

Fibrosarcoma arising after femur osteosynthesis with pin and plate placement in a cat

Fibrossarcoma em sítio de osteossíntese de fêmur com pino e placa em um felino

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Abstract

A 6-year old spayed female Siamese cat was presented with a history of left limb acute lameness. Radiographic imaging revealed a complete comminuted diaphyseal femur fracture, which was corrected through intramedullary pin placement along with plate osteosynthesis. After two months the animal returned to the hospital presenting severe diffuse enlargement of the left hindlimb, characterized as a non-delimited, solid, and firm plaque-like mass surrounding the pin and bone, which extended from the femorotibiopatellar joint to the pelvis, and infiltrated the adjacent musculature. Incisional biopsy exam indicated a highly malignant sarcoma. The entire limb was surgically removed and sent to histopathological evaluation. Microscopically, the mass was composed of spindle shaped cells, displaying high pleomorphism and cellular atypia. Abundant collagen production was evidenced through Masson's trichrome stain and strong cytoplasmic staining for vimentin. These results were consistent with fibrosarcoma. The cat went through one session of chemotherapy; however, tumor recurrence occurred 20 days later, and the animal was submitted to euthanasia. This is the first description of fibrosarcoma arising in the vicinity of an intramedullary pin and plate in a cat.

Key words: Feline. Mesenchymal. Osteosynthesis. Sarcoma.

Resumo

Uma gata, castrada, 6 anos de idade, da raça Siamês, apresentou histórico clínico de claudicação aguda do membro pélvico esquerdo. O exame radiográfico revelou uma fratura completa cominutiva diafisária do fêmur, a qual foi corrigida através de osteossíntese pela implantação de um pino intramedular, além de placa metálica. Depois de dois meses, o felino retornou ao hospital apresentando aumento severo e difuso do membro pélvico, caracterizada por uma massa não delimitada, sólida, como uma placa, circundando o pino e o osso, a qual se estendia da articulação femorotibiopatellar até a pelve, e infiltrava a musculatura adjacente. A biópsia incisional indicou sarcoma de alta malignidade. O membro inteiro foi cirurgicamente removido e enviado para análise histopatológica. Microscopicamente, a massa era composta por células fusiformes, demonstrando alto pleomorfismo e atipia celular. Abundante produção de colágeno foi evidenciada através da técnica histoquímica de tricrômico de Masson e forte

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imunomarcção citoplasmática para vimentina. Esses resultados foram consistentes com fibrossarcoma. O felino foi submetido a uma sessão de quimioterapia, porém houve recorrência tumoral 20 dias após a sessão, e o paciente foi submetido a eutanásia. Essa é a primeira descrição de fibrossarcoma que surgiu em local próximo a implantação de pino intramedular e placa em um felino.

Palavras-chave: Felino. Tumor mesenquimal. Osteossíntese. Sarcoma.

Feline injection site sarcoma (FISS), also known as vaccine-associated sarcoma, is a well-recognized pathological entity in the cat (DODDY et al., 1996). These tumors present mesenchymal origin, and are composed of spindle-shaped cells, and generally are classified as well-differentiated. Among the histological types, fibrosarcoma, malignant fibrous histiocytoma and chondrosarcoma are frequently observed (HENDRICK, 2017). FISS present unique characteristics related to their origin in the subcutaneous tissue, marked necrosis in the center of the tumor, high cellular pleomorphism, and mononuclear inflammatory infiltrate in the periphery of the neoplasm (DODDY et al., 1996; KASS et al., 2003; KLICZKOWSKA et al., 2015).

These neoplasms have high capacity to invade adjacent tissues, as muscular layers and dermis (KANG et al., 2017; HENDRICK, 2017). Even when macroscopically, the neoplasm presents a capsule, histologically, projections of the tumor can often be seen extending away from the mass along fascial planes (HENDRICK, 2017). FISS often originate in the subcutaneous tissue of interscapular and thoracic wall regions, where application of substances has been previously performed (KLICZKOWSKA et al., 2015).

Parenteral administration of a variety of substances, such as vaccines and medications, has been implicated as a triggering cause of sarcomas in domestic cats (KASS et al., 2003). This type of tumor is believed to develop as a result of a not well regulated and exacerbated inflammatory response in cats. Inherited individual characteristics may also play a fundamental role in the development of such neoplasms (NAMBIAR et al., 2001). In addition, the development of sarcomas in cats, mainly fibrosarcomas, has been documented secondarily to foreign bodies, like microchips (DALY et al.,

2008), non-absorbable surgical suture (BURACCO et al., 2002) and surgical sponges (HADDAD et al., 2010).

Fibrosarcomas induced by foreign bodies also demonstrate features that are similar to FISS, which suggests that a common pathogenesis may be involved (DALY et al., 2008; BURACCO et al., 2002). The objective of this study was to describe the clinical history, gross, cytological, histological, histochemical, and immunohistochemical findings of a case of fibrosarcoma presumably arising after intramedullary pin and plate placement during osteosynthesis of femur in a cat.

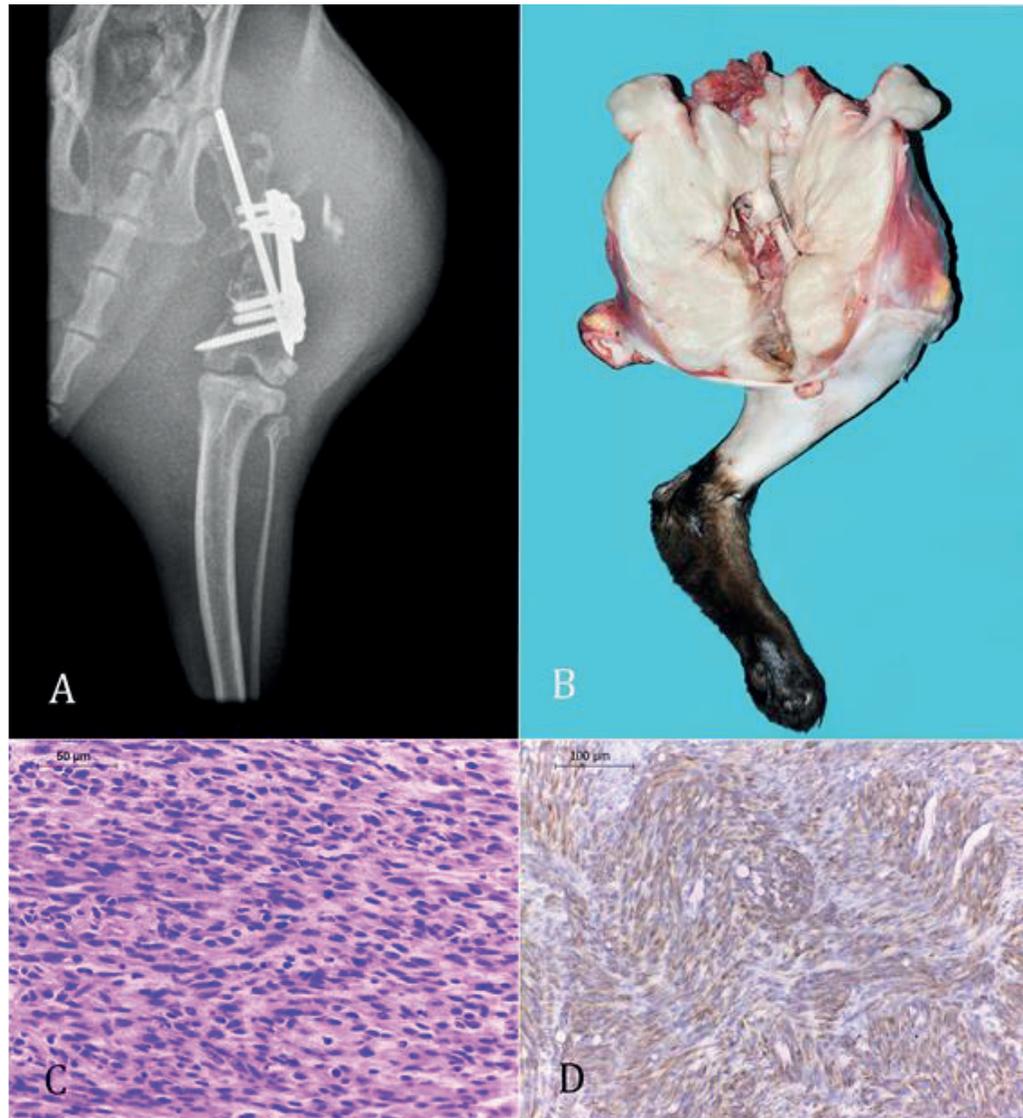
A 6-year-old spayed female Siamese cat was referred to the Veterinary Hospital of the Universidade Federal do Rio Grande do Sul, with a one-week history of severe acute left hindlimb lameness. According to the tutor, the animal was kept exclusively indoors. The cat had only received vaccines when it was around three months old, but the owner did not know the exact injection-site. Radiographic assessment of the affected limb revealed a comminuted complete diaphyseal fracture of left femur. There was no image consistent with neoplasia or bone alteration that could suggest a pathological fracture, such as diminished bone density. Blood analysis was performed and did not show any abnormality in complete blood count, or in the serum biochemistry values.

The cat was sent to surgery for osteosynthesis. An intramedullary pin was inserted in the femur and a plate was fixed to the lateral bone surface with six 2,4mm screws. During the surgical procedure, no indications of primary bone disease or neoplasia were observed. The cat returned to the veterinary hospital 13 days after the procedure for stitches removal presenting mild lameness; however, it was

able to bear weight in all limbs. Two months after the osteosynthesis procedure, the patient returned presenting a severely enlarged left pelvic limb. A mass of firm consistency on palpation, which extended from the stifle joint to the proximal region of the limb, was detected. Radiographic examination

of the area revealed soft tissue enlargement, loss of apposition, nonalignment between fracture fragments, proximal intramedullary pin migration, screw loosening, as well as absence of periosteal reaction/bone callus formation signs (Figure 1.A).

Figure 1. Fibrosarcoma in the pelvic limb of a cat. A- Radiographic examination two months after osteosynthesis, showing soft tissue enlargement, loss of apposition and alignment between fracture fragments, proximal intramedullary pin migration, screw loosening, and absence of periosteal reaction / bone callus formation signs. Craniocaudal projection. B- There is a whitish and firm mass, extending from the femoropatellar joint to the proximal portion of the limb, infiltrating and replacing the limb musculature. At the cut surface, the metal pin, the bridge plate, the metallic screws and bone spicules were observed in the center of the mass. C- Microscopic evaluation, mesenchymal cells displayed in bundles interspersed with abundant stroma. These cells present elongated nuclei and high mitotic count. Hematoxylin and eosin. Bar 50µm. D- Strong expression of vimentin by the neoplastic mesenchymal cells. Immunohistochemistry for vimentin. Bar, 100µm.



Fine needle biopsy aspirate was performed from several areas of the mass. At microscopic evaluation, the smears had low cellularity, and were represented by a small number of individualized, highly pleomorphic elongated cells, which showed marked anisocytosis and anisokaryosis, as well as some bizarre mitotic figures. An intense basophilic amorphous background with inflammatory cells, and cellular debris were also observed. Due to the cytological features, sarcoma was suspected; however, the small number of exfoliated cells and the presence of inflammation compromised the final diagnosis.

Incisional punch biopsy was then conducted. Histological evaluation revealed a fibrosarcoma, showing high malignancy and several characteristics of cellular atypia, such as variable cellular size and shape, pleomorphic nuclei with prominent nucleoli. There was also extensive areas of necrosis and lymphocytic inflammatory infiltrate. The limb was surgically removed by hemipelvectomy since the tumor was noted to be infiltrating into the left ischium. Excision of all macroscopic areas of the tumor and partial ostectomy of the ischium were performed, but it was not possible to obtain the desired adequate surgical margins.

The entire limb was referred for histopathological evaluation; permission for the usage of body parts of the referred animal for scientific purposes was obtained from the owner. Grossly, a white, firm, non-delimited, and non-encapsulated 16X12X8cm mass, extending from the femoropatellar joint to the proximal portion of the limb was observed infiltrating, and replacing the limb musculature. At the cut surface, the metal pin, the plate and the metallic screws were observed in the center of the mass (Figure 1.B). A large amount of bone spicules showing no macroscopic irregularities was also seen randomly scattered within the tumor; the bone marrow present in the osseous fragments did not show any macroscopic alterations.

Several tissue samples of the mass were collected and fixed in 10% neutral buffered formalin. Tissues

were processed routinely, embedded in paraffin wax, cut into 3-5 μ m sections and stained with Hematoxylin and Eosin (H&E) and Masson's trichrome. Histopathological evaluation revealed a non-demarcated, non-encapsulated neoplastic proliferation, located in the subcutaneous tissue and infiltrating in the musculature, composed by spindle shaped cells arranged in interwoven bundles. The cells presented scant cytoplasm, with indistinct cellular borders, elongated nuclei with finely stippled chromatin, and prominent nucleoli. There was marked anisocytosis and anisokaryosis, numerous karyomegalic cells, few multinucleated cells, and an average of three mitotic figures per high power field (x 40) (Figure 1.C). There was also extensive intratumoral necrosis, occupying about 50% of the neoplasm, as well as moderate inflammatory infiltrate of lymphocytes in the tumor periphery. Abundant collagen production was evidenced through Masson's trichrome stain. The histological pattern and morphology of the spindle cell population were consistent with fibrosarcoma. The limb was completely dissected; however due to the lack of normal architecture, lymph nodes were not found. The bone and bone marrow did not show any microscopic alterations.

Sections of the neoplasm were submitted to immunohistochemistry, using reagents specific for vimentin (1:200; Zymed, Carlsbad, California, USA), cytoqueratin (1:80; Dako, Santa Clara, California, USA), desmin (1:300; Zymed, Carlsbad, California, USA) and FeLV (1:500; Bio-Rad, Hercules, California, USA). Positive controls consisted of canine peripheral nerve sheath tumor (vimentin), canine skin (cytokeratin) and canine large intestine (desmin). For negative control, phosphate buffered saline was used to replace the primary antibodies in each IHC assay. For vimentin and cytokeratin, antigen retrieval was performed using Citrate buffer, pH 6.0, in a pressure cooker at 125°C for three minutes. Desmin antigen retrieval was conducted in a microwave oven, with citrate buffer, pH 6.0, in two cycles of five minutes each. Amplification was performed by using the

LSAB-Mach4 Universal kit (Dako) and labeling was possible using 3,3'-diaminobenzidine (DAB; Sigma, St. Louis, Missouri, USA). Sections were counterstained with Mayer's hematoxylin. The mesenchymal cells showed strong cytoplasmic positive expression of vimentin (Figure 1.D), and no labeling for cytokeratin. Weak positive labeling for desmin was observed, however the immunolabeling was restricted to the tumor periphery. There was no positive labeling for FeLV.

Postoperative treatment was recommended with radiotherapy and chemotherapy; however, the owner opted to conduct chemotherapy only. An intravenous application of doxorubicin at the dose of 1 mg/ kg was performed three weeks after the surgical procedure; however, tumor recurred in the surgical scar 20 days after chemotherapy. Due to the tumor recurrence and the poor prognosis, euthanasia was elected 53 days after the amputation surgery, and the owner did not authorize the necropsy.

Based on the clinical history, gross, cytological, histological, histochemical, and immunohistochemical findings, the diagnosis of fibrosarcoma was concluded. The main gross features of the tumor included its great size, marked invasion of adjacent tissues and the fact that the tumor surrounded and involved the intramedullary pin and the osteosynthesis plate. Microscopically, the tumor was composed of mesenchymal cells arranged in bundles, presenting severe cellular pleomorphism, high mitotic count and extensive areas of necrosis. Intense staining of collagen bands by Masson's trichrome, and strong positive vimentin immunostaining were detected.

The cat in this case was 6 years old, similarly to what has been documented by a previous FISS study (DODDY et al., 1996), in which animals that were diagnosed with such neoplasm were in the age group close to 8 years. These animals are considered younger than those affected by fibrosarcomas that do not originate at injection sites. Extremely rapid tumor growth and wide local invasiveness has been

frequently observed in cases of FISS (DODDY et al., 1996).

Cytological analysis is considered quite difficult to interpret in these cases, since fibrosarcomas usually exfoliate a low number of neoplastic cells. In addition, due to extensive tissue necrosis, inflammatory cells and debris frequently predominate in the smears, which may induce cellular dysplasia (HAUCK, 2003), leading to an inconclusive diagnosis, as the case reported.

For final diagnosis, histopathological evaluation is required (DODDY et al., 1996; HAUCK, 2003), and to confirm, its mesenchymal origin, the immunohistochemical technique for vimentin can be performed. Other markers can be used to exclude the possibility of other diagnosis, such as cytokeratin, that was used in the present case with this purpose. Weak labeling for desmin was observed in this case, but not in the tumor itself, only in its periphery. Such labelling was considered to represent cells of myofibroblast origin, which were surrounding the neoplasm. The histological features of FISS, mainly the one associated with a microchip implant (DALY et al., 2008), are very similar to the features of the present case, when considering intratumoral necrosis, cellular arrangement and presence of peritumoral mononuclear inflammatory infiltrate.

FISS pathogenesis is complex, multifactorial and not completely understood (DODDY et al., 1996). Current knowledge indicates that chronic inflammation along with uncontrolled fibroblastic proliferation may play a crucial role in the development of sarcomas; however, intrinsic features of the feline species are likely involved in the process (DODDY et al., 1996). It is believed that cells with neoplastic predisposition may be present in the tissue before external physical or chemical aggression, such as the introduction of a foreign body or substance in the organism (KIRKPATRICK et al., 2000; MOIZHESS, 2008). In cases of fracture-associated sarcoma, metal implants such as

intramedullary pin and plate, and their byproducts, may act as foreign bodies, playing an important role in carcinogenesis (KIRKPATRICK et al., 2000).

Similarly, chronic inflammation caused by parasitic infection has been associated as a cause of tumor development in several animal species, including canids, rodents, and humans (RANEN et al., 2004). *Spirocerca lupi* infection has been implicated with the development of esophageal fibrosarcomas and osteosarcomas in dogs (RANEN et al., 2004), which corroborates to the prolonged inflammation leading to tumorigenesis hypothesis. In such cases, it is believed that the parasites may act as foreign bodies, as the pin and plate in the case described. The implantation of a foreign body into tissues results in an inflammatory reaction, which can lead to emergence of a sarcoma in its vicinity (MOIZHESS, 2008).

The post-traumatic ocular sarcoma is also a well-known entity described in cats, which occurs after a traumatic event causing ocular injury, and can take place with chronic inflammation (DUBIELZIG et al., 1990). As in FISS cases, the cats that present these sarcomas generally have low survival time due to tumor infiltration and its sequels, such as blindness.

In the present case, the association between the intramedullary pin and plate implantation and the fibrosarcoma development was presumed. This is corroborated by the fact that the tumor arose few weeks after the orthopedic procedure, grew exponentially and obliterated normal limb morphology in about two months. Vaccine-site induced sarcoma could not be completely ruled out since the cat was immunized in a young age in an unknown place, and FISS are known to present an extremely variable latency period (DODDY et al., 1996).

As described in this case, as well as in cases of injection site sarcomas, ocular post-traumatic sarcoma and feline sarcomas associated with

foreign bodies, it seems that there is a common pathogenesis involving inflammation and wound healing in the development of these tumors (DUBIELZIG et al., 1990; VASCELLARI et al., 2006). Finally, the present report describes a unique case of fibrosarcoma presumably arising after intramedullary pin and plate placement during osteosynthesis of femur in a cat.

Conflict of Interest

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

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