may affect wild felids as well, there is poor information about the prevalence, histopathological characteristics, prognosis, and survival time of patients with ISS other than domestic cats. In the present case, *t*he tiger had received at least five subcutaneous vaccinations around the site of tumor formation during a period of 6 years. The number of vaccines given at a site, probably increases the risk of sarcogenesis. In one study, cats that received three to four vaccinations in the interscapular region were twice as likely to have ISS than cats that received one vaccine at that site(Kass et al. 1993).

Previous studies in cats showed the age of affected cats with FISS at the moment of the diagnosis ranges from 6 to 16 years,with a mean value of 11 ± 3 years without breed or sex predilection(Powers et al., 1995). The subcutaneous lesion in our case was measuring 20 cm in length and 15 cm in width. The results of clinical studies in cats showed that the size of the tumor at presentation time and prompt intervention is critical in determining the prognosis. Based on the results a cut-off point of 3.75 cm provides a sensitivity of 88% and a specificity of 85% in predicting tumor recurrence (Powers et al. 1995). However, it should be noted that probably the reported sizes and cut-off for ISS in cats do not have the same prognostic value for the larger animals such as lions and tigers. In cats with FISS that underwent radical surgery, the recurrence rate is up to 70%, and the disease-free survival time is approximately only 6 months. However, multiple factors such as surgeon experience and resecting tumors with clear margins, size of tumors before the first surgery, the *combination* of *surgery* and *radiation/chemotherapy* and the time between surgery and the start of radiotherapy, histological grading, and histological margin determination have key roles in a cat's survival time (Zabielska-Koczywąs et al., 2017). In the present case, the overall survival time was 18 months after the first surgery. Signs of systemic illness such as lethargy, anorexia, weight loss, vomiting, and increased respiratory rate may be noted in cats with metastasis. Metastasis is seen in up to 28% of cats with FISS, and regional lymph nodes and lungs are the most commonly reported sites (Kang et al. 2017). In the present case, lethargy, anorexia, and weight loss were the noticeable clinical signs. After the initial diagnosis of ISS was established, *further evaluation* by imaging for metastases was recommended to determine treatment options. However, the animal was in poor condition, and the owner refused to pursue further diagnostic procedures, and consent for necropsy was denied. Cytological examination of the fine needle aspirate, as an initial complimentary examination, is reliable in about 50% of FISS (Hendrick 2000). In this study, impression smears detected individualized plump oval cells with marked polymorphisms and moderate to high nuclear-to-cytoplasmic ratio, and nuclear pleomorphism with prominent nucleoli with *neutrophilic infiltrate were the main cytological findings.* The histopathology sections showed poor cellular differentiation, moderate mitotic figures, and mild necrosis, and the sarcoma was designated as grade III (range I-III) for overall findings. The presence of inflammation reaction as peritumoral lymphoid aggregation, smaller numbers of plasma cells, accentuated peripheral vascularity, variably distributed myofibroblast-like cells, and neoplastic multinucleated giant cells, are common features that may be seen in FISS. However, the presence of macrophages containing presumed adjuvant material, as in this case, is not always seen (Doddy et al., 1996, Couto et al. 2002). In the present study, moderate distribution of peritumoral lymphoid cells, and multi-nucleated giant cells, were present. With attention to severe pleomorphism of the undifferentiated spindle-shaped cells, a diagnosis of undifferentiated pleomorphic sarcoma was the most likely interpretation for this patient. Immunohistochemistry was performed to further investigate this case. The neoplastic cells were strongly positive for vimentin and weakly positive for SMA, but negative for, CK, S100 protein, and desmin. A study describing the immunohistochemical characteristics of FISSs reported that all tumors were immunoreactive for vimentin, and more than 60% of them were immunoreactive for SMA (Couto, Griffey et al. 2002). A second study of FISSs showed that all tumors were positive for vimentin and seventeen (81%) FISS were negative for desmin (Carneiro et al., 2019). Positive staining for vimentin and SMA and/or desmin, as muscle markers, is consistent with myofibroblast reactivity (Carneiro,

de Queiroz et al. 2019). In the present case, all histopathological findings were consistent with an injection site sarcoma in a white tiger. Our study had some limitations. The necropsy and imaging studies were denied by the owner. Based on the autopsy and imaging findings, additional information could be found about the tumor extension and behaviour.

Feline injection-site sarcoma is largely caused by vaccines, and similar to domestic cats, vaccination injection management can have a pivotal role in the disease management in wildlife cats. The *2013 AAFP Feline Vaccination Advisory Panel Report* included recommendations and management options for the reduction of FISS risk (Scherk et al., 2013). In this report, *avoidance of administrating unnecessary vaccines in cats,* administration of parenteral feline vaccines by the subcutaneous route, so as to facilitate early detection of tumor, consideration of the type of vaccine used, performing a biopsy according to the 3-2-1 rule, in order to evaluate the presence or absence of malignant tumor formation as soon as possible, staging and complete surgical removal of tumor are recommended. Furthermore, distal limbs have been suggested as the preferred sites for vaccination to facilitate clean margins if surgical amputation is required.

In conclusion, the history, clinical signs, cytological, histological, and immunohistochemical findings of this case were consistent with a subcutaneous injection site sarcoma in a white tiger (*Panthera tigris tigris*). The recurrence time after the first surgical removal of the mass was twelve months. Feline injection-site sarcoma is largely caused by vaccines, and so, as in domesticated cats, management options for the reduction of FISS risk in wildlife felids should be considered in vaccination programs.

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CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

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