ZOO ANIMALS

Subcutaneous fibrosarcomas with pulmonary metastases in a white tiger (*Panthera tigris*) and a lion (*Panthera leo*)

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SUMMARY

Two cases of recurrent subcutaneous fibrosarcomas in a white tiger and a lion were observed and the animals were euthanised humanely due to clinical deterioration. In both animals, postmortem examination revealed multinodular, white to fawn, firm to greasy, subcutaneous masses at the left side of the thorax infiltrating into the adjacent musculature. Furthermore, the tiger showed a single mass and the lion multiple masses in the lung. Histopathologically, the subcutaneous and pulmonary masses consisted of spindle-shaped neoplastic cells with necrotic areas, and infiltration with multinucleated giant cells and lymphocytes. Immunohistochemically, tumour cells labelled positive for vimentin and negative for desmin, factor VIII-related antigen, smooth muscle actin S100, CD31 and nerve growth factor receptor p75. Thus, the pulmonary tumours were diagnosed as metastases of subcutaneous fibrosarcomas. Like domestic cats, also large, non-domestic felids could be predisposed for metastasising fibrosarcoma, which may be associated with injections or trauma.

BACKGROUND

Fibrosarcomas represent, together with basal cell tumour, mast cell tumour and squamous cell carcinoma, the most common skin tumours of cats. In malignant mesenchymal skin tumours, fibrosarcomas are most common.[1 2] Contrarily, in large felids, fibrosarcomas are considered to be rare.[3] Large felids have a higher life expectancy in captivity than in wildlife. Thus, better possibilities for screening of non-neoplastic and neoplastic diseases are present. The development of sarcoma especially at injection sites is a phenomenon that occurs regularly in cats. To distinguish between an injection-site fibrosarcoma and a non-injection site fibrosarcoma, different microscopic features including subcutaneous localisation, necrosis, inflammatory cell infiltration, increased mitotic activity and pleomorphism have to be applied.[2] While non–injection-site sarcomas occur in the dermis, fibrosarcomas with association to injection sites can be found more frequently within the subcutis.[2] Also necrosis, rapid growth and local invasion represent more common features at injection sites.[2] However, despite the fact that the localisation, the occurrence of necrosis, rapid growth and invasion are characteristic for injection-site fibrosarcomas, the microscopic features do not guarantee a definite differentiation between injection-site and non–injection-site fibrosarcomas. Nevertheless, microscopic features are important in case of an insufficient vaccination history.

Associated with injection fibrosarcomas are part of feline injection-site sarcomas (FISS) and develop in 1–10 of 10,000 vaccinated cats.[4 5] FISS are mesenchymal neoplasms that include malignant fibrous histiocytomas, osteosarcomas, chondrosarcomas, rhabdomyosarcomas, liposarcomas and undifferentiated sarcomas.[6] For differentiation between the various neoplasms associated with FISS, different immunohistochemical markers are necessary. Regarding malignancy of FISS, they appear to recur frequently[7 8] whereas the occurrence of metastasis is discussed controversially and described for different organs including lungs, regional lymph nodes and skin.[8–10] For example, repeating excisions and subsequent recurrences may promote a more aggressive population of tumour cells and can lead to an increased ability to invade and metastasise.[8] Besides at injection-site localisation, fibrosarcomas in domestic cats can also occur spontaneously and solitary in older cats or in multiple localisations, as they can metastasise.[11] Moreover, feline fibrosarcomas can be induced by feline sarcomavirus[11] which is a mutated form of the feline leukaemia virus (FeLV).[11] In large, non-domestic felids, fibrosarcomas have only been reported in a few cases.[3 12 13] All fibrosarcomas were located in the subcutis. In one of these reports, the neoplasms were found in the interscapular region or on the left side of the thorax; however, metastasis could not be observed.[12] The present cases of a tiger and a lion demonstrate that hallmarks of injection-site fibrosarcomas including metastases, recurrence and high mitotic activity can also occur in large, non-domestic felids.

CASE PRESENTATION

In May 2018, a 12-year-old, male, white tiger (*Panthera tigris*) was presented with subcutaneous masses at the left elbow and at the right thoracic wall, which were surgically removed because a neoplastic process was suspected. The masses were composed of a skin sample sized 7.3×5×2.8 cm and several smaller resectates. They were surgically removed for diagnostics in concern for a neoplasia and a fibrosarcoma with inflammation was diagnosed. In

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September 2018, the animal exhibited a relapse associated with inflammatory skin lesions at the previously affected localisation leading to the decision to euthanise the animal.

In Summer 2015, an 11-year-old, male lion (*Panthera leo*) showed multiple, recurring subcutaneous tumours of variable sizes. The first occurred at the left thigh in the region of the hip bone which showed a size of 10 cm in diameter and appeared to decrease in size first. A fine-needle aspiration of this mass was performed in September 2016 which revealed high numbers of erythrocytes, neutrophils, eosinophils, macrophages and lymphocytes. A hypersensitivity reaction was suspected due to the presence of eosinophils with possibility of a response to foreign material or eosinophilic granuloma. Neoplastic cells were not found. Due to enlargement of the mass and a coarse consistency, a tumour was suspected and the 15-cm-diameter mass was removed in December 2016 for further diagnostics. Histological examination revealed a fibrosarcoma with surgical margins free of neoplastic cells. In June 2017, a 3-cm-diameter large mass located 1–2 cm cranial of the dorsal scar tissue remaining from the removal of the fibrosarcoma at the left thigh was detected and removed in July 2017. In December 2017, due to prolonged intermittent inappetence, the animal was anaesthetised for general examination and thoracic and abdominal radiography, which did not reveal any significant findings. Due to the suspicion of an inflammatory process and for improvement of the animal's condition, the lion received intravenous fluid therapy, prednisolone and an antibiotic therapy during anaesthesia. The administration of antibiotics was continued until January. In February 2018, the lion still showed intermittent inappetence and a mass was observed at the left thoracic wall. An examination under anaesthesia revealed that the mass infiltrated into the intercostal musculature. Based on these clinical findings, the animal was euthanised.

INVESTIGATIONS

After euthanasia, both carcasses underwent postmortem examination and a wide spectrum of organs was fixed in 4 per cent formalin overnight. After trimming, the organ samples were routinely embedded in paraffin and 4-µm-thick paraffin sections were cut and stained with H&E. Immunohistochemistry was performed using the avidin-biotin-peroxidase complex (ABC) method and the chromogen 3.3′-diaminobenzidine-tetrahydrochloride as described.[14 15] Sections were incubated with primary antibodies specific for vimentin, desmin, factor VIII–related antigen, smooth muscle actin (SMA), nerve growth factor receptor p75, CD31 and S100 at 4°C overnight. Further antibody details are listed in table 1. Histological and immunohistochemical slides were evaluated by light microscopy.

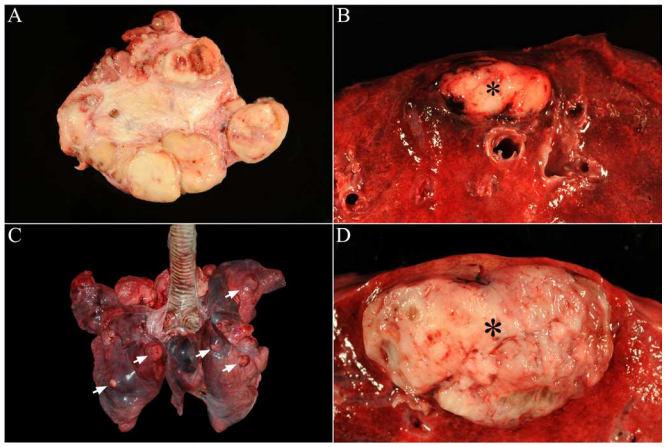

Figure 1 (A) Multinodular, partly firm and greasy, cut surface of subcutaneous mass from the region of the left elbow from a tiger. (B) Neoplastic mass (asterisk) similar to the subcutaneous masses infiltrating the lung parenchyma of the tiger. (C) Lung of a lion with multifocal, variable-sized neoplastic masses (arrows). (D) Cut surface of the metastasis in the lung of the lion (asterisk).

OUTCOME AND FOLLOW-UP

The macroscopic examination of the white tiger revealed a 20×13×6 cm large, multinodular, partly firm, partly greasy, and white to fawn subcutaneous mass at the left elbow with infiltration into the adjacent musculature (figure 1A). In addition, a 6-cm-diameter, poorly demarcated, immovable, subcutaneous mass with a central cavity was found in the subcutis of the left thoracic wall caudal of the left elbow infiltrating into the adjacent musculature. The right cranial lung lobe showed a 3-cm-diameter neoplastic mass (figure 1B) similar to the subcutaneous masses. Further coincidental macroscopic findings during postmortem examination included a focal cyst of the right kidney and a callus formation of the left, ninth rib probably due to an on old fracture. The lion had an 8-cm-diameter, round mass with a greasy cut surface and a central cavity containing small amounts of serosanguinous fluid in the subcutis of the left thoracic wall between the sixth and eighth rib. In the lung, multifocal, up to 13-cm-diameter neoplastic masses similar to the subcutaneous masses were present (figure 1C,D). No remnants of the previously removed fibrosarcoma at the left thigh were found.

Histologically, the tiger had multinodular, well-circumscribed, non-encapsulated, densely cellular neoplastic masses in the subcutis. Neoplastic cells were arranged in bundles and streams accompanied by moderate amounts of fibrovascular stroma. The neoplastic cells were up to 20 µm in diameter, spindle shaped, with distinct cell borders and moderate amounts of homogeneous, eosinophilic and fibrillar cytoplasm. They contained

Table 1 Primary antibodies used for immunohistochemistry

Antigen	Pretreatment	Dilution	Clonality	Supplier	Catalogue number
CD31	Microwave/citrate buffer	1:100	Polyclonal rabbit	Acris Antibodies, Herford, Germany	AP 15436PU-M
Desmin	None	1:100	Monoclonal mouse	Dako, Glostrup, Denmark	M 0760
Factor VIII–related antigen	Pronase	1:200	Polyclonal rabbit	Dako, Glostrup, Denmark	A 0082
Nerve growth factor p75	None	1:10	Monoclonal mouse	Abcam, Cambridge, United Kingdom	Ab 52 987
Smooth muscle actin (SMA)	None	1:200	Monoclonal mouse	Dako, Glostrup, Denmark	M 0851
S100	None	1:800	Polyclonal rabbit	Sigma-Aldrich, St. Louis, MO, USA	S-2644
Vimentin	None	1:100	Monoclonal mouse	Dako, Glostrup, Denmark	M 0725

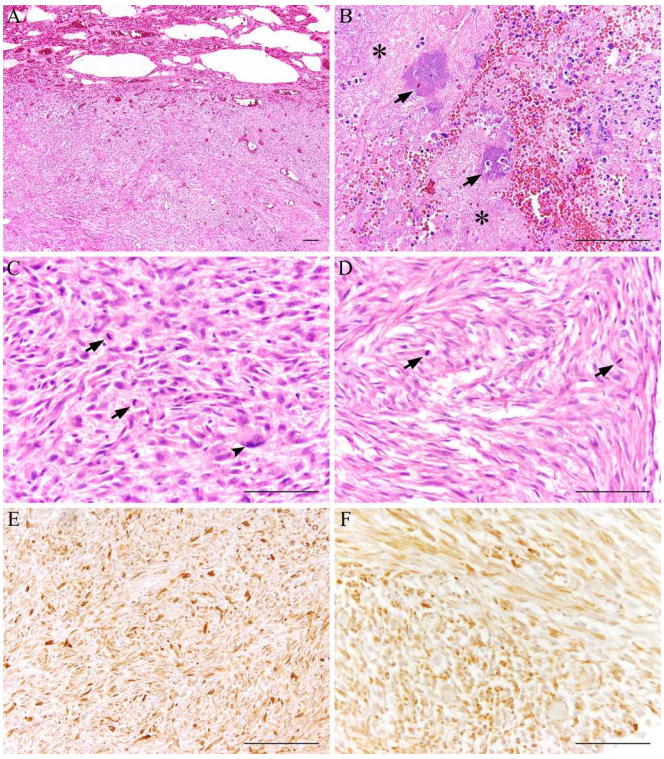

Figure 2 (A) Pulmonary metastasis of the tiger with invasion of the alveolar tissue, H&E staining. (B) Haemorrhage, bacterial colonies (arrows) and areas of necrosis (asterisks) in the tumour at the thorax from the lion, H&E staining. (C) Pulmonary metastasis of the tiger with numerous mitotic figures (arrows) and a multinucleated giant cell (arrow head), H&E staining. (D) Pulmonary metastasis of the lion with two mitotic figures (arrows) and neoplastic cells arranged in bundles and streams, H&E staining. (E) Neoplastic cells in the pulmonary metastasis of the tiger labelled positive immunohistochemically for vimentin, ABC method. (F) Positive labelling for vimentin in the pulmonary metastasis of the lion, ABC method. Bars 100 µm.

a central oval nucleus with finely stippled chromatin and one small nucleolus. Mild anisocytosis and anisokaryosis was present with a high mitotic rate of up to 10 mitoses per high-power field (HPF, x 400). Frequently multinucleated giant cells, mild lymphoplasmacytic inflammation and large areas of necrosis were present. The cellular origin of the multinucleated giant cells remains unclear since histiocytic cell markers were not applied. However, because these cells were located within areas of inflammation and had no criteria of malignancy, they were supposed to be histiocytic. Histological examination of the pulmonary mass revealed a similar appearance (figure 2). The pulmonary lymph nodes showed no infiltrations with neoplastic cells. In addition, an anthracosis of lung and pulmonary lymph nodes, an interstitial nephritis with hyalinisation and fibrosis, a lymphoplasmacellular gastritis, an eosinophilic enterocolitis and a hyperaemia of the spleen were present. Due to the low degree of expression, these findings were of minor relevance and not associated to the neoplastic event. In the subcutis and the lung of the lion multiple, well-circumscribed, non-encapsulated, densely cellular neoplastic masses with compression of the adjacent pulmonary tissue were present. The neoplastic cells revealed a similar spindle cell appearance and an arrangement in whorls, bundles and streams like the neoplastic masses of the tiger. The mitotic rate was high with 3 to 4 mitotic figures per HPF. Furthermore, the pulmonary tumours showed a moderate infiltration of macrophages at the borders of the neoplasia as well as mild multifocal, perivascular and peribronchiolar, lymphocytic infiltrates. Within the pulmonary as well as the subcutaneous neoplastic masses, a moderate, multifocal, central necrosis (figure 2B) with infiltration of neutrophils could be found. Besides, pathohistological findings included a lymphocyte depletion of the spleen and a lymphocytic infiltration of the stomach, which were unrelated to the neoplastic event and of minor clinical relevance. Immunohistochemically, approximately 95 to 100 per cent of the neoplastic cells of the subcutaneous tumours and the pulmonary metastases in both animals showed an intense cytoplasmic labelling for vimentin (figure 2E,F). There was no immunoreactivity of the neoplastic cells for antibodies to desmin, factor VIII–related antigen, SMA, nerve growth factor receptor p75, CD31 and S100.

DISCUSSION

The white tiger and the lion examined in this study developed subcutaneous sarcomas with metastases into the lungs. In domestic cats, subcutaneous sarcomas often develop after a vaccination or injection and are termed as FISS.[16] To date, the aetiology and pathogenesis of FISS remains unknown. It is assumed that local inflammatory processes after administration of vaccines especially against FeLV and rabies as well as other medications like steroidal and non-steroidal anti-inflammatories, microchip implantation, as part of a gossypiboma[17] or at sites of non-absorbable sutures lead to neoplastic transformation in domestic cats.[18] FISS show a rapid growth with a locally invasive behaviour resulting in a guarded to poor prognosis with recurrence rates as high as 45 per cent.[19] The median survival time after initial surgery varies between 324 and 576 days depending on the treatment regime, as especially after radical tumour excision longer survival times are reported.[8 16 20]

Histologically, FISS are usually characterised by strand-like proliferations of spindle-shaped tumour cells with necrotic areas and infiltrations of multinucleated giant cells.[6] In case of previous vaccinations, the giant cells can contain greyish material often interpreted as aluminium-based adjuvant.[7 21]

Due to the immunoreactivity of the subcutaneous and pulmonary neoplasms of the white tiger and the lion, the tumours were diagnosed as primary subcutaneous fibrosarcomas with pulmonary metastases. Fibrosarcomas show vimentin-positive, pleomorphic, spindle-shaped tumour cells with a high mitotic rate.[22] In domestic cats, fibrosarcomas metastasise with a frequency of 5 to 20 per cent and can occur in lung, skin and regional lymph nodes.[8–10] Furthermore, a single case of an ocular metastasis is reported.[9]

To the best of the authors' knowledge, this is the first report of subcutaneous fibrosarcomas metastasising into the lungs in large, non-domestic felids. Despite the fact that clinical information concerning the exact localisation of previous injections is lacking, a possible association of the primary neoplasms with injections cannot be excluded. Inflammatory reactions as a consequence of local injuries are also discussed as a possible trigger for malignant transformation.[16] The fibrosarcomas of the tiger and the lion share several similarities with FISS.[11] Whereas it is unknown if there was an injection previously in both animals, the tiger and the lion were middle aged as also described in FISS in cats.[11] Moreover, the primary tumours of both animals showed a central cavity due to necrosis which is also typical in FISS.[11] Furthermore, both primary fibrosarcomas and their metastases revealed a high mitotic rate (tiger: up to 10 mitoses/HPF; lion: 3 to 4 mitoses/HPF). Contrarily,

non–injection-associated fibrosarcomas usually have a low or variable mitotic rate.[11] Multinucleated giant cells can be present in both non–injection-associated fibrosarcomas and in FISS[11] and were only present in the tiger. Intra vitam diagnostic in both animals first revealed an eosinophilic and lymphoplasmacytic (tiger) or an eosinophilic and lymphohistiocytic (lion) inflammation. In both spontaneous fibrosarcomas and FISS, an associated inflammation is described.[11] Macrophages with adjuvant material of vaccines, which can be found in FISS,[11] were neither detected in the tiger nor in the lion. Non–injection-associated fibrosarcomas can reveal recurrence, whereas in FISS recurrence is common.[11] Recurrence of the fibrosarcomas was found in both animals. In FISS, metastasis can occur[11] like in the current cases in the tiger and the lion. Summarised, the development of a fibrosarcoma due to an injection should be considered in both animals.

Previous studies report general pathological findings[13] and neoplasia[13] in large felids. Junginger *et al* found neoplasia in half of the examined animals (19 of 38) arising most often from the endocrine and genital system.[23] Moreover, neoplasia was present in the lympho-haematopoietic and alimentary organs.[23] Nevertheless, fibrosarcomas were not present in the examined animals. A total of 108 captive large felids with neoplasia, most often affecting the reproductive, the endocrine and the integumentary system, were examined.[13] Mammary carcinomas were found most often. Integumentary neoplasms contained squamous cell carcinoma, soft-tissue sarcoma, fibrosarcoma, myxosarcoma, haemangioma, malignant melanoma and sebaceous adenoma.[13] A metastasis of a fibrosarcoma was reported, but the localisation was not described.[13] Summarised, except from mammary tumours, the number of integumentary tumours was small.[13] In wild large felids, integumentary tumours seem to be rare with a description of a cutaneous squamous cell carcinoma at a paw of a wild lion.[24]

In summary, the results of this report like those of other authors[12] show that large, non-domestic felids could be predisposed for the development of fibrosarcomas like domestic cats. In domestic cats, a genetic predisposition for the development of injection site sarcomas is assumed.[16 25 26] A connection of germline polymorphisms in the p53 gene and the development of vaccine-associated feline sarcomas is suspected.[26] p53 arbitrates different cellular functions in humans and wildlife animals including cell cycle arrest, DNA repair and apoptosis.[27] Due to similarities in the tiger genome to the cat (95.6 per cent) and the genetic similarities of tigers and lions,[28] similar predispositions for the development of fibrosarcomas could be considered. Nevertheless, until now the role of predisposing gene alterations promoting the development of malignancies in large felids remains uncertain. Thus, further studies are needed. Furthermore, the findings demonstrate that in cases of recurring, inflamed malignant spindle cell tumours, fibrosarcomas should always be considered as differential diagnoses and the possibility of metastases after surgical excision of such tumours should be kept in mind.

Learning points

- Large, non-domestic felids could be predisposed to the development of fibrosarcomas like domestic cats.
- Injections or trauma can promote the development of fibrosarcomas.
- Fibrosarcomas in large, non-domestic felids can metastasise into the lung.

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