GUIDELINE for Feline Injection-Site Sarcoma

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Key points

- In cats, invasive sarcomas (mostly fibrosarcomas), so called “feline injection-site sarcomas” (FISS), are the most serious adverse effects following vaccination. They develop at sites of vaccination or injection. They have characteristics that are distinct from those of fibrosarcomas in other areas and behave more aggressively. The rate of metastasis ranges from 10 to 28%.
- The pathogenesis of these sarcomas is not yet definitively explained. According to the most widely accepted hypothesis, chronic inflammatory reactions are considered a trigger for subsequent malignant transformation. As the pathogenesis of FISS is not yet fully understood, the following ABCD recommendations on how best to avoid their occurrence are sometimes based only on expert opinion.
- Adjuvanted vaccines induce intense local inflammation, and it has been suggested that they would be linked to the development of FISS. The risk is likely lower for modified-live and recombinant vaccines, but no vaccine is risk-free. Injection of cold vaccines also has been associated with a higher risk of FISS. Moreover, injections of long-acting drugs (such as glucocorticoids, and others) also have been associated with sarcoma formation.
- Aggressive, radical excision is required to avoid tumour recurrence. The prognosis improves if additional radiotherapy and/or immunotherapy (such as recombinant feline IL-2) and chemotherapy are used in addition to surgery.
- For prevention, injection of any irritating substance should be avoided. Vaccination should be performed as often as necessary, but as infrequently as possible. Vaccines should be brought to room temperature prior to administration. Non-adjuvanted, modified-live or recombinant vaccines should be preferred over adjuvanted vaccines, if available and proven equally effective. Injections should be given at sites at which surgery would likely lead to a complete cure; the interscapular region should generally be avoided. Postvaccinal monitoring should be performed.

Epidemiology

Vaccination of cats always has received scientific and public attention following the supposition that a range of rare adverse effects can arise following vaccination (Moore and HogenEsch, 2010). In cats, the most serious of these adverse consequences is the occurrence of invasive sarcomas (mostly fibrosarcomas), so called “feline injection-site sarcomas” (FISS), that can develop within the skin at sites of previous vaccination (Fig. 1 and 2). A task force has been instituted and is regularly updated in the USA to help veterinarians to understand, manage, and prevent these tumours (Morrison and Starr, 2001; Vaccine-Associated Feline Sarcoma Task Force, 2005). Despite extensive research on the pathogenesis of these sarcomas, there is no definitive causal relationship that explains their

occurrence and the direct link to vaccination. The most accepted hypothesis suggests that chronic inflammatory reaction at the site of injection provides a trigger for subsequent malignant transformation (Hartmann et al., 2015).

Fig. 1. Cat with feline injection-site sarcoma. Courtesy of Tadeusz Frymus, Warsaw University of Life Sciences, Warsaw, Poland

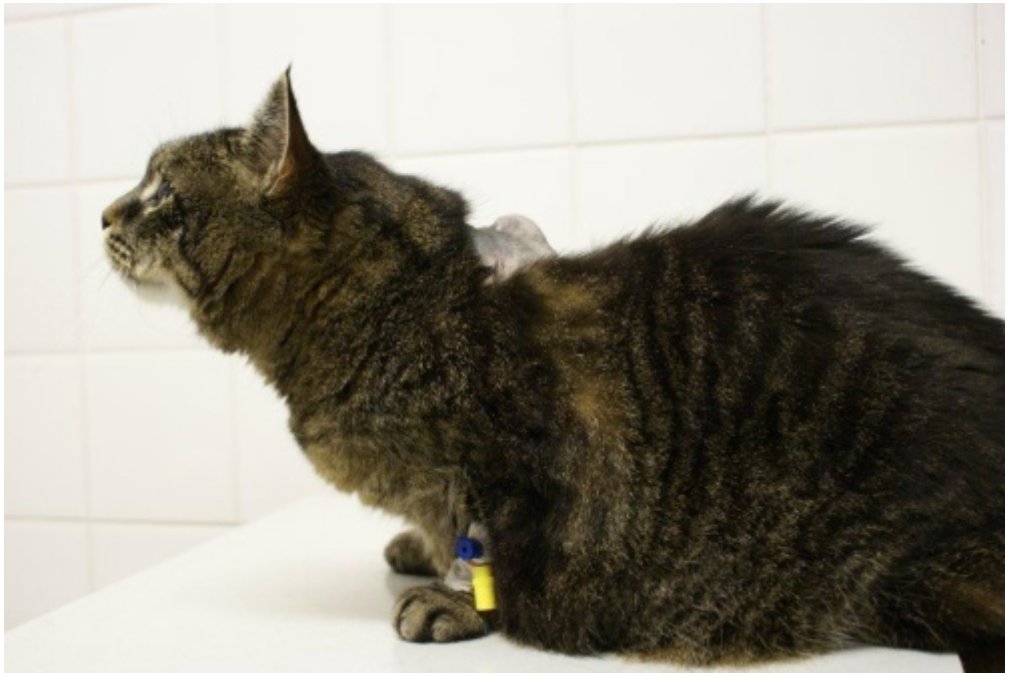

Fig. 2. Cat with feline injection-site sarcoma. Courtesy of Johannes Hirschberger, Ludwig Maximilians University, Munich, Germany

In 1991, an increased incidence of tumours in cats that developed at injection sites was first reported in the United States (Hendrick and Dunagan, 1991). This observation was connected to an increased use of rabies and feline leukaemia virus (FeLV) vaccinations (Hendrick and Goldschmidt, 1991; Kass et al., 1993). As a consequence, these tumours were first called feline “vaccine-associated sarcomas”. However, the subsequent finding that also other non-vaccinal injectables can be associated with this type of tumour has led to reclassification of these neoplasms as “feline injection-site sarcomas” (FISS). These tumours seem to be relatively unique to cats (Carroll et al., 2002), although comparable tumours have been reported in ferrets (Munday et al., 2003) and very occasionally in dogs (Vascellari et al., 2003). Still, FISS are highly similar to soft tissue sarcomas in dogs and humans, at the level of gene expression (Wei et al., 2019).

FISS occur at sites typically used for vaccination and injections, such as the interscapular region (Fig. 3), the lateral thoracic or abdominal wall, the lumbar regions, and in the area of the semimembranosus and semitendinosus muscles in the hind limbs. FISS are

most commonly located in the subcutis, but also can occur intramuscularly (Dubielzig et al., 1993; Hendrick and Brooks, 1994; Graf et al., 2018). FISS can develop as early as four months and up to two to three years after an injection (Srivastav et al., 2012).

Fig. 3. Cat with feline injection-site sarcoma. Courtesy of Johannes Hirschberger, Ludwig Maximilians University, Munich, Germany

In the last 20 years, an epidemiological association has been demonstrated between vaccinations and the later development of FISS (Hendrick et al., 1992, 1994; Kass et al., 1993, 2003; Macy, 1995; Dean et al., 2006). The incidence of FISS has been estimated at 1 to 4 in every 10,000 vaccinated cats in USA (Coyne et al., 1997; Gobar and Kass, 2002; Ladlow, 2013), and the ratio of injection-site to non-injection-site sarcomas increased from 0.5 in 1989 to 4.3 in 1994 (Doddy et al., 1996). In one study in the USA, reported rates were 0.3 FISS per 10,000 vaccines and 11.8 postvaccinal inflammatory reactions per 10,000 vaccinations in cats (Gobar and Kass, 2002). If inflammatory reactions are a necessary prelude to FISS, then these rates suggest that 1 in 35 to 40 inflammatory reactions develop into FISS. In Poland, the prevalence of FISS was estimated to be 16 in 10,000 cats in general practice and 85 in 10,000 cats in practices specialising in oncology (Kliczkowska et al., 2015). In the UK, the incidence of FISS seems to be relatively low (incidence risk of FISS per year was estimated to be 1 in 16,000-50,000 cats registered by practices, 1 in 10,000-20,000 cat consultations, and 1 in 5,000-12,500 vaccination visits) (Dean et al., 2013). One reason for the low rate might be that rabies vaccination is not a routine procedure for cats in UK. One study in Canada compared the annual prevalence of feline postvaccinal sarcomas among 11,609 feline skin mass submissions from 1992 to 2010 and revealed no decrease in disease prevalence or increase in age of affected cats in response to change in vaccination formulation or recommended changes in feline vaccination protocols (Wilcock et al., 2012). In contrast, studies in Switzerland demonstrated a marked drop in the relative frequency of fibrosarcoma diagnoses (Graf et al., 2015, 2016, 2018).

Pathogenesis

Despite extensive research, there is no definitive proof of the pathogenesis of FISS. The most widely accepted hypothesis suggests that a chronic inflammatory reaction at the site of an injection acts as a trigger for subsequent malignant transformation. The mechanism by which the inflammatory reaction causes tumour formation is not fully understood. Growth factors can promote proliferation, can induce malignant transformation, and also can be involved in the regulation of angiogenesis. Overexpression of growth factors and oncogene activation have been demonstrated in cats with FISS and are suspected to play a role in tumour development (Hendrick, 1998, 1999; Nieto et al., 2003). Fibroblasts can undergo neoplastic transformation through different mechanisms, such as activation of oncogenes and inactivation of tumour suppressor genes. Chronic inflammation can induce production of free radicals and metabolites that cause DNA damage and mutations, acting as an initiator of carcinogenesis. The environment provided by chronic inflammation, coupled with a genetic predisposition, alters the susceptibility to carcinogenic injuries (O'Byrne and Dalgleish, 2001). One study showed that cyclooxygenase-2 (COX-2) is expressed within FISS and that there is a close relationship between COX-2 expression and the degree of inflammation (Santelices Iglesias et al., 2018). Cyclooxygenase-2 participates in the synthesis of arachidonic acid derivatives, including prostaglandin E2, which is related to carcinogenic processes (Williams et al., 1999; O'Byrne and Dalgleish, 2001). Overexpression of

COX-2 is associated with tumour proliferation and invasion, inhibition of apoptosis, suppression of immune surveillance, and angiogenesis (Williams et al., 1999). Activation of the nuclear factor-kappa B- (NF-κB-) signaling pathway can target genes associated with tumour progression and up-regulate expression of tumour-promoting cytokines and survival genes in tumours (Hsueh et al., 2019). Additionally, high expression rate of nuclear NF-κB p65 in FISS and dose-dependent inhibitory effects on the growth of FISS primary cells treated with NF-κB inhibitor suggested a role of NF-κB in FISS development (Hsueh et al., 2019).

It has been suggested that especially adjuvanted vaccines would be linked to the development of FISS due to the more intense local inflammation associated with such products. Adjuvants, such as aluminium, has been identified in histological or ultrastructural studies of FISS biopsy samples (Hendrick et al., 1992; Madewell et al., 2001; Deim et al., 2008). In most inactivated vaccines, an adjuvant is added to enhance the inflammation at the site of injection, which is intended and necessary when applying a killed agent in order to trigger the necessary immune response. However, this inflammation might potentially lead to malignant transformation. Traces of adjuvants can be seen in the inflammatory reaction, specifically accumulated within macrophages or multinucleate giant cells, and later in histological sections of FISS in the transformed fibroblasts (Hendrick et al., 1992). Intracellular crystalline particulate material was found in an ultrastructural study in five of 20 investigated FISS, and in one of the five cases was identified as aluminium-based (Madewell et al., 2001). Although no specific vaccine or adjuvant has been incriminated (Kass et al., 2003), local irritation from adjuvant is thought to potentially stimulate fibroblasts to the point that malignant transformation could occur.

At first, only rabies and feline leukaemia virus (FeLV) vaccines were identified as risk factors (Kass et al., 1993; Hendrick et al., 1994; Coyne et al., 1997), but subsequently other vaccines, including vaccines against feline panleukopenia virus (FPV), feline herpesvirus (FHV), and feline calicivirus (FCV) also were found to be involved in the development of FISS in some cases (Hendrick et al., 1994; Lester et al., 1996; Burton and Mason, 1997; Coyne et al., 1997; DeMan and Ducatelle, 2007). In addition to vaccines, injections of long-acting drugs, e.g., glucocorticoids, penicillin, lufenuron (Esplin et al., 1999; Gagnon, 2000; Kass et al., 2003; Srivastav et al., 2012), cisplatin (Martano et al., 2012), or meloxicam (Munday et al., 2011) have been associated with sarcoma formation. In one study, the frequency of administration of long-acting corticosteroid injections (dexamethasone, methylprednisolone, and triamcinolone) was significantly higher in cats with FISS in the interscapular region than in control cats (Srivastav et al., 2012). Fibrosarcomas were also reported at the site of a deep, non-absorbable suture in one cat (Buracco et al., 2002); derived from a granulomatous inflammation caused by a foreign body in one cat; around a surgical swab in the abdomen of one cat (Haddad et al., 2010); adjacent to the site of microchip implantation in two cases (Daly et al., 2008; Carminato et al., 2011); and associated with a subcutaneous fluid port device (McLeland et al., 2013). This suggests that all inflammatory reactions, theoretically, have the potential to lead to the development of FISS through triggering uncontrolled proliferation of fibroblasts and myofibroblasts, which, in some cases, results in malignant transformation.

Although many causes of inflammation are associated with FISS development, the risk seems to be higher for vaccines compared to other injections; amongst vaccines, the risk seems to be higher when adjuvanted vaccines are used. One study in Switzerland showed a marked decrease in the number of FISS in the recent years, and the authors of that paper conclude that the introduction of a non-adjuvanted FeLV vaccine in 2007 might be the reason for the decrease (Graf et al., 2018); however, even if there is an association it is not proof of a causal relationship and other factors were not addressed (e.g., awareness of good vaccination practices, such as not applying cold vaccines and vaccination only if necessary). One study compared associations between vaccine types and other injectable drugs with the development of FISS in a case-control study of 181 cats with soft tissue sarcomas (cases), 96 cats with tumours at non-vaccine regions (control group I), and 159 cats with basal cell tumours (control group II). There was an association between the administration of various types of vaccines and other injectable products (e.g., long-acting corticosteroids) and FISS development. Of 192 sarcomas, 101 had vaccinations at the site of tumour development during the preceding three years, and 23 had received other injections (Srivastav et al., 2012). This study also showed that adjuvanted inactivated vaccines were significantly more commonly associated with FISS development than other vaccines; of 35 vaccinated cats with sarcoma on the hind limb, 25 had received adjuvanted vaccines, seven cats had received modified live virus (MLV) vaccines (FPV, FHV-1 and FCV), and only one cat had received a recombinant vaccine. However, these findings also indicate that no vaccines were risk-free and that other factors also can be associated with the development of FISS (Srivastav et al., 2012).

Although an epidemiologic association has been demonstrated between FeLV vaccination and a higher risk of FISS, the possible role of FeLV and its mutant feline sarcoma virus (FeSV) in the development of FISS has not been demonstrated by the presence of either FeLV or FeSV in tumours (Ellis et al., 1996). One study from Brazil reported positive immunohistochemistry staining for FeLV p27 antigen in nine of 21 FISS (Carneiro et al., 2019); however, the method used (polyclonal antibodies for histology) has most likely low specificity and thus the significance of the results must be doubted.

Furthermore, no other viruses, including feline immunodeficiency virus, feline foamy virus, polyomaviruses or papillomaviruses, were detected in tumour tissues (Kidney et al., 2000, 2001a, 2001b, 2002). No evidence was found for the replication or expression of endogenous retroviruses being involved in FISS formation (Kidney et al., 2001a, 2001b).

The observation that not all cats develop FISS after vaccination suggests that there might be a genetic predisposition. It has been proposed that there is a higher incidence of FISS in siblings of affected cats, and that some cats tend to develop more than one FISS.

Alterations with unknown relevance, such as hyperploidy (Kalat et al., 1991), translocations (Mayr et al., 1996) and triploidy (Mayr et al., 1991) of oncogene and tumour suppressor loci have been found on extra chromosomes and monosomic chromosomes in affected cats. Mutations have been identified in the tumour suppressor gene p53, which is implicated in cancer initiation and progression in sarcoma tissue of cats with FISS (Mayr et al., 1995, 1998; Nambiar et al., 2000, 2001; Banerji and Kanjilal, 2006). A case-control study (50 domestic shorthair cats with a confirmed diagnosis of FISS and 100 disease-free matched controls) investigating a possible association between polymorphisms in the genomic sequence of the feline p53 gene and a predisposition to FISS, found a strong association between FISS and the presence of specific nucleotides at two of the polymorphic sites (Banerji et al., 2007). However, another study, conducted in Germany could not reproduce these findings and observed no association with the polymorphisms described (Mucha et al., 2014).

Clinical signs

FISS are tumours characterized by invasive local growth in the subcutis (rarely within the muscles), often with spread along fascial planes (Hirschberger and Kessler, 2001). Most FISS are fibrosarcomas (Doddy et al., 1996), but other malignancies, such as osteosarcomas (Esplin et al., 1993), chondrosarcomas (Hendrick and Brooks, 1994), rhabdomyosarcomas (Hendrick and Brooks, 1994), malignant fibrous histiocytomas (Esplin et al., 1993; Hendrick and Brooks, 1994), and myofibroblastic sarcomas (Dubielzig et al., 1993) have also been described. FISS are usually firm, indolent, seemingly well-circumscribed, subcutaneous masses that are often not freely moveable.

FISS behave more aggressively than sarcomas at other sites (Hendrick et al., 1994). The rate of metastasis ranges from 10% to 28% (Couto and Macy, 1998; Hershey et al., 2000). The lung is the most common site of metastasis, followed by regional lymph nodes and abdominal organs, such as kidney, spleen, intestine and liver (Sandler et al., 1997; Kobayashi et al., 2002).

Diagnosis

In contrast to fibrosarcomas in other areas, FISS have different histological characteristics with typical perivascular infiltration of lymphocytes and macrophages at the tumour periphery, a central area of necrosis, inflammation and local infiltration of tumour cells (Fig. 4) (Doddy et al., 1996; Madewell et al., 2001).

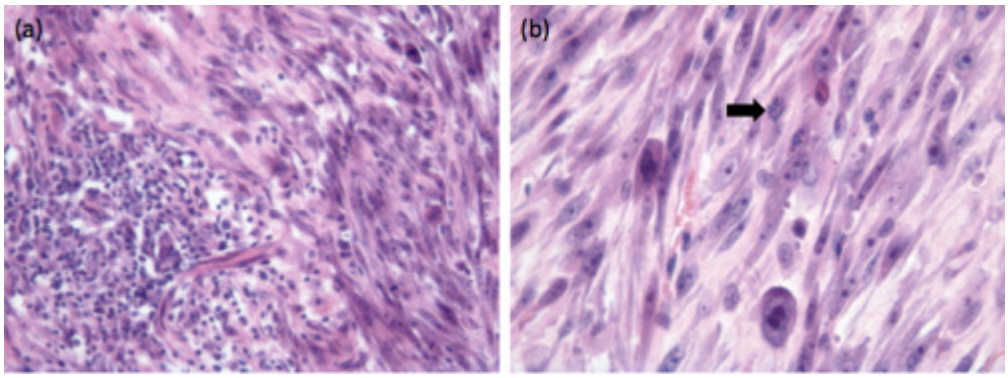

Fig. 4. Histological sections of a 2 cm diameter mass removed from the lateral thorax of a 13-year-old domestic shorthair cat. A similar interscapular mass had been removed from this cat 2 months previously. (a) A focus of lymphoplasmacytic inflammation is contained within the surrounding sarcoma. (b) Higher magnification of the neoplastic tissue reveals a pleomorphic population of neoplastic spindle cells with occasional giant nuclei and irregular mitotic activity (arrow). Haematoxylin and eosin stain. Courtesy of Michael Day, School of Veterinary Sciences, University of Bristol, United Kingdom

In CT, common features of FISS are a marked local invasiveness of the musculature and heterogeneity of the tissue in the periphery of the neoplasia (Travetti et al., 2013).

Treatment

Multimodal therapy, incorporating combinations of surgery, radiation therapy, and sometimes chemotherapy or immunotherapy, is recommended (Saba et al., 2017). Appropriate treatment should most importantly first include staging and careful planning of the surgery (Ladlow, 2013), because aggressive, radical excision is crucial to avoid tumour recurrence. The prognosis improves if, in addition to radical surgery, additional treatments such as radiotherapy, chemotherapy or immunotherapy are used. Preoperatively,

(contrast-enhanced) computed tomography (CT) or magnetic resonance imaging (MRI) should be obtained for staging and to determine the extent of the tumour and the size of the radiation field required to maximize the chance of a successful outcome (Rousset et al., 2013). CT or ultrasound can also assess peritumoural inflammation (Zardo et al., 2016) and CT angiogram and MRT can aid to target peritumoural lesions (Nemanic et al., 2016; Löhr et al., 2021), but neither CT angiography nor MRI imaging features can predict the tumour type and grade of FISS (Fleming et al., 2019). Using CT, a dynamic approach has been suggested for tumour volume measurement to optimally evaluate invasiveness of FISS (Longo et al., 2018). It was shown that the actual size of tumours determined by CT can be up to twice the size estimated at physical examination (McEntee, 2000; Martano et al., 2011; Ferrari et al., 2017), and the size of the tumour is considered one of the most important prognostic markers (Porcellato et al., 2017).

Surgeons should attempt to achieve complete, *en bloc*, surgical tumour resection with at least 3 cm (better 5 cm) margins (Phelps et al., 2011) and the removal of one fascial plane underlying the tumour, because incomplete resection can result in recurrence as early as two weeks after surgery (Lester et al., 1996; Scherk et al., 2013). Tumour-free margins are very important to achieve a longer disease-free interval, which was 700 days when complete tumour excision was accomplished in addition to adjuvant radiation therapy, but only 112 days for incomplete resection in addition to adjuvant radiation therapy (Cronin et al., 1998). However, even with clean surgical margins, the recurrence rate can be as high as 50% (McEntee and Page, 2001; Giudice et al., 2010). A retrospective analysis of cats with FISS located on the chest or abdominal wall or the interscapular region using a standardized radical resection technique with 3 cm lateral margins found that cats operated on for recurrent tumours were significantly more likely to die from tumour-related reasons compared with patients with *de novo* tumours, and that cats operated on for tumour recurrences had a significantly higher chance of another recurrence. In addition, tumour bed biopsies that did not contain tumour cells were associated with a significantly lower recurrence rate compared with those with tumour cells (Müller and Kessler, 2018). It has to be considered that significant decreases in surgical margin length occur following excision and subgross evaluation of tumour-free margins overestimates the actual (histologic) tumour-free margins (Terry et al., 2017), while shrinkage of FISS volume following excision and formalin fixation seems to be negligable (Terry et al., 2016).

Treatment using surgical excision alone has a recurrence rate of up to 70%, with tumour regrowth usually occurring in the first six months after surgery (Hendrick et al., 1994), but preoperative or postoperative radiation therapy significantly decreases recurrence rates and prolongs remission times (Cronin et al., 1998; Kobayashi et al., 2002; Steger-Lieb et al., 2002; Eckstein et al., 2009; Mayer et al., 2009). One study evaluating 22 cats retrospectively suggested that iridium-192 brachytherapy might also be useful as adjuvant treatment (Bloch et al., 2020), but larger prospective studies are necessary.

On the other hand, chemotherapy in addition to surgery is less effective. However, two studies showed some benefits of chemotherapy. In one study, a certain efficacy of three epirubicin doses before and after surgery was demonstrated, compared to outcomes of historical controls (Bray and Polton, 2016). Doxorubicine was also suggested as adjuvant treatment (Petznek et al., 2014). In a randomized multicenter study, liposome-encapsulated doxorubicin (LED) and doxorubicin (DOX) treatment in addition to surgery (but without radiation) led to a prolonged median disease-free interval when compared to surgery alone, with no difference in efficacy between LED and DOX (Poirier et al., 2002). New approaches, such as combining doxorubicin (DOX)-loaded phosphatidyldiglycerol-based thermosensitive liposomes with local hyperthermia, also hold some promise (Zimmermann et al., 2016). One study used both bleomycin and cisplatin as an adjuvant electrochemotherapy (ECT) protocol and demonstrated superior rates of tumour-free survival and disease-free interval (Spugnini et al., 2020). On the other hand, the tyrosine kinase inhibitor toceranib, which is licensed for the treatment of canine mast cell tumours, did not lead to a clinical response in cats with FISS (Holtermann et al., 2017). FISS tissue γH2AX immunohistochemistry can indicate DNA damage, and γH2AX expression might be useful to determine chemosensitivity (Kang et al., 2017), but further studies are needed. Thus, chemotherapy mainly remains an option for palliative treatment in cats with non-resectable FISS, when radiation therapy is not available (Zabielska-Koczywąs et al., 2017).

Additional immunotherapy appears to be a promising option (Jahnke et al., 2007; Huttinger et al., 2008; Jas et al., 2015). Results of prospective randomized controlled studies on cytokine gene transfer techniques for adjuvant-immunological treatment of FISS showed reduced recurrence rates. In cats receiving gene therapy by the peritumoural administration of histo-incompatible Vero cells expressing human interleukin-2 in addition to surgery and radiation therapy, only five of 16 FISS cats (31%) had FISS recurrence, while eleven of 16 control cats (69%) that only had surgery and radiation therapy, but no immunotherapy, had FISS recurrence within 16 months (Quintin-Colonna et al., 1996). Use of neo-adjuvant gene therapy using a non-viral vector that expresses feline granulocyte-macrophage colony-stimulating factor (GM-CSF) or a combination of the feline genes GM-CSF, IL-2, and interferon-γ (IFN-γ) was well tolerated by cats (Jahnke et al., 2007; Huttinger et al., 2008) and showed promising results. Recombinant feline IL-2 is now commercially available in Europe for the treatment of FISS in combination with excision and radiation therapy. In a randomised controlled clinical trial, administration of a recombinant canarypox virus expressing feline IL-2 was well tolerated and resulted in a significantly longer median time to relapse and a significant reduction in the risk of relapse at one year and two years (Jas et al., 2015).

ABCD recommends that **cats that have or have had a FISS** should no longer receive any injectable vaccine (while otherwise limit the risk of infections) or any other avoidable injections.

Prognosis

Most importantly, size of the tumour influences prognosis (Porcellato et al., 2017), and radical excision is crucial for a favourable outcome, and thus, prognosis is primarily influenced by the tumour site and its accessibility to surgery and the possibility of obtaining tumour-free margins (Lester et al., 1996; Cronin et al., 1998; Phelps et al., 2011; Scherk et al., 2013). Therefore, pre-operative diagnostic imaging (contrast-enhanced CT and/or MRI) is extremely important to determine the true extent of the tumour (Rousset et al., 2013). Prognosis is better if, in addition to radical surgery, further therapeutic options such as radiotherapy (Cronin et al., 1998; Kobayashi et al., 2002; Steger-Lieb et al., 2002; Eckstein et al., 2009; Mayer et al., 2009) or immunotherapy (Jahnke et al., 2007; Huttinger et al., 2008; Jas et al., 2015) are applied. In cats undergoing postsurgical curative or coarse-fractionated radiation therapy, factors predictive of a better outcome included lack of visible mass as opposed to macroscopic disease, adjuvant chemotherapy for gross disease, and a smaller number of surgeries preceding radiation therapy (Eckstein et al., 2009). In another study looking at prognostic parameters, it was determined that neutrophil-to-lymphocyte ratio, white blood cell count and neutrophil count were prognostic parameters for local recurrence of FISS, with white blood cell count being the parameter with the greatest impact (Chiti et al., 2020).

Prevention

Prevention consists of three general considerations (Table 1). First, injections in cats should always be given at sites at which surgery (such as amputation of a limb or excision of lateral abdominal skin) would likely lead to a complete cure with the least complicated surgical procedure. Second, general recommendations to reduce the inflammatory reaction at injection sites should be followed, such as avoiding the administration of irritating substances. And third, it is advised to vaccinate as often as necessary, but as infrequently as possible (e.g., according to the principles of current vaccination guidelines, such as avoiding FeLV vaccination in already FeLV-infected cats or feline panleukopenia vaccination in cats with pre-existing antibodies against FPV).

In general, injecting distally in a leg aids in the treatment of subsequent sarcomas (by amputation of the leg) because these tumours are very difficult to excise completely and often recur after resection (Macy, 1995). Administration of vaccines (or other injections) between the scapulae is generally contraindicated because tumour resection is almost impossible in this location. To assess the acceptance of the Vaccine-Associated Feline Sarcoma Task Force of the American Association of Feline Practitioners (AAFP) recommendation (published in 1999) by veterinarians, a study including 392 cats with FISS compared the anatomical locations of tumours between cases with FISS diagnosed before and after the publication of these recommendations (Vaccine-Associated Feline Sarcoma Task Force, 1999). Comparing the prevalence of cases arising before and after the publication of the vaccination recommendations, the proportions of FISS significantly decreased in the interscapular (53% to 40%) and right and left thoracic (10% to 4% and 9% to 1%, respectively) regions, whereas the proportions of FISS significantly increased in the right thoracic limb (1% to 10%) and the combined regions of the right pelvic limb with the right lateral aspect of the abdomen (13% to 25%) and the left pelvic limb with the left lateral aspect of the abdomen (11% to 14%). Thus, despite publication of the vaccination recommendations, a high proportion of tumours still developed in the interscapular region. There was also an increase in lateral abdominal FISS, which could be attributable to aberrant placement of injections intended for the hind limbs. Thus, veterinarians are complying with vaccination recommendations to some extent, but only the administration of vaccines as distally as possible on a limb would allow for complete surgical margins if limb amputation is required (Shaw et al., 2009). Data in Europe show a similar situation. In a study examining the location of FISS in cats presented to the oncology service at the University teaching hospital in Munich, most FISS still occurred between the scapulae (40%), followed by the right (19%) and left thoracic walls (13%) (Haas, 2009). A study from Brazil, investigating anatomical location of biopsy samples compatible with FISS submitted between 2007 and 2017, found that 34.9% of the tumours were located on the thoracic wall, 29.2% in the flank, 21.3% in the interscapular region and 14.6% in the limbs (Cecco et al., 2019).

A cross-sectional study in the UK determined cat owners' attitudes towards surgical treatments of different anatomical regions. However, less than half of the owners (39%) would pursue surgery regardless of tumour site. One percent would not pursue surgery. Of the remainder, respondents would not allow amputation of the forelimb (20%), hindlimb (15%) or tail (15%). On the other hand, the majority of respondents were willing to travel up to 100 miles for radiotherapy or chemotherapy (66 and 69%, respectively) (Carwardine et al., 2014). Thus, owner education by the veterinarian explaining optimum treatment options is important.

Unfortunately, there is still a lack of information to provide evidence-based vaccine site recommendations. The majority of safety and efficacy data comes from licensing studies in which vaccines are administered subcutaneously in the interscapular region, which should not be used for any injection in field cats. Current research indicates that radical surgical resection of injection-site sarcomas including margins of at least 3 cm, but preferably 5 cm (Phelps et al., 2011), is associated with the highest response rate and long-term survival (Hershey et al., 2000). With this in mind, the AAFP panel on vaccination guidelines conducted an informal survey of veterinarians whose practices focused on radiation (12), surgical (36), and medical (44) oncology for opinions on what the preferred vaccination sites should be (Scherk et al., 2013). These experts agreed that distal to the stifle followed by distal to the elbow were their preferred sites. Nearly as popular was the tail. Respondents frequently commented that vaccines should be administered as low on the leg as possible. They

added that vaccination of cats resting in a crouched position often results in inadvertent injection of the skin fold of the flank, resulting in tumours that are difficult to resect (Scherk et al., 2013). This is reflected in the recent paper that found an increase in lateral abdominal injection-site sarcomas since the publication of the Vaccine Associated Feline Sarcoma Task Force vaccination recommendations in 1999 (Phelps et al., 2011). Based on this, the AAFP recommends in their guidelines, that vaccines against FPV, FHV-1, and FCV should be administered below the right elbow, FeLV vaccines should be administered below the left stifle, and rabies vaccines should be administered below the right stifle (Scherk et al., 2013). So far, vaccination in the tail was not considered a practical option. However, a pilot study demonstrated that vaccination in the tail is well tolerated by cats and that the tail-vaccinated cats developed an antibody response comparable to that observed following injection of the vaccine distally in the leg (Hendricks et al., 2014), and so further studies should be performed to confirm that this would be an alternative option leading to equal protection rates.

In addition to considering appropriate injection sites, post vaccination monitoring plays an important role. Vaccination sites should be recorded (Day et al., 2016), and veterinarians should instruct their clients to monitor vaccination (or injection) sites for swelling or lumps in order to detect potential sarcomas early and at a time while they still can be removed successfully.

Veterinarians should be aware that **any skin or subcutaneous mass** in a cat requires further diagnostic and interventional approaches. At the very least, owners should follow the "**3-2-1**"-rule: Incisional wedge biopsies or total removal and histological examination of any mass is warranted if the mass is still present **three** months after vaccination or if the mass becomes larger than **two** cm in diameter or if the mass is increasing in size **one** month after vaccination. In general, diagnostic investigation is warranted when any cutaneous mass is noted in a cat. FISS are usually firm, indolent, seemingly well-circumscribed, subcutaneous masses that are often not freely moveable.

Concerning general recommendations to prevent inflammatory reactions at injection sites, there are a few rules to follow. Generally, cats should receive as few subcutaneous injections as possible. Intramuscular injections in cats should be avoided because intramuscular tumours develop with a similar frequency, but are more difficult to detect early. Whenever feasible, cats should receive drugs orally or intravenously. The subcutaneous injection of long-acting irritating substances (such as long-acting glucocorticoids) should be avoided.

One study examined potential risk factors when administering vaccines (Kass et al., 2003), and few factors were associated with the development of FISS. It was observed that the size of the needle and the syringe, the velocity of injection, and whether manual pressure was applied after injection or not, played no role. In contrast, the temperature of the vaccine made a significant difference, with cold vaccines being associated with a higher risk of FISS development than vaccines at room temperature (Kass et al., 2003). Thus, vaccines should be taken out of the refrigerator about 15 minutes before injection, but not much longer to avoid reduction in vaccinal efficacy. In addition, multi-dose vaccine vials, i.e. 10 doses of the same vaccine within one vial, that are only rarely used in cats nowadays, were associated with a higher risk of FISS development (Kass et al., 2003).

Regarding the risk of FISS development, intranasal vaccines are to be preferred over injectable vaccines in cats. However, in most countries, only injectable vaccines are available. Therefore, vaccines are preferred that cause the least subcutaneous inflammatory reaction. Currently, there is insufficient information to make definitive recommendations on the vaccine type (Kass, 2018), however it is the consensus of the ABCD expert group that, with the current state of knowledge, vaccines without adjuvants should be preferred over adjuvant-containing vaccines, if these are available and as long as they have been proven to be equally effective.

It has been shown that recombinant canarypox-vectored vaccines cause less inflammation at the injection site by an extensive study investigating the subcutaneous tissue response following the administration of a single dose of multi-component vaccines. Three groups of 15 cats were injected with one of three vaccines or saline as a negative control; cats in group A received a non-adjuvanted recombinant canarypox-vectored FeLV vaccine; cats in group B received a FeLV vaccine with a lipid-based adjuvant; cats in group C were vaccinated with a FeLV vaccine adjuvanted with an alum-Quil A mixture. On days 7, 21 and 62 post-vaccination, significantly less inflammation was associated with administration of the non-adjuvanted recombinant canarypox-vectored vaccine. The inflammation was most severe in cats receiving aluminium-based adjuvant. Cats receiving adjuvanted vaccines had evidence of residual adjuvant material accumulated within macrophages even at 62 days post-vaccination (Day et al., 2007). As described earlier, in a case-control study comparing associations between vaccine types with development of FISS, adjuvanted inactivated vaccines were significantly more commonly associated with FISS development than other vaccines; of 35 vaccinated cats with sarcoma on the hind limb, 25 cats had received adjuvanted vaccines, seven cats had received MLV vaccines (FPV, FHV-1, and FCV), while only one cat had received a recombinant canarypox-vectored vaccine (Srivastav et al., 2012).

Finally, to prevent development of FISS, cats should be vaccinated only as frequently as necessary. Therefore, long vaccination intervals should be applied in adult animals where possible, vaccines (such as rabies vaccines and FPV vaccines) that are licensed for three- or even longer boosters should be preferred, no FeLV or rabies vaccinations should be administered to indoor-only cats, and immune cats should not be vaccinated (e.g., if presence of antibodies, e.g., against FPV, is detected). This confirms the necessity of individual vaccination schedules.

TABLE 1: IMPORTANT CONSIDERATIONS TO AVOID FELINE INJECTION-SITE SARCOMA (FISS) DEVELOPMENT
Vaccination of cats provides essential protection and should not be stopped because of the risk of FISS.
Vaccines are not the only injectable medical products associated with FISS.
A tailored vaccination schedule is important for each cat. Cats should be vaccinated only as often as necessary in accordance with current guidelines.
Appropriate sites for injections should be selected. The interscapular region as well as the lateral thoracic wall should generally be avoided for any injection. Vaccines should be injected at a site where a mass can be easily surgically removed, preferably distally in a limb.
Vaccines should be brought to room temperature prior to administration, but should not be kept unrefrigerated for hours.
Multi-dose vaccine vials should not be used in cats.
Subcutaneous injection is preferred to intramuscular injection.
Non-adjuvanted vaccines are generally preferred over those containing adjuvant. Thus, modified-live vaccines or recombinant vaccines, that usually do not contain an adjuvant, are preferred over inactivated vaccines that typically contain adjuvant, if available and have proven equal efficacy. Vaccines with a long duration of immunity should be preferred over those with a short duration of immunity.
Generally, any skin or subcutaneous mass in a cat requires further diagnostics. Specifically, thorough post-vaccination monitoring should be performed. Any lump at the site of injection that is still present three months after vaccination, or that is larger than two cm in diameter, or that is increasing in size one month after vaccination, should be surgically removed and investigated by histopathology.

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