UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE VETERINÁRIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS

ASPECTOS HISTOPATOLÓGICOS E IMUNO-HISTOQUÍMICOS DOS SARCOMAS DE APLICAÇÃO EM FELINOS DOMÉSTICOS

BIANCA SANTANA DE CECCO

PORTO ALEGRE
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Dissertação apresentada como requisito para a obtenção do grau de Mestre em Ciências Veterinárias na área de concentração em Patologia Animal e Patologia Clínica, da Universidade Federal do Rio Grande do Sul.

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22 de Fevereiro de 2018.

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CIP - Catalogação na Publicação

de Cecco, Bianca Santana
Aspectos histológicos e imuno-histoquímicos dos sarcomas de aplicação em felinos domésticos / Bianca Santana de Cecco. -- 2018.
47 f.
Orientadora: Luciana Sonne.

Coorientadora: Fernanda Vieira Amorim da Costa.

Dissertação (Mestrado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Veterinária, Programa de Pós-Graduação em Ciências Veterinárias, Porto Alegre, BR-RS, 2018.

1. Patologia Veterinária. 2. Felinos. 3. Sarcomas de Aplicação. 4. Oncologia. 5. Vacinal. I. Sonne, Luciana, orient. II. da Costa, Fernanda Vieira Amorim, coorient. III. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

RESUMO

O sarcoma de aplicação felino (SAF) é uma neoplasia mesenquimal que se origina de fibroblastos e miofibroblastos. É caracterizado pela alta capacidade invasiva que mesmo após exérese cirúrgica radical a taxa de recidiva pode chegar aos 80%. Esse trabalho foi divido em dois artigos. No primeiro, o objetivo foi contribuir para a caracterização histológica, histoquímica e imuno-histoquímica (IHQ) dos SAF no intuito de auxiliar o diagnóstico. Foram avaliadas 91 amostras de pele submetidas a exame histológico, subsequente coloração de tricrômico de Masson e IHQ para vimentina e Ki-67. A localização anatômica mais predominante foi em tórax, seguida por flanco, região interescapular e membros. O fibrossarcoma foi diagnosticado em 90% dos casos. A contagem mitótica foi avaliada e comparada com a marcação de Ki-67. Ambos índices se apresentaram baixos. No segundo artigo, descreve-se um caso de sarcoma associado a colocação de pino intramedular em um felino com fratura de fêmur. Dois meses após a cirurgia, retornou com fibrossarcoma envolvendo todas estruturas do membro. Os sarcomas de felinos também podem ser induzidos por agentes e materiais, como por exemplo por colocação de pino-intramedular e placas após fratura de fêmur. Este fibrossarcoma induzido apresenta um comportamento biológico muito semelhante aos SAF de origem vacinal, demonstrando extensas áreas de necrose e pleomorfismo celular acentuado, além de rápido crescimento. Observou-se forte marcação para tricrômico de Masson e na realização de IHQ apresentou forte imunomarcação para vimentina.

Palavras-chave: gatos, vacinal, Ki-67, mesenquimal, oncologia.

ABSTRACT

Feline injection-site sarcoma (FISS) is a mesenchymal neoplasm that originates from fibroblasts and myofibroblasts. It is characterized by the high invasive capacity that even after radical surgical excision the relapse rate can reach 80%. This work was divided into two articles. In the first, the objective was to contribute to the histological, histochemical and immunohistochemical (IHC) characterization of FISS in order to aid the diagnosis. We evaluated 91 skin samples submitted to histological examination, subsequent masson trichrome staining and IHC for vimentin and Ki-67. The most predominant anatomical location was in the thorax, followed by the flank, interscapular region and limbs. Fibrosarcoma was diagnosed in 90% of cases. The mitotic count was evaluated and compared with Ki-67 labeling. Both indices were low. In the second article, we describe a case of sarcoma associated with intramedullary pin placement in a feline with a femoral fracture. Two months after surgery, he returned with fibrosarcoma involving all limb structures. Feline sarcomas can also be induced by agents and materials, such as by intramedullary pin and plate after femoral fracture. This induced fibrosarcoma presents a biological behavior very similar to the FISS of vaccine origin, showing extensive areas of necrosis and marked cellular pleomorphism, besides fast growth. Strong labeling was observed for Masson's trichrome and in the IHC assay it showed strong immunostaining for vimentin.

Keywords: *cats, vaccinal, Ki-67, mesenchymal, oncology.*

SUMÁRIO

1. INTRODUÇÃO

Os sarcomas de aplicação felino (SAF) são neoplasias de origem mesenquimal que apresentam comportamento biológico agressivo e alta taxa de invasão tecidual (DODDY *et al*., 1996). Mesmo após décadas de pesquisa e muito investimento, técnicas precisas que avaliam prognóstico e tratamentos curativos ainda são desconhecidos, tornando estes sarcomas em desafios aos médicos veterinários (KIRPENSTEIJN, 2006). Apesar de serem realizadas cirurgias radicais somadas a tratamentos adjuvantes como quimioterapia e radioterapia, as taxas de recidivas são altas e os custos para esses tratamentos são muito elevados (MORRISON; STARR, 2001).

A denominação sarcoma vacinal já foi substituída pela nomenclatura de sarcoma de aplicação pela possibilidade de outros químicos além das vacinas, como medicamentos, e alguns materiais já terem sido descritos como causas de origem de desenvolvimento tumoral (ESPLIN; McGILL, 1999; BURACCO *et al*., 2002; DALY *et al*., 2008). Neste estudo, utilizaremos a terminologia sarcoma de aplicação felino (SAF).

O estudo do comportamento biológico dos SAF e a pesquisa por fatores prognósticos são essenciais para a melhoria na escolha e no desenvolvimento de tratamentos mais eficazes (KIRPENSTEIJN, 2006; CARNEIRO *et al.*, 2008).

Este trabalho tem como objetivo auxiliar na caracterização histológica, histoquímica e imuno-histoquímica dos sarcomas de aplicação felinos. Além disso, também há o relato de caso de um fibrossarcoma originado próximo da colocação de pino intramedular e placa de metal para osteossíntese em um felino após fratura cominutiva de fêmur.

2. REVISÃO BIBLIOGRÁFICA

2.1 HISTÓRICO E EPIDEMIOLOGIA

O SAF foi descrito primeiramente por Hendricks e Goldschimidt em outubro de 1991, após o aumento do aparecimento de tumores no subcutâneo de felinos em local de aplicação de vacinas. Esses autores relacionaram o crescente aumento do número de casos dessa neoplasia com a obrigatoriedade da vacinação antirrábica, a qual é produzida com adjuvante a base de alumínio.

As vacinas da raiva e da FeLV foram as mais implicadas como indutoras de SAF (HENDRICK *et al*., 1994; KASS *et al*., 1993). Entretanto, já foi demonstrado que tanto o tipo da vacina quanto a marca não interferem no desenvolvimento de neoplasias (KASS *et al*., 2003; WILCOCK *et al.*, 2012). Essas vacinações eram realizadas geralmente no tecido subcutâneo da região dorsal interescapular, aumentando os casos de neoplasias nessas regiões (DODDY *et al.*, 1996).

A partir desta observação, foi criada a *Vaccine Associated Feline Sarcoma Task Force* (VAFSTF), em novembro de 1996, com o objetivo de identificar métodos de prevenção, diagnóstico e possibilidades de tratamento (STARR, 1998). Essa entidade que já foi desmembrada, e funcionava como um guia para os médicos veterinários para a padronização de localização adequada para a realização da vacinação, sugerindo que essa seja realizada em região distal de membros, para facilitar assim a exérese cirúrgica ampla, no caso amputação (VAFSTF, 2005).

As recomendações da VAFSTF eram que a vacinação antirrábica fosse realizada no membro pélvico esquerdo, o mais distal possível; e a vacina polivalente fosse feita abaixo do cotovelo direito, também o mais distal possível (MORRISON; STARR, 2001). Um estudo realizado por SHAW *et al*. (2009), demonstrou que houve diminuição no número de SAF na região interescapular desde que começou a disseminação da informação da VAFSTF, e aumento da ocorrência dessas neoplasias em membros. Entretanto também houve aumento destes tumores na região abdominal lateral, o qual poderia ser explicado pela tentativa de vacinação do membro pélvico, porém atingindo o subcutâneo do abdômen (SHAW *et al*., 2009).

No ano de 2003, ainda eram observados mais SAFs em região torácica do que em região de membros, e isso ocorreu devido a demora de disseminação da informação e das recomendações da força tarefa, porém também deve ser levada em consideração a

questão do período de latência do tumor, ou seja, o intervalo de tempo entre a aplicação até o desenvolvimento tumoral (SHAW *et al*., 2009).

Os estudos epidemiológicos relatam uma incidência de sarcoma de aplicação em 0,63 a 3 sarcomas para cada 10.000 felinos vacinados (GOBAR & KASS; 2002). De acordo com NAMBIAR *et al*. (2001), a baixa prevalência de felinos afetados em relação a todos felinos vacinados sugere que o desenvolvimento do neoplasma seja uma característica inerente a cada animal.

A realização de frequentes vacinações no mesmo local anatômico aumenta as chances de desenvolvimento de tumores, sendo em torno de 127% maior com duas aplicações e 175% com três ou mais aplicações (KASS *et al*., 1993).

A média de idade dos felinos afetados pelo SAF geralmente é menor do que a dos felinos afetados por sarcomas não relacionados à aplicação, de 8-9 anos e de 10-12 respectivamente (DODDY *et al*., 1996; KASS *et al*., 1993).

2.2 PATOGENIA

A patogenia dos SAF ainda é obscura. Porém, a hipótese mais aceita atualmente sugere que os fatores de crescimento não apenas promovem proliferação celular, mas também induzem à transformação neoplásica e a regulação da angiogênese (O'BYRNE; DALGLEISH, 2001). Devido à intensa relação entre sistema imune e transformação tumoral, sugere-se que a inflamação crônica associada ao estresse oxidativo causado pela aplicação de substâncias exógenas, além dos fatores inerentes de cada felino, levem a transformação maligna de fibroblastos e miofibroblastos (MACY *et al*., 1996 O'BYRNE; DALGLEISH, 2001). Muitas pesquisas relacionam a inflamação crônica como uma grande contribuidora da carcinogênese através de diversos mecanismos (COUSSENS; WERB, 2002).

Novos casos demonstraram que a aplicação medicamentosa (KASS *et al*., 2003), implantação de microchips (DALY *et al*., 2008), sutura não absorvível (BURACCO *et al*., 2002), colocação de cateter para fluidoterapia (McLELAND *et al*., 2013) e lufenuron (ESPLIN; McGILL, 1999) também pode desencadear transformação neoplásica em fibroblastos imaturos e ocasionar sarcomas. Em relação às vacinas, KASS *et al*. (2003) observou que o único fator que aumentava o risco de desenvolvimento de SAF era a temperatura da vacina no momento da aplicação, em que houve correlação positiva entre a menor temperatura e o maior risco.

Vacinas com adjuvantes de alumínio foram comprovadamente associadas à gênese neoplásica (ESPLIN; McGILL, 1999). De acordo com ABDELMAGEED *et al.* (2017), a exposição ao alumínio pode tanto iniciar quanto promover o desenvolvimento neoplásico na ausência de uma resposta inflamatória.

Há grande especulação envolvendo a susceptibilidade genética dos felinos que desenvolvem os SAF, e muitos autores definem a patogenia como uma associação de fatores que se iniciam com características inerentes do felino e o trauma (HAUCK, 2003; KASS *et al*., 1993). A inflamação crônica ocasiona a transformação neoplásica por parte dos fibroblastos e miofibroblastos envolvidos no processo de reparação tecidual (ESPLIN; McGILL, 1999; KASS *et al*., 1993).

No passado, alguns autores sugeriram a oncogene viral como uma possível facilitadora da ocorrência de SAF. Porém, de acordo com KIDNEY *et al.* (2000), que analisaram 130 amostras de SAF através de técnicas moleculares e imuno-histoquímicas em busca do vírus da leucemia felina (FeLV), não foram observados casos positivos. Também foram realizados testes em relação a outros agentes comuns em felinos como o herpesvírus, poliomavírus e papilomavírus, que também não resultaram em casos positivos, diminuindo a possibilidade do envolvimento viral na patogênese dos SAF (KIDNEY *et al*., 2000; 2001a; 2001b).

Mais recentemente, observou-se que os SAF demonstram uma superexpressão proteica bem como um aumento na expressão e mutação do *gene p53*, além de diversos fatores de crescimento e metaloproteinases (NIETO *et al*, 2003). Entre esses fatores de crescimento, podem ser incluídos a expressão anormal do fator de crescimento plaquetário (PDGF) e fator de crescimento fibroblástico básico (FGF-b) (KIRPENSTEIJN, 2006).

2.3 DIAGNÓSTICO

Para a realização de um diagnóstico definito do sarcoma de aplicação é necessária a realização do exame histológico, pois mesmo que seja realizado o exame citológico, este pode não ser conclusivo devido a abundante quantidade de componente inflamatório presente na amostra, além de extensas áreas de necrose e áreas císticas (HAUCK, 2003). No caso de citologias com observação de células neoplásicas, o diagnóstico deve ser sugestivo de sarcoma (MAULDIN, 1997).

A possibilidade de se tratar de um SAF deve ser considerada a partir do esquema 3-2-1, isto é, quando a massa permanecer após três meses de vacinação, se apresentar aumento de volume maior que 2cm de diâmetro e/ou está apresentando aumento de volume em um mês após a vacinação (HARTMANN *et al*., 2015). A suspeita deve ser confirmada através da combinação do local anatômica do neoplasma, diagnóstico histológico e histórico clínico do felino (SÉGUIN, 2002).

2.3.1 ACHADOS MACROSCÓPICOS

O SAF é caracterizado clinicamente pelo aparecimento de uma massa solitária geralmente localizada no subcutâneo, que ao corte apresenta-se firme, muitas vezes aderida aos planos mais profundos, em local onde houve aplicação prévia de fármacos ou vacinas (OGILVIE; MOORE, 2002; HARTAMANN *et al*., 2015). A maioria dessas massas se apresentam como não dolorosas e geralmente císticas, o que pode confundir o clínico por serem muitas vezes semelhantes a granulomas de aplicação (MORRISON; STARR, 2001; KIRPENSTEIJN, 2006;). Em comparação com sarcomas não originados em local de aplicação, os SAF são maiores, mais agressivos e com maior taxa de recidiva (HENDRICK *et al*., 1994).

Quando se trata da localização anatômica, esses tumores são mais frequentes na região torácica dorsal, principalmente entre as escápulas, seguidas pela região de flanco, membros e região lombar (KASS *et al*., 2003).

Mesmo que haja associação de cirurgias radicais e tratamentos adjuvantes, as taxas de recidivas são de até 80% no intervalo de quatro meses (OGILVIE; MOORE, 2002; SÉGUIN, 2002). Metástases podem ocorrer em até 22% dos casos e são, geralmente, localizados primariamente em pulmões, linfonodos regionais, mediastino, pericárdio, assim como em outros sítios como fígado, pâncreas, intestino, baço e olhos (CRONIN *et al.*, 1998; SÉGUIN, 2002; VAFSTF, 2005).

2.3.2 ACHADOS HISTOLÓGICOS

Na histologia esses neoplasmas são de origem mesenquimal, e são, em sua maioria, fibrossarcomas. Outras variantes também são observadas como o fibro-histiocitoma, rabdomiossarcoma, mixossarcoma, condrossarcoma e osteossarcoma (COUTO *et al*., 2002; HENDRICK *et al*., 1994). O SAF tem características de alta malignidade como a acentuada atipia celular, elevada taxa mitótica e invasão tecidual

periférica (COUTO *et al*., 2002; GIUDICE *et al.*, 2010). Independente do tipo histológico destes tumores, eles são caracterizados por centro necrótico, infiltrado linfocítico peritumoral e um comportamento biológico agressivo e infiltrativo (COUTO *et al*., 2002; DODDY *et al*, 1996; SÉGUIN, 2002).

Em medicina humana, o sistema de graduação histopatológico é de enorme auxílio para o estabelecimento do prognóstico, porém em medicina veterinária a credibilidade do grau histológico é questionada (GIUDICE *et al*., 2010).

A localização histológica mais comum é o tecido subcutâneo, por vezes se estende à musculatura, em contrapartida com os sarcomas não relacionados à aplicação, que se apresentam mais comumente na derme (DODDY *et al*., 1996; SÉGUIN, 2002). Os SAF apresentam alta invasão de tecidos periféricos, com a formação de projeções digitiformes (SÉGUIN, 2002), dificultando a excisão cirúrgica.

COUTO *et al*. (2002) descreveu o SAF como um neoplasma com características histológicas únicas, quando comparado com sarcomas de origem não relacionados à aplicação, tanto pelo infiltrado linfoide peritumoral predominantemente do tipo T, assim como pela abundante vascularização periférica, células miofibroblásticas originárias do tecido de reparação e células gigantes multinucleadas.

Muitas vezes os SAF possuem em região peritumoral macrófagos com citoplasma espumoso, por vezes com grânulos azulados a acinzentados que através de análise com auxílio de microscopia eletrônica e análise radiográfica realizada nas massas tumorais, foi possível identificar como alumínio proveniente do adjuvante vacinal e oxigênio (MADEWELL *et al*., 2001).

2.3.3 CARACTERIZAÇÃO HISTOQUÍMICA E IMUNO-HISTOQUÍMICA

Por vezes é possível se utilizar de exames complementares para a definição da origem celular, do comportamento biológico e de auxílio ao prognóstico. Em questão desses exames complementares podemos citar a histoquímica e a imuno-histoquímica (COUTO *et al*., 2002).

Quando levado em consideração a produção de colágeno, a técnica histoquímica de Tricrômico de Masson se torna útil, pois auxilia na diferenciação de tumores produtores de colágeno daqueles de origem muscular (EHRHART *et al*., 2013). Os fibrossarcomas mais diferenciados possuem uma excelente marcação para esta coloração. Porém, quanto mais anaplásica a célula mais provável que ela produza menos matriz (EHRHART *et al*., 2013).

Como os SAF possuem como característica o infiltrado mononuclear peritumoral, a utilização de técnicas para determinar o tipo linfocítico se torna interessante para o estudo do papel da inflamação na oncogênese tumoral. Como já descrito por COUTO *et al*. (2002), a marcação para CD3 tende a ser mais dominante pela maior quantidade de linfócitos T nos sarcomas por aplicação.

O papel do anticorpo anti-vimentina se dá pela pesquisa da origem celular da neoplasia. A vimentina é um marcador de células mesenquimais, um marcador geral para sarcomas, auxiliando na exclusão de neoplasias que não façam parte desse grupo (WERNER *et al*., 2005).

O antígeno Ki-67 é uma proteína nuclear expressada desde o início da fase G1 até o final do ciclo celular, e sua imunomarcação se relaciona com a atividade proliferativa tumoral (SCHOLZEN; GERDES, 2000). O índice Ki-67 pode ser relacionado diretamente com o tamanho tumoral, invasão vascular e necrose, porém, CHOONG *et al*. (1994) não demonstrou interferência do tipo histológico do sarcoma com a número de células marcadas positivamente para Ki-67.

2.4 TRATAMENTO

Alguns estudos já comprovaram que a excisão ampla do tumor com margens cirúrgicas limpas parece aumentar o intervalo de tempo livre da doença (HERSHEY *et al*., 2000). Entretando, devido ao comportamento infiltrativo do SAF, nem sempre a excisão cirúrgica com margem ampla é possível, com isso utilizam-se terapias adjuvantes, nas quais os resultados mais promissores provêm da utilização de terapia multimodal, a qual é caracterizada por diversas modalidades de radioterapia, quimioterapia e/ou imunoterapia (KIRPENSTEIJN, 2006).

Segundo OGILVIE; MOORE (2002), a excisão cirúrgica deve ser agressiva, possuir margens mínimas de 3cm, com retirada de qualquer estrutura que entre em contato com a neoplasia, incluindo músculos e ossos.

Quando o tumor se localiza na região interescapular, é necessário que, além da incisão ampla, os processos espinhosos vertebrais dorsais também sejam retirados, bem como seja feita escapulectomia parcial para aumentar o intervalo livre da doença. Porém, a realização de tal cirurgia radical aumenta as chances de deiscência de pontos, infecções secundárias e a mortalidade (WITHROW *et al.*, 2007).

2.5 PROGNÓSTICO

Os fatores de prognósticos dos SAF ainda não estão padronizados, porém algumas características do tumor podem auxiliar a formação de um prognóstico, como exemplo o tamanho do tumor. Tumores que medem menos de 2cm de diâmetro apresentam prognóstico mais favorável que tumores maiores. A mobilidade tumoral também deve ser avaliada para estabelecimento da invasão tecidual adjacente. (DILLON *et al*., 2005). O prognóstico para um animal com SAF recorrente ou inoperável é de reservado a desfavorável (KIRPENSTEIJN, 2006).

Observou-se significativamente que felinos com excisão cirúrgica com margens livres da neoplasia tiveram maior tempo de sobrevida. Com isso, é possível afirmar que animais que desenvolveram o SAF em local de fácil retirada, como membro, possuem maior chances de obterem margens limpas (HERSHEY *et al.*, 2000).

2.6 MEDIDAS PREVENTIVAS

A VAFSTF sugere que a aplicação de qualquer substância com probabilidade de causar sarcoma seja realizada na região distal de membros, pela maior facilidade de retirada cirúrgica completa, mesmo que a taxa de recidiva nestes locais seja considerável, pois a probabilidade de controle da doença a longo prazo seguindo a amputação é alta (STARR, 1998; VAFSTF, 2005). HENDRICKS *et al.* (2013) concluiu que a vacinação e aplicação medicamentosa em terço distal da cauda é uma localização bem aceita pelos felinos e aplicável pelos médicos veterinários, o que tornou essa região uma alternativa, pois se o felino desenvolver sarcoma, a região é de fácil amputação.

Segundo KIRPENSTEIJN (2006), nos felinos que forem vacinados, deve ser realizado um arquivo detalhado sobre local da aplicação, data, tipo de vacina e dose. Após a aplicação, qualquer nódulo ou sinal de reação inflamatória deve ser monitorado atentamente. Felinos que apresentarem granuloma vacinal por mais de quatro meses ou nódulo com características de malignidade, sugere-se que realizem cirurgia radical para retirada do tumor (GOBAR; KASS, 2002). A VAFSTF recomenda que sejam realizadas reavaliações trimestrais, durante o mínimo de um ano, após a remoção cirúrgica de um SAF (MORRISON; STARR, 2001).

ARTIGOS

Neste item são apresentados os artigos:

Artigo 1: "**Aspectos histopatológicos e imuno-histoquímicos relacionados aos sarcomas de aplicação em felinos domésticos**" a ser submetido ao periódico Ciência Rural.

Artigo 2: "**Fibrosarcoma induced by pin and plate placement"** a ser submetido a um periódico ainda não definido.

Caracterização anatomopatológica e imuno-histoquímica dos sarcomas de aplicação em gatos no Rio Grande do Sul

Anatomopathological and immunohistochemical characterization of sarcomas of application in cats in Rio Grande do Sul

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ABSTRACT

Feline application sarcomas (FISS) are neoplasms of mesenchymal cells, with marked invasion of peripheral tissues and high malignancy characteristics. They originate in places where there has been previous application of substances or materials in cats. The objective of this study was to contribute to the study of histological and immunohistochemical evaluation of FISS. Ninety-one samples of cutaneous biopsies that were diagnosed with FISS were selected. These samples were reevaluated in histology, then Masson's trichrome histochemistry and immunohistochemistry (IHC) were performed for vimentin and Ki-67. Histological diagnoses in descending order of occurrence were fibrosarcoma (89%), malignant fibrous histiocytoma (8%), myxosarcoma (1%) and chondrosarcoma (1%). The mitotic count of the neoplasms was low, with 83.8% of the tumors with up to 9 mitoses / 10 fields. In IHC for vimentin of well differentiated tumors, light (30%), moderate (50%) and marked (20%) cell markers were observed. The Ki-67 cell proliferation index was also considered low in the more anaplastic tumor with a mean of 2.38%, being the mean of the control with 5,80%.

Key words: cats, tumor, vaccine, fibrosarcoma.

INTRODUCTION

Feline injection-site sarcoma (FISS) is a mesenchymal neoplasm, particularly aggressive that courses with high recurrence caused by its capacity to invade adjacent tissues (DODDY et al, 1996; HENDRICK, 2017). These sarcomas have their origin in the subcutaneous tissue in regions where there was previous vaccination, drug application or surgical material implantation (DALY et al, 2008). FISS can affect cats only three years old, however its median age is 8,1 years, which characterizes the affected cats as younger than those with non-application related sarcomas (DODDY et al, 1996; HENDRICK, 2017).

The most accepted pathogenesis theory is that growth factors not only promote cellular proliferation, but also induce neoplastic transformation and angiogenesis regulation, added to unique characteristics from each cat (O'BYRNE & DALGLEISH, 2001). This study objective was to contribute to clinical parameters, histological and immunohistochemical characterization of FISS diagnosticated in the metropolitan region of Rio Grande do Sul.

MATERIALS AND METHODS

Ninety-one biopsies were selected with the clinical and histopathological diagnosis of feline sarcoma, compatible with FISS and its histological variants, received in the period of 2007 and 2017 by the veterinary pathology department of Universidade Federal do Rio Grande do Sul. Information related to breed, age, sex, anatomical localization, tumor size and peripheral tissue adhesion were obtained through clinical data and gross morphology of the samples.

The neoplasms compatible with FISS were selected based on anatomical location used for vaccine and drug application, histological characteristics and patient history. Fibrosarcomas in non-injection sites, such as face and foreskin, were used as control samples. For anatomical classification, the interscapular and thoracic regions, flank and members were

considered. In relation to the size, the tumors were standardized in tumors smaller than 2cm (T1), tumors between 2 and 5cm (T2), and greater than 5cm (T3).

The paraffin-embedded tissue samples were cut into sections of 3 μm thick, and routinely stained with Hematoxylin and Eosin (HE) for histological reassessment. During the evaluation the tumor differentiation was observed, the samples were characterized as well differentiated, moderately differentiated and anaplastic. Relative to the histological location of the neoplasm, the samples were standardized in subcutaneous, muscular and/or dermis. The presence of lymphocytes in the periphery of the neoplasm was graded according to its intensity in discrete, moderate and accentuated. Similar graduation was performed for multinucleated giant cells and macrophages.

Posterior to histological analysis, 30 neoplasms (10 of each differentiation degree) and 10 control neoplasms not related to FISS were randomly submitted to histochemical technique of Masson's trichrome and immunohistochemical (IHC) staining for vimentin and Ki-67. Five FISS samples with accentuated lymphocytes infiltrate in the tumor periphery were selected to IHC to differentiate between lymphocytes type B (CD79α) and T (CD3).

The peroxidase-labeled universal polymer method (MACH 4, Universal HRP-Polymer, Biocare Medical) was used for the four tests and the sections were stained with Harris haematoxylin, positive and negative controls were used for each antibody. The immunohistochemical antibodies and protocols applied are detailed in Table 1.

Table 1 – Antibodies and immunohistochemical protocols applied in sarcoma samples of application in domestic felines.

Antibodies	Clone	Recovery	Chromogen
Vimentin (Zimed)	V9	Citrate buffer pH6,0 125ºC 3 min.	DAB*
CD79αcy (Dako®)	HM57	EDTA buffer pH 9,0 96ºC 20 min.	AEC**
CD3 (Dako®)	policlonal	Protease XIV 15 minutes	AEC**
Ki-67 (Dako®)	MIB-1	Citrate buffer pH 6,0 125ºC 7 min.	AEC**

*diamino benzidina

**3-amino-9-etil- carbazol

For both the Masson's trichrome, vimentin, CD3 and CD79α the slides were evaluated and classified according to the intensity of labeling: group I weak immunostaining (less than 30% of labeled cells), group II moderate (30-75% labeled cells) and group III marked (more than 75% labeled cells).

The Ki-67 cell proliferation index was determined by counting a mean of 1000 cells in 40X objective, where the percentage of labeled nuclei was determined. The core count was performed manually using Image J® software with the CellCounter tool.

RESULTS

The age of affected felines varied from two to 16 years (mean of 9.8 years). As for sex, 61.5% were females and 38.5% males. Of the 84 cats from which it was possible to obtain information about the breed, 86.8% were non-breed, 4.4% were Siamese and 1 were Persian (1.1%).

The anatomical location of the neoplasms corresponded to 34% in the thoracic region (31/91), followed by 29.7% in the flank (27/91), 18.6% in the interscapular region (17/91) and 17.7% in the members (16/91). Information regarding the size of the neoplasms at the time of clinical care was obtained in 61 cases. Of these 10% were T1, 24.5% belonged to the T2 group and 65.5% to T3. In 54 (59,4%) cases there was invasion of peripheral tissues, including muscle and sometimes dermis, while in the other 37 cases (40,6%), the neoplasm was restricted to the subcutaneous.

The samples diagnosed as FISS were histological mostly compatible with fibrosarcoma, in a total of 89,2% (81/91), follow by malignant fibrous histiocytoma with 8,8% (8/91), and myxosarcoma and chondrosarcoma both with 1% (1/91) (Figure 1). All neoplasms showed necrosis areas, classified as discrete (29,6%), moderate (37,4%) and

accentuated (33%). The mitotic count was evaluated, and the simples were classified according to its intensity in grade I (83,5%), II (11%) e III (5,5%).

Lymphocytes in peripheral region of the tumor were observed in all cases of FISS and were consistent with discrete amount (40,6%), moderate and accentuated (29,7%). Macrophages were observed in all samples analyzed, which were also classified as discrete infiltrate (90,1%), moderate (8,8%) and accentuated (1,1%). In four neoplasms was possible to observe an amphiphilic material in the macrophages cytoplasm. Multinucleated giant cells were seen in 35 of 91 FISS evaluated, and were classified in discrete amount (60%), moderate (17,2%) and accentuated (22,8%).

In the histochemical analysis was possible to graduate Masson's trichrome intensity in discrete equivalent to 20% of the samples, 36,6% were moderate and 43,4% accentuated. Vimentins labeling was discrete in 26,6% of samples, 33,4% was moderate and 40% was accentuated. In the five samples analyzed for T lymphocytes (CD3) moderate labeling was observed (1/5) and accentuated (4/5). Regards to CD79α, there was discrete labeling (3/5) and accentuated (2/5). The immunohistochemical labeling for Ki-67 showed that the median labeling was of 3%, in contrast to the control samples that remained in 5,8%.

DISCUSSION

The mean age of the felines included in this study was 9.8 years of age, which presents somewhat older animals as described by DODDY et al. (1996), which reports the average age of felines affected by FISS as close to 8.1 years. However, it is consistent with the retrospective study by SHAW et al. (2009), which showed an increase in the age of cats diagnosed with FISS from the first reports of the late 1990s to the year 2006, when the animals also had an average of 9.8 years, however there is no conclusive explanation for this age increase. There is no sex or breed predisposition. Mixed breed cats are the most common felines attended in the clinical routine in teaching hospitals.

Most FISS were in the thoracic region, flank and interscapular, all localities that make it difficult to perform radical surgery, which is the recommended treatment in the case of FISS. In any of these places, surgery involves not only removal of the tumor with three to five centimeters wide margins in each direction, but also is added partial or total removal of other adjacent structures such as musculature and bones (HARTMANN et al., 2015). When it comes to the interscapular region the situation worsens due to extensive excision, and removal of ventral spines and scapulectomy processes (WITHROW et al., 2007).

It is also observed that only 17.7% of the samples from this study were removed from the limb region, which demonstrates that the recommendations made by the Vaccine Associated Feline Sarcoma Task Force (VAFSTF), a task force created in 1996 with the intention of identifying methods of prevention and treatment, besides standardizing the application of vaccines to be performed in the most distal parts of the limbs in preference to the thoracic and interscapular region, is still not widely disseminated by feline clinicians in the Metropolitan Region of Porto Alegre. Besides lack of information, it must be considered that not only vaccines, but also drugs and clinical and surgical materials also can induce sarcoma formation (BURACCO et al., 2002; DALY, 2008). The application in the distal third of the tail has been increasingly widespread by feline veterinarians, because in the case of sarcoma development, radical curative surgery is more feasible and easy to perform, as well as the visualization of the neoplasm by the owner of the cat (HENDRICKS et al., 2013).

Most of the FISS analyzed in this study (90%) were classified in the groups related to size T2 and T3, which corresponds to that proposed by KASS et al. (1993), where FISS present themselves as large masses, generally larger than those not related to injection-site sarcomas. Variation in tumor size depends significantly on the time between visualization of tumor and surgical removal of it. The anatomical site of FISS, when in an interscapular region that normally has excess skin and subcutaneous fat, makes it difficult for the owner to observe

the tumor, which makes the distal region of the tail and local limbs more propitious for brief visualization, since any increase volume is easy to perceive.

In addition, fibrosarcoma was the most prevalent histological type described in this study, as already cited by other authors (DODDY et al., 1996; HENDRICK et al., 2002). Characterized as neoplasm of spindle-shaped cells arranged in interwoven and supported by collagen bundles (HENDRICK, 2017). Although FISSs are generally classified as well differentiated tumors according to histological pattern, they tend to present higher cell pleomorphism, with cells showing high variability in size and shape (HENDRICK, 2017).

Other histological types may also be included in FISS, such as malignant fibrous histiocytoma, myxosarcoma, and chondrosarcoma (DODDY et al., 1996; HENDRICK, 2017). COUTO et al. (2002) suggests that sarcomas that fit the clinical and histopathological features of FISS should be diagnosed only as an injection-site sarcoma, due to the large pleomorphism of this neoplasm, to alert clinical veterinarians about the possibility of neoplasm being more aggressive and infiltrative than expected. Obtaining a detailed clinical history of the feline plus the histopathological characteristics allow the definitive diagnosis of FISS.

The most common histological site is the subcutaneous region as already discussed by DODDY et al. (1996), because the applications are generally performed in this region. This histological localization also helps in the differentiation between sarcomas after application of non-induced sarcomas, which mostly originate in the dermis (COUTO et al., 2002). In large part of the samples (59.4%) it was possible to observe invasion of adjacent structures such as dermis and musculature. The high capacity of invasion of peripheral tissues is one of the most important characteristics of FISS, and it is directly related to the high relapse rate that is close to 80% (OGILVIE; MOORE, 2002; SÉGUIN, 2002).

All the neoplasms evaluated, presented significant areas of necrosis, around 37.4% of the samples had moderate and 33% had marked areas of necrosis, many due to their large

size, as already suggested by COUTO et al (2002), the high rate of growth of FISS associated with insufficient angiogenesis leading to a lack of blood supply is the main cause of intratumoral necrosis.

All neoplasms also had variable intensities of peritumoral inflammatory infiltrate composed predominantly of lymphocytes, plasma cells and occasional macrophages. The most accepted hypothesis is that myofibroblasts activated by the tumor prevent the infiltration of these mononuclear cells by the formation of mechanical protection, however allowing them to be in the periphery of the tumor (COUTO et al., 2002). Inflammatory cells act in conjunction with tumor growth rate and allow the occurrence of intratumoral necrosis, a common finding in injection-site sarcomas (O'BYRNE & DALGLEISH, 2001). The common presence of inflammatory cells is usually the cause of the decrease in the accuracy of the cytological examination as a diagnostic tool, since the areas of necrosis tend to form cystic cavitations, and the collected material can only result in necrotic debris and inflammatory cells, leading to the inconclusive diagnosis (EHRHART et al., 2013).

Tumors that were tested for CD3 and CD79α antibodies had marked peritumoral infiltrate of lymphocytes. Significant CD3 labeling suggests that most of these lymphocytes are T-lymphocytes, as already presented by COUTO et al (2002), but the role of lymphocytes in the neoplastic formation is still unclear.

The higher production of collagen is expected in more differentiated tumors (EHRHART et al., 2013), due to its physiological competence in the production of support and repair collagen. Not only tumors well differentiated, but also the mild and anaplastic samples presented moderate to accentuated labeling for Masson’s trichrome, which represents that even in more anaplastic cells have the ability to produce collagen. In the case of vimentin, moderate labeling prevailed with 33,4%, with no significant difference between the differentiation groups.

Ki-67 antigen is a nuclear protein expressed from the start of the G1 phase to the end of the cell cycle, and its immunostaining is related to tumor proliferative activity (SCHOLZEN & GERDES, 2000). The markers for Ki-67 in this study were irregular, not relating the mitotic count to the proliferative index, which was also observed by PORCELLATO et al. (2017). Some factors directly influence the marking of Ki-67 mainly when related to the time of exposure to formaldehyde samples.

CONCLUSION

FISSs affected felines with a mean age of 9.8 years. The neoplasm was observed mainly in the thoracic and interscapular region. The most diagnosed histological type was fibrosarcoma, and all had peritumoral mononuclear inflammatory infiltrate and areas of necrosis, which ranged from moderate to marked intensity. The anatomopathological characteristics of a subcutaneous neoplasm in injection-site location, with high cellular pleomorphism, intratumoral necrosis and peritumoral mononuclear inflammatory infiltrate, added to compatible clinical history a definitive diagnosis of FISS can be made. Masson's trichrome and vimentin labeling showed strong positivity, demonstrating the fibroblastic origin of these tumors and their capacity to produce collagen. Most of the neoplasms presented in this study invaded adjacent structures, which reinforces the knowledge of the invasive nature of FISS.

DECLARATION OF CONFLICTING INTERESTS

We have no conflict of interest to declare.

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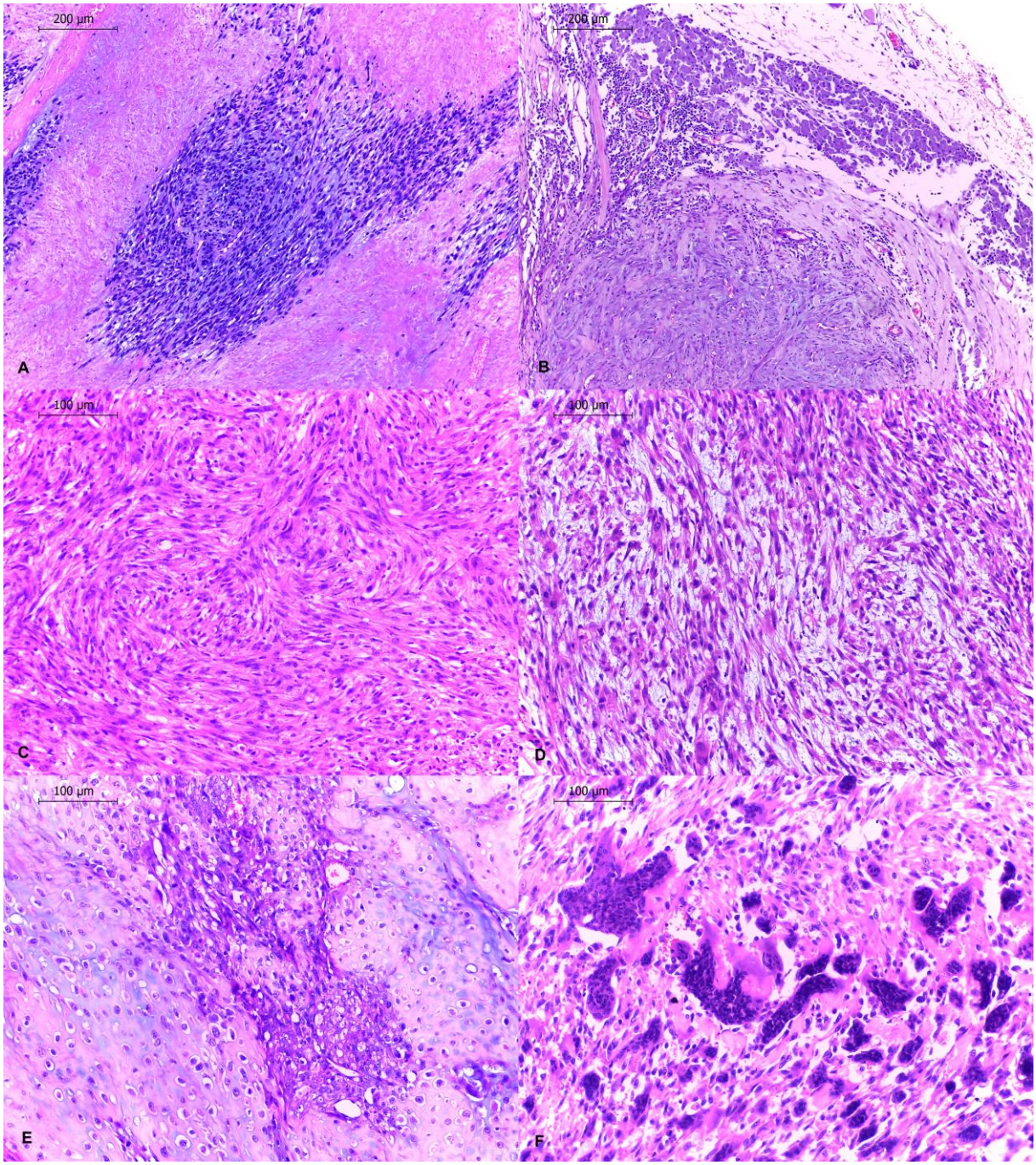

Figure 1 - Characteristics and histological types related to FISS. A-Injection-site sarcomas presenting extensive areas of necrosis. HE Obj.10X. B- Fibrosarcoma with abundant infiltrate of lymphocytes and macrophages in the periphery of the tumor. HE Object 10X. C- Fibrosarcoma with fusiform cells arranged in bundles in different directions and supported by moderate fibrovascular matrix. HE Obj. 20X. D- Myxosarcoma with fusiform cells organized in bundles supported by abundant loose basophilic myxoid matrix. HE Obj. 20X. E- Chondrosarcoma with spindle-to-polygonal cells in the middle of a well-differentiated chondroid matrix. HE Obj. 20X. F- Malignant fibrous histiocytoma represented by large amount of multinucleated giant cells in medium to abundant fibrovascular matrix. HE Obj. 20X.

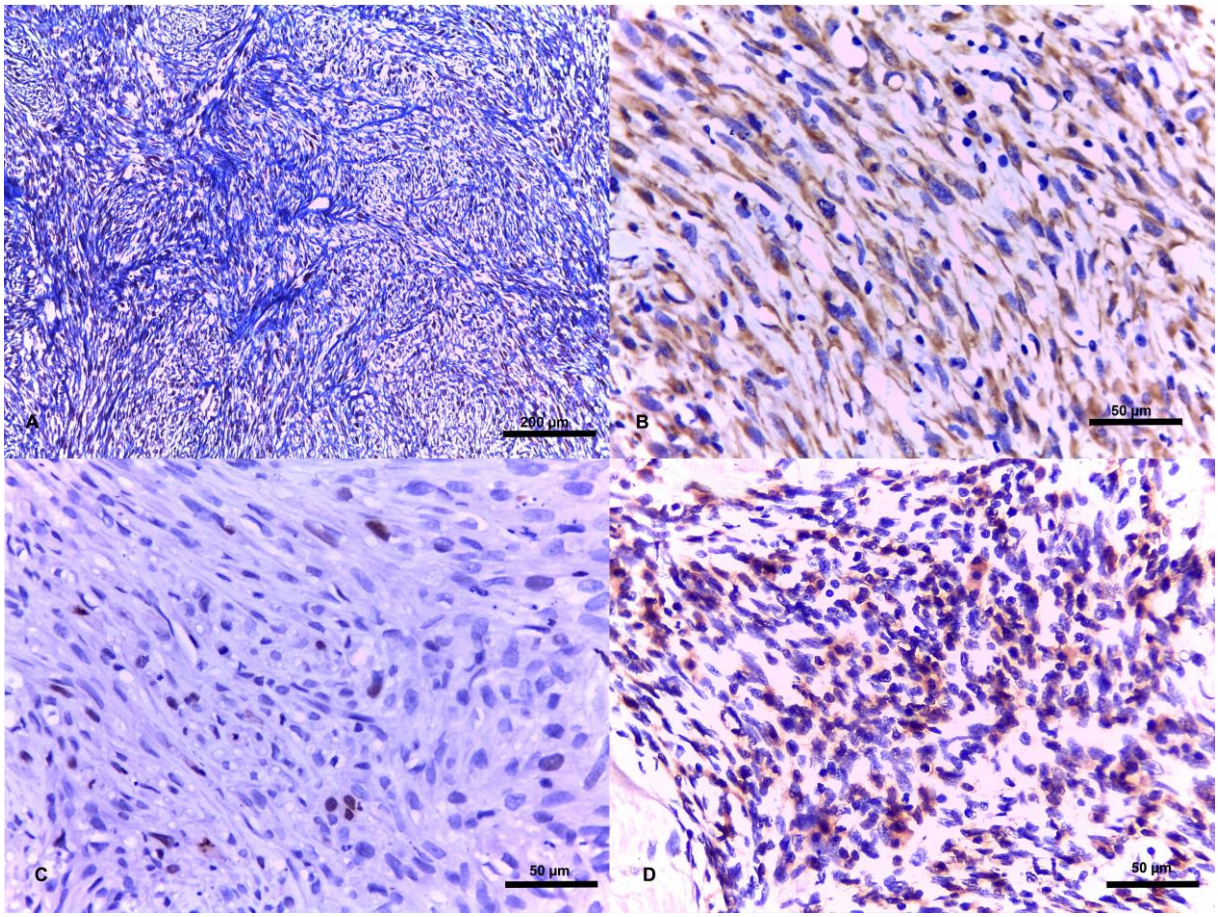

Figure 2 - Histochemical and immunohistochemical characteristics related to FISS. A- Spindle mesenchymal cells in medium to abundant collagenous matrix. Masson's Trichrome, Ob. 10X. B- Cytoplasmic marking for mesenchymal cells. IHQ Vimentin Obj. 40X. C- Nuclear marking in cells with high cell proliferation. IHQ Ki-67 Obj. 40X. D- Immunohistochemistry of peritumoral T lymphocyte infiltrate with cytoplasmic labeling. IHQ CD3 Obj. 40X.

CASE REPORT

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

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FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Summary

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 femorotibiopatelar 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.

Keywords: feline, osteosynthesis, sarcoma, mesenchymal.

Feline injection site sarcoma (FISS), also known as vaccine-associated sarcoma is a well-recognized pathological entity in the cat, which was first described in 1991 (Hendricks & Goldschmidt, 1991). Parenteral administration of a variety of substances, such as vaccines and medication, 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; Carminato *et al.*, 2011), subcutaneous fluid tube (McLeland *et al*., 2013) and surgical sponges (Haddad *et al.*, 2010).

FISS have unique characteristics related to their origin in the subcutaneous tissue, marked necrosis in the centre of the tumor, high cellular pleomorphism, and abundant amount of mononuclear inflammatory infiltrate in the periphery of the neoplasm (Doddy *et al.*, 1996; Kass *et al*., 2003). Fibrosarcomas induced by foreign bodies also demonstrate these features, which suggests that a common pathogenesis is involved (Daly *et al.*, 2008; Carminato *et al.*, 2011).

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 indoors and had no access to the street. The cat only received vaccines when kitty, but the owner did not know the exact injection-site. Radiographic assessment of the affected limb revealed a communitive complete diaphyseal fracture of left femur. There was no image consistent with neoplasia or bone alteration that could suggest a pathological fracture. Blood analysis was performed and did not show any abnormality in complete blood count, or in the serum biochemistry values.

The cat was forwarded 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, and nonalignment between fracture

fragments, proximal intramedullary pin migration and screw loosening, and absence of periosteal reaction/bone callus formation signs (Figure 1).

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 was 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 associated with extensive necrosis. The limb was surgically removed by hemipelvectomy because 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. 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 centre of the mass (Figure 2). A large amount of bone spicules showing no 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 3-5µm 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 3 to 4 mitotic figures per high power field (x 40) (Figure 3). There was also extensive intratumoral necrosis (Figure 4), 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 (Figure 3). 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 localized.

Sections of the neoplasm were submitted to immunohistochemistry, using reagents specific for vimentin (1:200; Zymed, Carlsbad, California, USA), desmin (1:300; Zymed, Carlsbad, California, USA) and FeLV (1:500; Bio-Rad, Hercules, California, USA). 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 haematoxylin. The mesenchymal cells showed strong cytoplasmic positive expression of vimentin (Figure 4) and weak positive labeling for desmin. 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 later. 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 study (Doddy *et al.*, 1996), in which animals that were diagnosed with FISS were in the age group close to 8 years, and 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). In the present case, the time elapsed between the original lesion and the observation of a massive neoplasm was only 2 months, identical to that reported in the case of fibrosarcoma arising adjacent to microchip implantation (Carminato *et al.*, 2011).

Cytological analysis is considered quite difficult to interpret in such 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. The histological features of FISS, mainly the ones described associated with microchip implant (Daly *et al.*, 2008; Carminato *et al.*, 2011), 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; Nieto *et al.*, 2003; Carminato *et al.*, 2011). 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).

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). In rats, fibrosarcomas arising from hepatic cysts of *Cysticercus fasciolaris* have been reported (Hanes, 1995), 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 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, 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, and FISS are known to present an extremely variable latency period (Doddy *et al.*, 1996). In addition to that, fracture itself could cause a strong inflammatory stimulus, which may predispose the formation of fibrosarcomas in felines (Aminkov & Manov, 2005).

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, 1990; Vascellari *et al*., 2006; Kidney, 2008). Still, it is not clear if the fracture itself or the intramedullary pin placement led to neoplastic transformation. 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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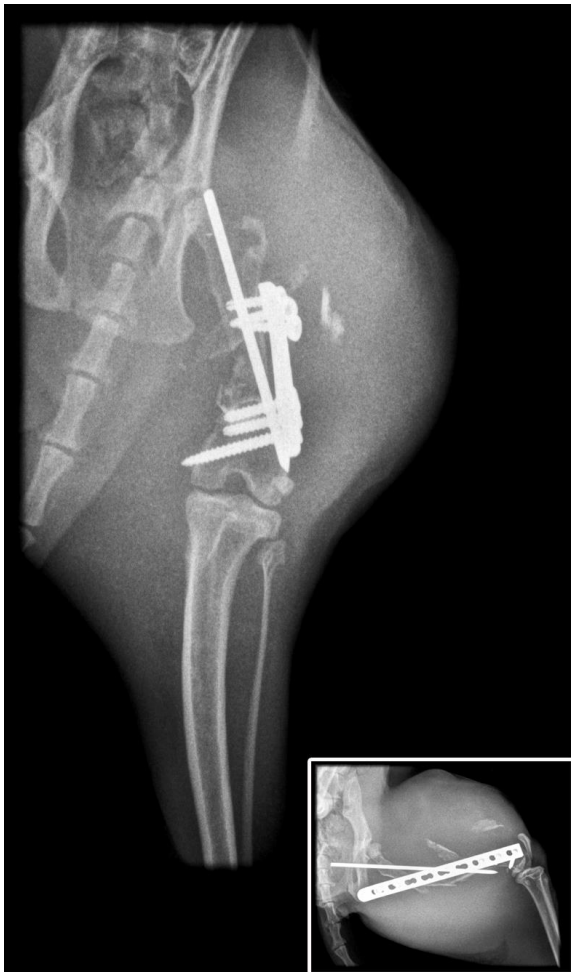

Figure 1 - Radiographic examination of the left hindlimb two months after osteosynthesis, showing soft tissue enlargement, loss of apposition and alignment between fracture fragments, proximal intramedullary pin migration, screw loosening, and absence of periosteal reaction / bone callus formation signs. Craniocaudal projection. Inset: mediolateral projection showing the metal plate complete extension.

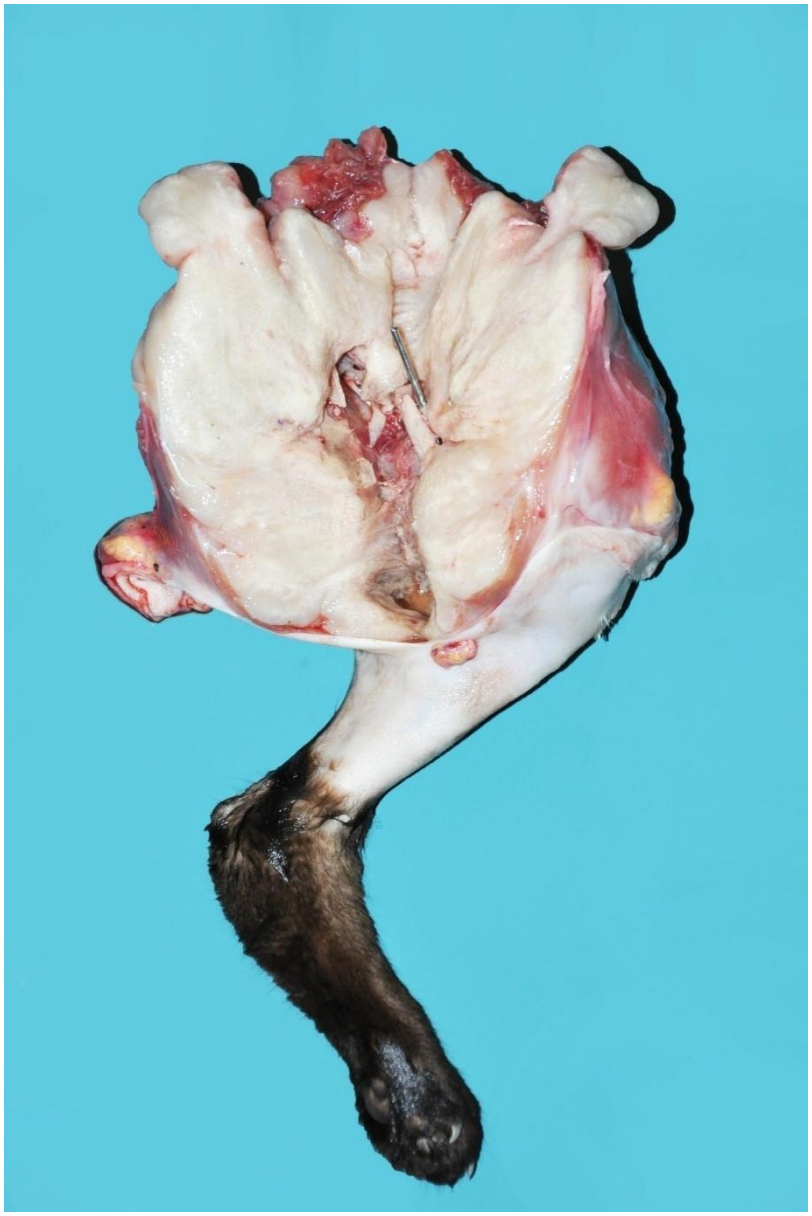

Figure 2 – Fibrosarcoma in the pelvic limb of a cat. There is a whitish and firm mass, extending from the femoropatellar joint to the proximal portion of the limb, infiltrating and replacing the limb musculature. At the cut surface, the metal pin, the bridge plate, the metallic screws and bone spicules were observed in the centre of the mass.

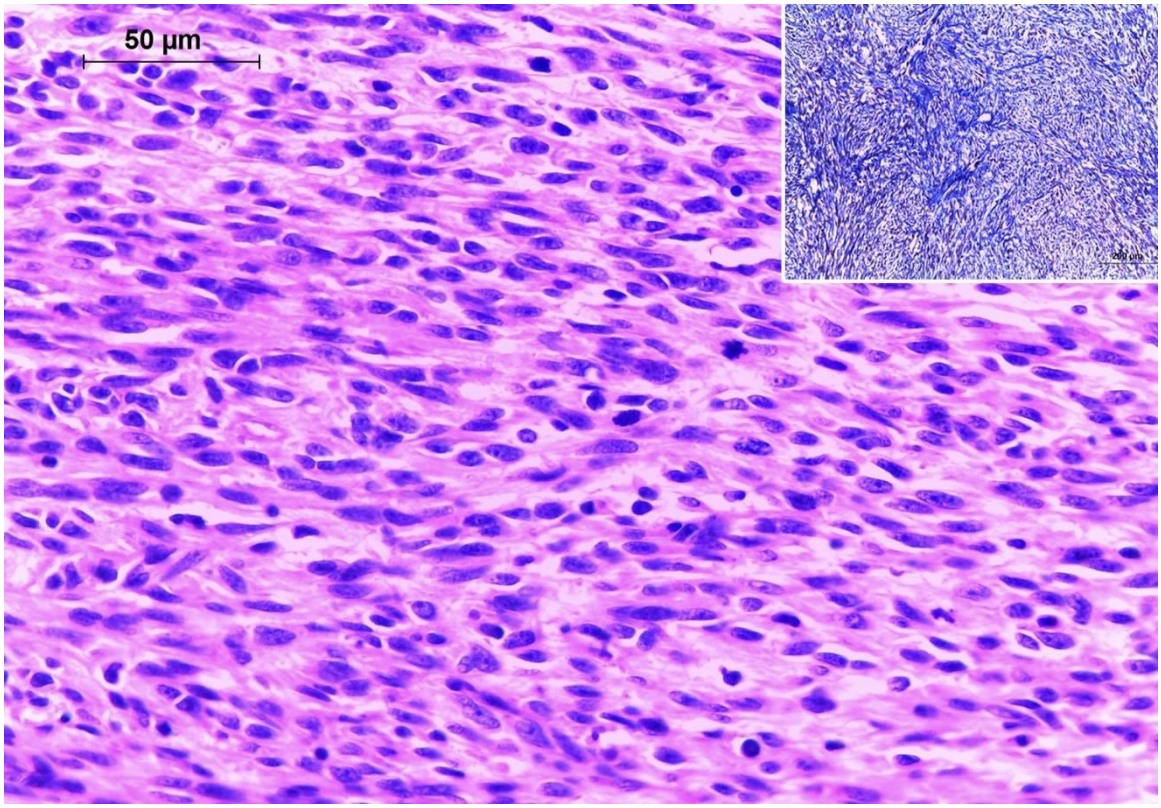

Figure 3 – Microscopic appearance of the fibrosarcoma showing mesenchymal cells displayed in bundles interspersed with abundant stroma. These cells present eloganted nuclei and high mitotic count. HE Bar 50 µm. Inset: strong positive staining of collagen. Masson's trichrome Bar 50 µm**.**

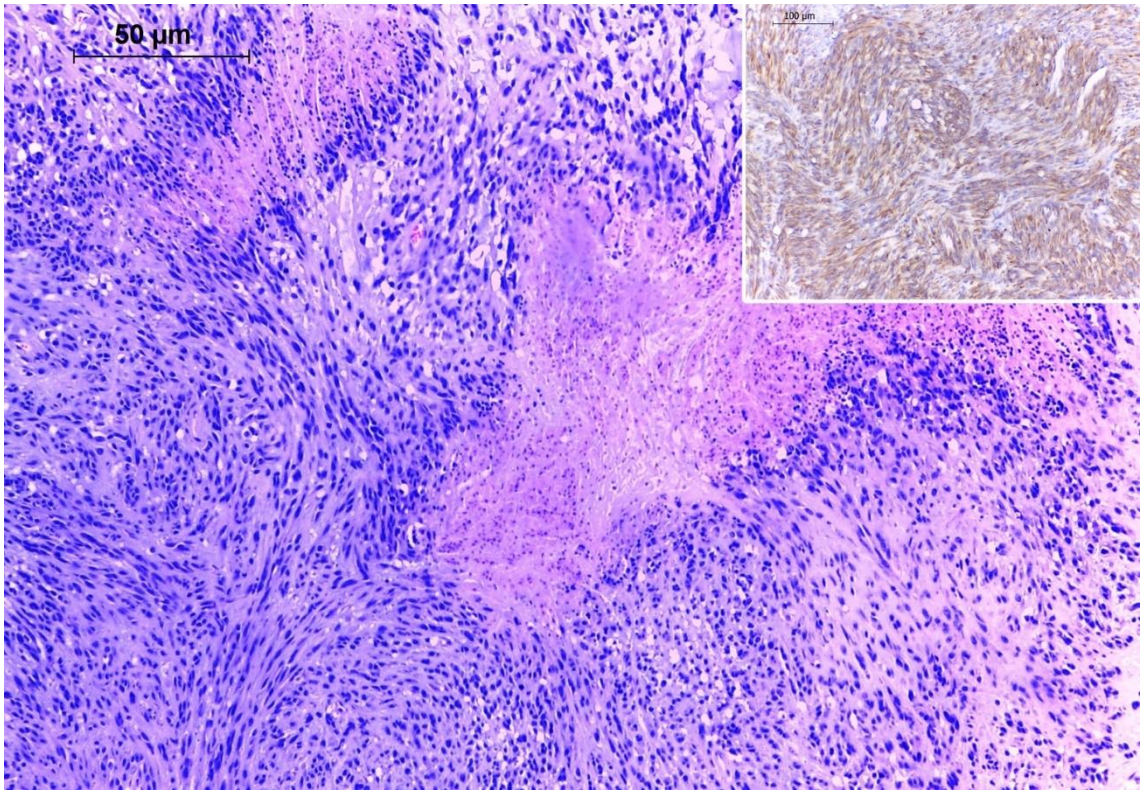

Figure 4 – Microscopial appearance of the tumor showing extensive areas of necrosis, fusiform cells in bundles arranged in multiple directions. HE Bar 50 µm. Inset: Expression of vimentin by the neoplastic mesenchymal cells. IHC, Bar 100 µm

2. CONSIDERAÇÕES FINAIS

Os SAF afetam felinos com a idade média de 9,8 anos, sem predileção por raça ou sexo. Acometem a região torácica, seguido de flanco, região interescapular e membros. Geralmente se originam no subcutâneo e se aderem aos tecidos musculares adjacentes. Os tumores classificados nos grupos T2 e T3 foram mais prevalentes, somando 90% das amostras.

Todos os casos classificados como SAF nesse estudo apresentaram infiltrado inflamatório mononuclear em região peritumoral, principalmente de linfócitos T, como caracterizados pela imuno-histoquímica. A associação do infiltrado inflamatório mononuclear peritumoral com as extensas de áreas de necrose observadas na maioria dos tumores e o acentuado pleomorfismo celular foram características essenciais para aferir o diagnóstico de sarcoma de aplicação nos casos pesquisados.

Os diagnósticos histológicos em ordem decrescente foram fibrossarcoma (89%), fibrohistiocitoma (8%), mixossarcoma (1%) e condrossarcoma (1%).

Tanto a marcação da produção de colágeno através da técnica de tricrômico de Masson como a imunomarcação para vimentina permitiram uma melhor análise da origem da neoplasia.

A análise da contagem mitótica e marcação imuno-histoquímica pelo Ki-67 foram baixos quando comparados ao grupo controle.

Também pode ser observado que não apenas medicações e vacinas ocasionam fibrossarcomas em felinos, mas também estes podem ser associados a colocação de pino intramedular e placa em osteosíntese de fratura de fêmur, com características muito semelhantes ao SAF em relação a invasão tecidual e acentuado pleomorfismo celular.

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