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Marina Martano *, Emanuela Morello, Paolo Buracco

School of Veterinary, Department of Animal Pathology, via Leonardo da Vinci 44, 10095 *Grugliasco, Italy*

*Corresponding author. Tel.: +39 011 6709064; fax: +39 011 6709165. *E-mail address:* marina.martano@unito.it

Keywords:
Feline injection-site sarcomas
Surgery
Radiotherapy
Chemotherapy
Inflammation

A B S T R A C T

Feline injection-site sarcomas (FISS) have been known since the early 1990s. After an initial correlation with rabies and feline leukaemia virus vaccination, subsequent studies have demonstrated that an abnormal reaction of feline tissues to chronic inflammation was mainly responsible for the disease. The low incidence of FISS in the population is explained by its multifactorial aetiology, since individual genetic characteristics are also implicated.

FISS is an infiltrative tumour with low metastatic potential but local recurrence is common. Multi-modal treatment (extensive surgery, radiotherapy, chemotherapy) is recommended. The use of sophisticated imaging techniques can improve diagnosis and help in surgical planning. After the initial enthusiasm in understanding the disease, only few advances have been made in the last few years. New promising therapies may arise from a better knowledge of the molecular pathogenesis of FISS and the successful development of drugs modulating the immune system.

Introduction

Since the first description by Hendrick and Goldschmidt (1991), feline injection-site sarcomas (FISS) have been described almost all over the world. An association between new recommendations for feline vaccination (especially for rabies) and an increased incidence of FISS was initially observed in the United States, leading to the term *'vaccine-associated sarcomas'*. However, recent studies investigating the pathogenesis of FISS have shown that in addition to vaccines, other foreign material injected in the subcutis or muscle of a predisposed cat can induce a chronic inflammatory response and ultimately neoplastic transformation. Therefore, these neoplasms are now referred to as *'injection-site sarcomas'*.

FISS are characterised by a variable and sometimes long latency period, a rapid growth and a local malignant behaviour in spite of a low metastatic rate. Effective therapeutic strategies have not yet been found, but a multimodal approach is recommended. New therapeutic advances may develop from a better knowledge of the molecular basis of the disease. In order to fund research for a better understanding of the epidemiology, aetiology and treatment of FISS, and to draw guidelines to avoid the spread of this iatrogenic tumour, a task force was instituted in 1996 (Vaccine-Associated Feline Sarcoma Task Force - VAFSTF[1]). Its work concluded in 2005 with a roundtable and a document capturing the updated knowledge on FISS (VAFSTF, 2005). The aim of this review is to provide an overview of the current knowledge on FISS.

Epidemiology and aetiopathogenesis

The initial description and a retrospective epidemiology study was published by Kass et al. (1993) on 345 cats for which a diagnosis of fibrosarcoma was made and for which the vaccination history (as well as the site of development of the tumour) were known. The authors observed that those animals that developed a tumour at the vaccination site were younger than those with similar tumours in other body areas, and had a bimodal distribution of age with a peak at 6-7 years and a second at 10-11 years. A strong relation between feline leukaemia virus (FeLV) and rabies vaccination and tumour development was evident. The risk increased with the number of injections administered at the same site, and was 50% higher following a single

vaccination compared to cats receiving no vaccination, 127% higher after two injections, and 175% higher after three or more vaccinations.

The histological finding of particles of grey-brown material in the necrotic centre and within the cytoplasm of macrophages (Hendrick and Dunagan, 1991; Hendrick and Brooks, 1994) was consistent with an inflammatory reaction elicited by foreign material that could have been caused by vaccination. The overall frequency of the malignancy in the cat population was low (approximately 20/100,000 cats). Since then, contradictory data on incidence have been reported, varying from 1/1000-1/10,000 (Esplin et al., 1993; Lester et al., 1996) to 0.63/10,000 vaccinated cats (Gobar and Kass, 2002). In some European countries the incidence may be higher, although no documented information exists. The latency period from the time of injection to tumour development can vary from 3 months to 3-10 years (McEntee and Page, 2001; Seguin, 2002), which makes the determination of incidence challenging and may also explain why a specific vaccine brand has not been implicated (Hendrick et al., 1994; Kass et al., 2003).

Histologically, FISS are mesenchymal tumours of different histotypes. The most frequently observed is fibrosarcoma, but malignant fibrous histiocytoma, osteosarcoma, chondrosarcoma, rhabomyosarcoma and undifferentiated sarcoma are also reported. All seem to develop from proliferating fibroblasts and myofibroblasts at the site of chronic inflammation (Hendrick and Brooks, 1994). Myofibroblasts probably represent a transitional stage through which fibroblasts and macrophages pass during the process of wound healing, and their prevalence in FISS is consistent with an abnormal response to a traumatic insult. A similar mechanism has been described by Martins-Green et al. (1994) in chickens infected by Rous sarcoma virus (RSV), where a tumour develops at the site of virus injection and at the site of wounding in infected animals. If the inflammatory response is inhibited, the sarcoma never develops. Cytokines, such as trans-forming growth factor-b (TGF-b), acidic fibroblast growth factor (FGF-a) and basic fibroblast growth factor (FGF-b), that are released by the inflammatory cells, are considered responsible for this phenomenon. The same mechanism may be relevant to FISS, since FGF-b and TGF-a have been detected in the majority of 50 FISS samples examined by immunohistochemistry (Nieto et al., 2003). These cytokines are involved in the promotion of malignant transformation by the stimulation of division and migration of vascular endothelial cells and by activating DNA synthesis in mesenchymal cells.

FISS have also been described after the injection of long acting antibiotics or steroids (Kass et al., 2003), the benzoylurea pesticide lufenuron (Esplin and McGill, 1999), non-absorbable suture material (Buracco et al., 2002) and (presumably) microchip implants (Daly et al., 2008). The low incidence of FISS is explained by its multifactorial aetiology. Chronic inflammatory stimulation can elicit fibroblast and myofibroblast proliferation and transformation through multiple cytokines and growth factors, such as platelet-derived growth factor (PDGF), in addition to over-expression or mutations of oncogenes and tumour suppressor genes (Hendrick, 1998). In particular, the alteration (Banerji and Kanjilal, 2006; Banerji et al., 2007) and/or cytoplasmic location (Hershey et al., 2005) of p53 has been found to be prognostic for recurrence time of FISS. Over-expression of oncogenes, such as c-Kit, involved in the pathogenesis of malignant tumours in dog and man, has also been detected in FISS, although a relationship with histological grade, survival or specificity has not been demonstrated (Smith et al., 2009). Similar results have been obtained with matrix metalloproteinases (MMP), a family of endopeptidases that play an important role in wound healing, regenerative and inflammatory processes, although no significant differences in expression have been identified when compared with non-injection-site sarcomas (Sorensen et al., 2004).

The immune system can be involved in the neoplastic transformation. In humans, chronic immune stimulation due to infectious or non-infectious causes (such as cigarette smoke or asbestos fibres) can lead to malignant transformation (O'Byrne and Dalgleish, 2001). In FISS, although only a few reports on escape mechanisms from immune surveillance are available (Jelinek, 2003; Cerruti et al., 2007), initial preliminary results from immunotherapy have been promising (King et al., 1995; Hampel et al., 2007).

Other factors that may contribute to enhance tissue trauma have been investigated (Macy, 1999; Kass et al., 2003). Administration of vaccines at colder temperatures seemed to be significantly associated with a higher risk, while the type of syringe used, massaging the area, intramuscular injection, as well as FeLV and feline immunodeficiency virus (FIV) status or other feline virus infection did not demonstrate any correlation.

Diagnosis

The diagnosis of FISS starts with the signalment, with particular attention to the injection history. FISS are usually rapidly-growing masses that develop at sites commonly used for injection; although the time from the last injection to tumour development can be very long, once the process is initiated the mass can usually reach several centimetres in diameter within a few weeks. Rapid growth leads to central necrosis. A viscous transparent to light brown fluid may be present, especially in larger or more rapidly growing tumours. As fine needle aspiration and cytology evaluation is diagnostic only in about 50% of cases, VAFSTF recommends that every mass that (a) persists for more than 3 months after injection, (b) becomes larger than 2 cm, and (c) increases in size 1 month after an injection, should be biopsied (3-2-1 rule) (AVMA, 1999).

Incisional biopsy is preferred to Tru-cut biopsies, since the tumour can be heterogeneous and can be misdiagnosed as a granuloma from small tissue samples. Biopsies should be performed at sites that can be easily excised without further extending the surgical field. The histology of FISS was first characterised by Doddy et al. (1996) on 165 tissue samples. These tumours were characterised by peripheral infiltration by inflammatory cells (lymphocytes and macrophages; Fig. 1), granulation tissue and multinucleated tumour cells. Areas of osteoid, chondroid or myxomatous matrix could be observed. Tumour cells express a smooth muscle actin, desmin and/or vimentin by immunohistochemistry (Couto et al., 2002). The histological finding of amorphous to globular grey-brown material in necrotic central areas and within the cytoplasm of macrophages is

thought to represent remnants of the vaccine it-self or aluminium salts used as adjuvant (Hendrick and Dunagan, 1991; Hendrick and Brooks, 1994). Features of malignancy (like necrosis, high number of mitotic figures, cellular pleomorphism) are more common in these tumours compared to sarcomas not linked to injections (Fig. 1).

A further characterisation was made by Couto et al. (2002) who proposed a scoring system (I-III, increasing in malignancy) based on overall differentiation, mitotic rate, and presence of necrosis. Multinucleated neoplastic giant cells were present in the majority of the more aggressive, grade III tumours, while no correlation was found between the presence or intensity of inflammation, tumour vascularity and histological grade. A similar grading system was proposed by Kuntz et al. (1997) for prognostic purposes in canine soft tissue sarcomas; these criteria have been suggested for FISS, but the real applicability to this specific neoplasm is still debatable.

Once a histological diagnosis has been reached, staging is completed by haematology, serum biochemistry profile, FIV and FeLV status, urinalysis and chest radiographs (with at least the two lateral views). Depending on tumour location, abdominal ultrasound may be performed. When possible, computed tomography (CT) or magnetic resonance imaging (MRI) of the lesion and the thorax are indicated (Fig. 2). This is now widely available and the most reliable method for evaluating tumour extension. In fact, the infiltrating nature of FISS is most challenging in planning treatment. Recurrence rates are as high as 45%, even after wide surgical excision (Cronin et al., 1998). Distant metastases (to lung, regional lymph nodes, mediastinum, pericardium, liver, pelvis) (Esplin and Campbell, 1995; Esplin et al., 1996) are a less important problem, since they are reported in 0-28% of FISS (Hershey et al., 2000; Bregazzi et al., 2001).

Treatment

An effective cure for FISS has not been found, but it is now recognised that a multimodal approach can lead to better results. The mainstay of therapy, however, is wide surgical excision of the primary tumour, meaning a margin of 3-5 cm of macroscopically healthy tissue and at least one facial plane beneath the tumour (Fig. 3). Radical excision including spinous vertebral process amputation, partial or total scapulectomy, rib resection or limb amputation may be necessary. When these criteria were applied to 45 cats with or without subsequent radiation therapy, the overall median disease-free interval (DFI) was 10 months (Davidson et al., 1997), but cats with complete excision had a significantly longer median DFI (16 months vs. 4 months). DFI can be significantly improved when a complete first excision is performed by experienced surgeons (Hershey et al., 2000).

The combination of wide surgical excision and radiotherapy with or without chemotherapy has been evaluated by several authors with similar conclusions (Cronin et al., 1998; Cohen et al., 2001; Kobayashi et al., 2002; Eckstein et al., 2009; Mayer et al., 2009). Overall DFI ranged from 398 to 810 days; overall survival (OS) from 520 to 1290 days; local recurrence rate from 41% to 45%, and rate of metastasis from 12% to 21%. The absence of neoplastic cells at the surgical margins is not always predictive of a better prognosis. In a recent study on recurrence, tumour grading and surgical margins using 48 cases Giudice et al. (2010) reported that tumours with infiltrated mar-gins recurred 10 times more frequently compared to those with clean margins, even though recurrence occurred also in 19% of the clean cuts. No significant correlation was observed between grading (Kuntz et al., 1997) and local recurrence. The tumour recurred in 42% of cases with histologically clean margins also in an-other study (Kobayashi et al., 2002); this may relate to the unique pathogenesis of FISS and questions the interpretation of margins in veterinary tumour histopathology.

There are no prospective studies that have robustly evaluated chemotherapy for FISS. However, the most commonly used drugs are doxorubicin, carboplatin, and cyclophosphamide. A study by Barber et al. (2000) evaluated the effect of doxorubicin alone in 12 non-resectable FISS, but despite an initial reduction >50% in tumour volume in 50% of the animals, the response was not durable and all cats showed progressive disease. Median overall survival (OS) was significantly longer for cats that responded to therapy. Poirier et al. (2002) demonstrated that both doxorubicin and liposome-encapsulated doxorubicin were efficacious in the treatment of FISS, even though the latter had more side effects. A positive prognostic effect of chemotherapy was also reported by Kobayashi et al. (2002) and Eckstein et al. (2009). Martano et al. (2005) reported a median disease-free interval (DFI) of 771 days in 20 cats treated with surgery alone; the median DFI was not reached in 49 animals treated by a combination of wide surgery and doxorubicin, but the DFI ranged from 460 to 1687 days. No statistically significant differences were noted between the two groups.

Imatinib mesylate, a tyrosine kinase inhibitor (TKI), has been shown to block the PDGF/PDGF receptor signalling pathway effectively in FISS cell lines and to inhibit FISS growth in a murine mod-el (Katayama et al., 2004). TKI are new drugs currently being evaluated for the treatment of many neoplasms in veterinary medicine. A more thorough investigation of their use in FISS is desirable. In a phase II study ifosfamide demonstrated an antitumour effect against FISS, but its nephrotoxic potential makes it undesirable for routine administration to cats (Rassnick et al., 2006). Immunotherapy with recombinant feline interferon-x has been investigated for safety (Hampel et al., 2007), but no clinical results on improvement of DFI have been reported.

According to a roundtable convened by VAFSTF (2005), even if a definitive conclusion has not been reached, a multimodal approach, combining wide surgical excision, radiotherapy (both in a neoadjuvant or adjuvant setting) and chemotherapy, is recommended. Fig. 4 shows the current work up of FISS used at the authors' institution.

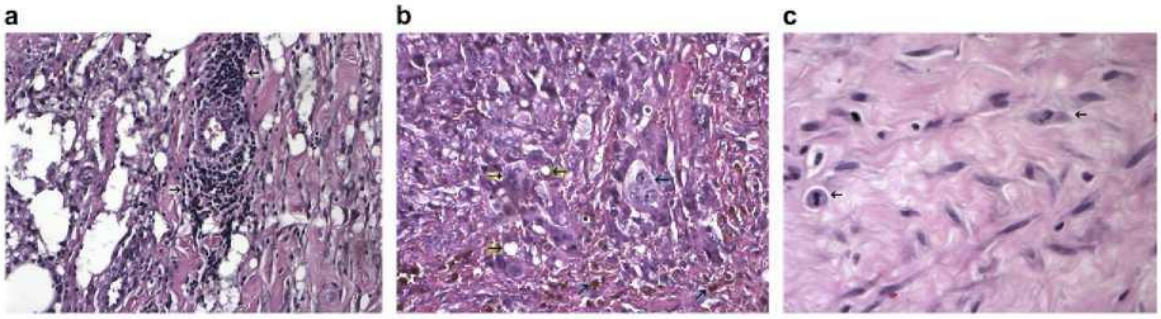

Fig. 1. Histopathological appearance of a FISS. (a) A perivascular and multifocal lymphocytic infiltrate is evident at the periphery of the tumour (arrows). On the left side of the slide areas of panniculitis are evident, whilst on the right side neoplastic tissue can be observed. (Haematoxylin and eosin stain, 20x). (b) Cellular pleomorphism is evident with multinucleated cells (horizontal arrow on blue background), atypical mitotic figures (dotted arrow), areas of haemorrhage (diagonal arrows on blue background), and intracellular vacuoli (horizontal arrows on yellow background). (H&E, 200x). (c) Mitotic figures, sometimes atypical, are evident; note the abundant extracellular matrix (H&E, 400x). (Courtesy of Dr. Selina Iussich.)

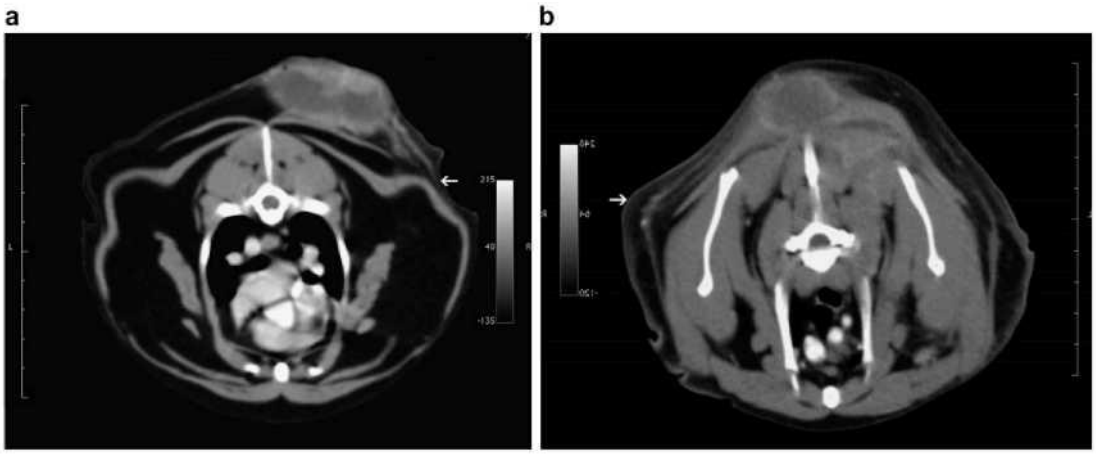

Fig. 2. Contrast enhanced *CT* images of FISS. (a) This imaging technique allows better evaluation of the extent of the tumour. The lateral spread of the lesion (arrow) was not apparent clinically. The main mass has a fluid content. (b) This particularly wide tumour consisted of multiple nodules, one of which becomes evident on the right lateral aspect of the scapula (arrow). The spinal process is very close to the tumour mass and has to be resected.

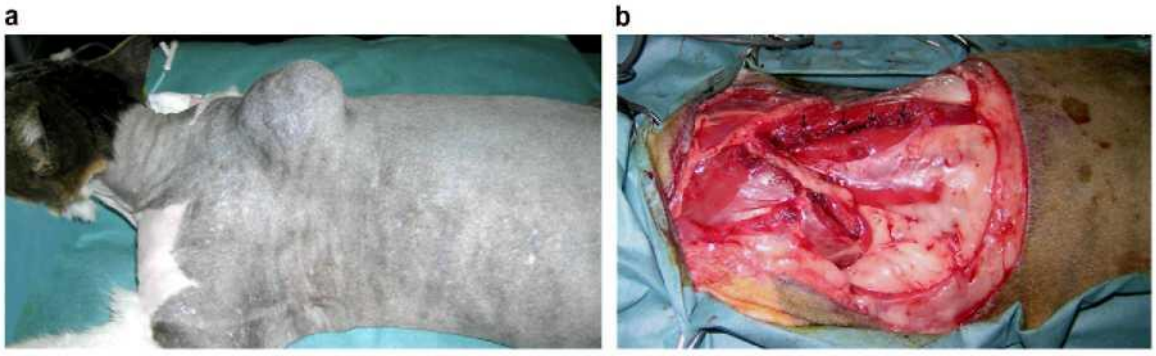

Fig. 3. Resection of an interscapular FISS. (a) Pre-operative view. The area prepared for surgery needs to be wide. (b) Intraoperative view. The spinous spinal processes have been resected (arrows) along with the superficial muscular layers.

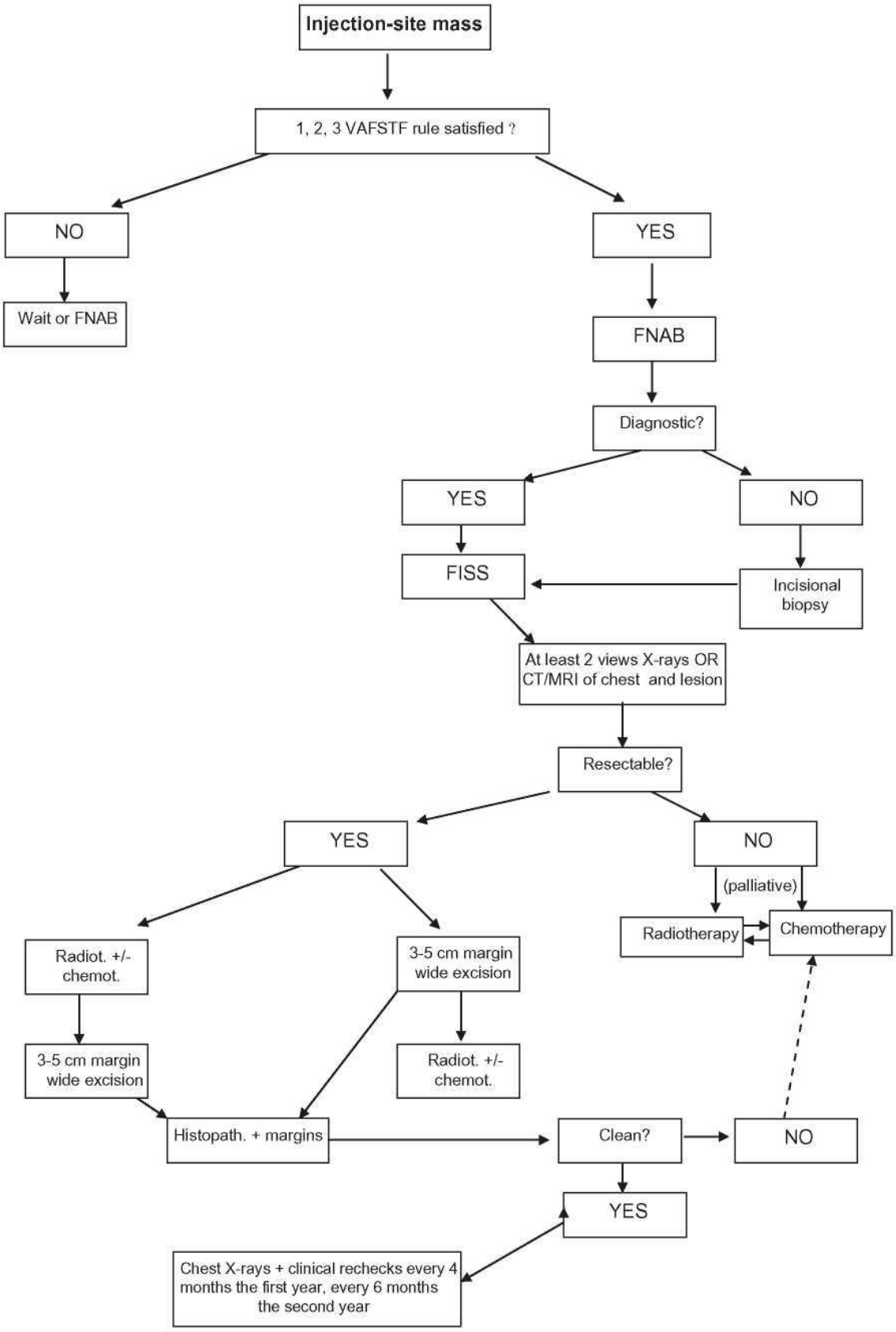

Fig. 4. Algorithm of the work up for FISS.

Prevention

To better understand which substances are implicated in FISS formation, VAFSTF has drawn up guidelines for veterinary practitioners for the administration of injectable drugs. The current standard is to administer FeLV vaccination in the left rear leg, as distal as possible, the rabies vaccine in the right rear leg, as distal as possible, and the four-in-one vaccine FVRCP ± [1]C in the right shoulder (AVMA, 1999; Morrison and Starr, 2001).

A survey on 392 cats with FISS and treated before and after December 31, 1996 (when the VAFSTF was founded) reported a change in tumour development during that period (Shaw et al., 2009). After December 1996, the number of FISS detected in areas of the body cranial to the diaphragm progressively decreased, while a greater number of tumours were detected in the posterior part of the body. In 2006 there was an approximately equal distribution of tumours in the body. According to VAFSTF guidelines (and not considering other substances as responsible for FISS formation) rabies vaccination was found to cause 51.7% of new cases, FeLV 28.6% and FVRCP ± C 19.7%. Nothing has been reported about the incidence of the disease over the same period. Although retrospective, this study reveals that the guidelines had been followed by most practitioners, confirming that FISS is perceived as a problem. Moreover, these data confirmed that injections do represent the cause of the disease.

It would be interesting to evaluate whether the incidence of FISS will decrease following the 2005 vaccination policy advised by VAFSTF (2005): 'to *vaccinate the largest possible number of individuals in the population at risk, vaccinate each individual no more frequently than necessary, and vaccinate only against infectious agents to which individuals have a realistic risk of exposure and sub-sequent develop disease*'. While rabies immunization is controlled by national regulation, for FeLV and FVRCP ± C the duration of immunity could be longer than reported by the manufacturers. Therefore some authors have suggested that 3 years could be a safe vaccination interval (Scott and Geissinger, 1997, 1999). For other drugs administered by subcutaneous or intramuscular injection, their use should be limited and the distal part of the limbs or the lateral part of the body (i.e., away from the vertebral column) should be preferred as sites of administration.

Conclusions

Research on FISS has reached a plateau and new efforts are needed to improve tumour control. More sophisticated imaging techniques (such as CT) are valuable tools in planning the excision field, but the infiltrating nature and the pathogenesis of the tumour demand new approaches to treat the disease. The lack of correlation between completeness of excision and local tumour recurrence questions the validity/utility of the current grading system, of margin evaluation, and of current treatment modalities. In the last few years the literature on FISS has focused on treatment and on the evaluation of gene expression. Future studies evaluating new drugs (such as TKIs), or new approaches (such as stimulating the host immune system against the tumour) may revolutionise our approach toward this neoplasm.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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[1] These vaccines include feline viral rhinotracheitis (FVR), calicivirus (C), panleukemia (P), and feline chlamydia (-C).

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