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. These tumours develop at sites of vaccination or of injection of other substances. 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 fully understood. There is neither definitive causal relationship that explains their occurrence nor a direct link with vaccination. The most widely accepted hypothesis suggests that chronic inflammatory reaction at the site of injections provides a trigger for subsequent malignant transformation.
- Aggressive, radical excision is required to avoid tumour recurrence.
- The prognosis improves if additional radiotherapy and/or immunotherapy and/or chemotherapy are used in addition to surgical excision.
- To prevent FISS development, several recommendations should be followed:
  - Although vaccinations can be associated with FISS formation, immunisation of cats with various vaccines provides essential protection and should not be stopped because of the risk of FISS. It is important to realize that vaccines are not the only injectable medical products associated with FISS.
  - However, an individually tailored vaccination schedule is important for each cat. Cats should be vaccinated only as often as necessary in accordance with current guidelines.
  - Mucosal intranasal vaccines are preferred over injectable vaccines, if available.
  - Injectable 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.
  - Vaccines providing a long duration of immunity are preferred over those with a short duration of immunity.
  - 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 sites where any subsequent mass could be easily surgically removed, preferably distally in a limb.
  - Generally, any skin or subcutaneous mass in a cat requires further diagnostics. Specifically, thorough post-vaccination monitoring should be performed considering the “3-2-1”-rule: Any lump at the site of injection
that is still present three months after vaccination, or that is larger than 2 cm in diameter, or that is increasing in size one month after vaccination should be surgically removed and investigated through histopathology.

- ABCD recommends that veterinarians carry out a risk-benefit analysis for each vaccine to avoid unnecessary vaccination of cats, to monitor the injection site, and to report all vaccine-associated adverse events to the manufacturer and/or to their competent authority.

- Considering injectable vaccines, there has been much discussion of whether non-adjuvanted vaccines should be generally preferred over those containing adjuvant, if available and with proven similar efficacy. However, currently, there is insufficient information to make definitive recommendations on the vaccine type. The current knowledge is as follows:
  - It is a fact that every injectable vaccine can cause FISS.
  - It is a fact that vaccines containing adjuvant cause more inflammation than vaccines without adjuvants.
  - It has been reported that inflammation might contribute to FISS development.
  - There are conflicting data on whether vaccine adjuvants truly are associated with increased risk of FISS development.
  - It is currently difficult to truly compare vaccine efficacy studies because of differences in study designs.
  - Therefore, there is insufficient information to make definitive recommendations on the vaccine type and it is important to consider efficacy data. Where efficacy studies have shown higher protection rates in cats vaccinated with adjuvanted vaccines versus non-adjuvanted vaccines, the former are preferable in cats at high risk of infection. Otherwise, given the current state of knowledge, if vaccines without adjuvants have been proven to be equally effective as adjuvant-containing ones, it is reasonable to suggest that vaccines without adjuvants should be preferred, at least until the pathogenesis of FISS is better understood.

Epidemiology

Vaccination of cats always has received scientific and public attention following the supposition that a range of rare vaccine-associated adverse events (VAAEs) 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) (Hosie et al., 2013; Hartmann et al., 2023; ABCD Guideline for Adverse Reactions to Vaccination). A task force has been instituted and is regularly updated in the USA to help veterinarians understand, manage, and prevent these tumours (Morrison and Starr, 2001; Vaccine-Associated Feline Sarcoma Task Force, 2005). In fact, it was mainly the occurrence of FISS that increased the concern among cat owners about the safety of vaccines in general and rekindled the discussion on VAAEs in cats. Despite extensive research on the pathogenesis of these sarcomas, there is neither proof of definitive causal relationship that explains their occurrence nor of a direct link with 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).
Around 1990, an increased incidence of tumours in cats that developed at injection sites was first reported in the USA (Hendrick and Dunagan, 1991; Hendrick and Goldschmidt, 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 other non-vaccinal injectables could also be associated with this type of tumour has led to reclassification of these neoplasms as “feline injection-site sarcomas” (FISS). It has been suggested that injections in cats induce repeated local irritation with chronic inflammation, which can act as a precursor to local neoplastic transformation of stromal cells. 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 rarely 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 hindlimbs. 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. Provided by Katrin Hartmann, LMU, 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; Kass et al., 1993; Hendrick and Brooks, 1994; Macy, 1995; Kass et al., 2003; 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 from 1998 to 2000 in the USA and Canada, the reported rates were 0.3 cases of FISS per 10,000 vaccines and 11.8 post-vaccinal 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. During 2008–2013 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 specialised in oncology (Kliczkowska et al., 2015). In the UK, the incidence of FISS seems to be relatively low (in 2007, the 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 post-vaccinal 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 formulations (introduction of recombinant non-adjuvanted rabies (and later FeLV) vaccines) or recommended changes in feline vaccination protocols (e.g., calling for more selective use of FeLV vaccination and less frequent rabies vaccination) (Wilcock et al., 2012). In contrast, studies in Switzerland demonstrated a marked drop in the relative frequency of fibrosarcoma diagnoses from 2005 to 2014 (Graf et al., 2018).

Pathogenesis

Despite extensive research on the pathogenesis of FISS, there is no definitive causal relationship that explains their occurrence nor a direct link with vaccination. The most widely accepted hypothesis suggests that chronic inflammatory reaction at the site of an injection provides a trigger for subsequent malignant transformation (Day et al., 2007). The mechanism by which the inflammatory reaction might cause 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). Generally, COX-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-kB) signalling 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-kB p65 in FISS and dose-dependent inhibitory effects on the growth of FISS primary cells treated with NF-kB inhibitors suggested a role of NF-kB in FISS development (Hsueh et al., 2019). Activation of the Janus kinase signal transducer and activator of transcription 3 (STAT3) also might play an important role in the tumorigenesis of FISS (Shih et al., 2022). Interestingly, it was shown via immunohistochemistry that the expression of estrogen receptors in FISS, but not the expression of progesterone receptors, correlated with clinical and histopathological aspects (mitotic index and degree of pleomorphism) and thus, estrogen receptors expression could influence tumour growth (Zanuncio et al., 2021).

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. However, in a prospective multi-center case-control study from 2003, no specific vaccine or vaccine brand could be incriminated in the formation of FISS (Kass et al., 2003). Adjuvants are mostly added to enhance a pro-inflammatory reaction at the site of injection, which is intended and a necessity when applying a killed agent in order to trigger the required immune response. Adjuvants, such as aluminium, have been identified in histological or ultrastructural studies of FISS biopsy samples (Hendrick et al., 1992; Madewell et al., 2001; Deim et al., 2008), and it was thought that these adjuvants originated from adjuvant-containing vaccines. Traces of adjuvants can be seen in the inflammatory reaction, specifically accumulated within macrophages or multinucleate giant cells, as well as 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 material (Madewell et al., 2001). One study investigated the degree of inflammation after vaccination with adjuvant-containing vaccines versus vaccines without adjuvants. It showed that multi-component vaccines containing the recombinant canarypox-vector FeLV cause less inflammation at the injection site than multi-component vaccines with FeLV and an adjuvant. Three groups of 15 cats were injected with a single dose of one of the three the vaccines or saline as a negative control; cats in group A received a non-adjuvanted recombinant canarypox-vector FeLV vaccine; cats in group B received an inactivated FeLV vaccine with a lipid-based adjuvant; cats in group C were vaccinated with an inactivated 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-vectorized vaccine in group A compared to groups B and C. The inflammation was most severe in cats of group C receiving the aluminium-based adjuvant. Those cats receiving adjuvanted vaccines had evidence of residual adjuvant material accumulated within macrophages even at 62 days post-vaccination (Day et al., 2007). In 2020, a new 0.5 ml presentation of the non-adjuvanted recombinant canarypox-vectorized FeLV vaccine was registered in Europe. One study (Haist et al., 2023) assessed the local reactions for three months after subcutaneous injection of this 0.5 ml vaccine in comparison to a vaccine adjuvanted with aluminium-hydroxide and Quillaja saponaria (AO/H/Quil A) by physical examination, histopathology, and by non-invasive computed tomography (CT). Swelling reactions were more frequent, more pronounced, and long-lasting in the adjuvanted vaccine group. Microscopically, moderate to severe inflammatory reactions were observed in the adjuvanted vaccine group on days 7 and 21 post-injection and reactions were still present on day 84, while mild inflammatory lesions were observed in the non-adjuvanted vaccine group only on days 7 and 21. With the adjuvanted vaccine, inflamed areas were measurable by CT in all cats on days 7 and 21, whereas they were detected only on day 7 and only in 20% of cats from the non-adjuvanted vaccine group. On day 7, the mean inflamed volume was nearly 300 times larger in adjuvanted vaccine group (Haist et al., 2023). Thus, an association between adjuvants and more pronounced tissue inflammation has been shown; however, the association between inflammation and tumour formation is more difficult to demonstrate. It has been suggested that inflammatory cells have an impact on cancer development by increasing the invasive capacity, angiogenesis, and motility of tumour cells (Coussens and Werb, 2002). One paper evaluating metallothionein expression in FISS, demonstrated an association between Ki67 index (a marker of cell proliferation) and tumour grade and inflammatory score in FISS, suggesting that inflammation plays an important role not only in pathogenesis but also in tumour progression (Mikiewicz et al., 2023). At first, only rabies and 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; Srivastav et al., 2012). 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), and 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); at the site of a retained surgical sponge 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 (McLeod et al., 2013). This suggests that any inflammatory reactions independent of the stimulus, theoretically, could 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.

Many causes of inflammation could be associated with FISS development, and FISS can occur in animals following vaccination with inactivated, recombinant or modified live vaccines as well as with non-vaccine injections; e.g., in the UK, the Veterinary Medicines Directorate (VMD) received notifications of FISS in cats associated with different vaccine types. However, these reports did not take the number of injected vaccines into account (Davis et al., 2013, 2015, 2016; Dyer et al., 2007, 2008, 2009, 2010, 2011, 2012). In a prospective case-control study conducted at the University of California, Davis, USA, it was demonstrated that, among injections, vaccines were more frequently associated with FISS development compared to other compounds; amongst vaccines, the risk was significantly higher when adjuvanted vaccines were used (Srivastav et al., 2012). One study in Switzerland showed a marked decrease in the number of FISS from 2005 to 2014, when compared to older studies in the same region (Graf et al., 2015, 2016), and the authors of the paper concluded that the introduction of a non-adjuvanted FeLV vaccine in 2007 might have accounted for the decrease (Graf et al., 2018). However, the decrease in FISS incidence was in no relation to the non-adjuvanted vaccines sold at the time, and the timespan that is necessary for FISS development was not considered. Other factors were not addressed in the study, such as increased awareness of good vaccination practice, not injecting cold vaccines, and vaccinating only if necessary (e.g., Switzerland became rabies-free in 1999).

One study (Srivastav et al., 2012) 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 received vaccinations at the site of tumour development during the preceding three years, and 23 had received other injections at that site (Srivastav et al., 2012). This study also showed that adjuvanted-inactivated rabies vaccines were significantly more commonly associated with FISS development than recombinant rabies vaccines in the broad hindlimb region; of 35 vaccinated cats with sarcoma on the hindlimb, 25 had received inactivated vaccines (predominantly rabies), seven cats had received modified live virus (MLV) vaccines (FPV, FHV and FCV), and only one cat had received a recombinant rabies 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). The latter finding is also supported by the data from the UK VMD receiving notifications of FISS in cats vaccinated with MLV, inactivated, and recombinant vaccines (Davis et al., 2013, 2015, 2016; Dyer et al., 2007, 2008, 2009, 2010, 2011, 2012).

A possible role of FeLV and its mutant feline sarcoma virus (FeSV) in the development of FISS was not demonstrated by 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) had, most likely, a low specificity and thus, the significance of the results is doubtful. 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). Additionally, no evidence was found for the replication or the expression of endogenous retroviruses being involved in FISS development (Kidney et al., 2001a, 2001b).

The observation that only few 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). Thus, the significance of these polymorphisms in FISS development is unknown.

Clinical signs

FISS are tumours characterized by invasive local growth in the subcutis (rarely within the muscle), often with spread along fascial planes (Hirschberger and Kessler, 2001). Most FISS are fibrosarcomas (Dodd 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 rarely 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, intestines, and liver (Sandier 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).

In CT, common features of FISS are a marked local invasiveness into 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, 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) 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 (Roussset et al., 2013). CT or ultrasound can also assess peritumoural inflammation (Zardo et al., 2016), and CT angiogram and MRI can aid to target peritumoural lesions (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 (preferably 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 negligible (Terry et al., 2016). New techniques, such as optical coherence tomography (OCT), an imaging technology that uses light waves to generate real-time views of tissue architecture, can help to assess surgical margins correctly by allowing fast, high-resolution scanning of margins, even for less experienced people (Coleman et al., 2021).

Treatment using surgical excision alone has a recurrence rate of up to 70%, with tumour regrowth usually occurring within 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. In one case series including three cats, histotripsy, a non-invasive focused ultrasound therapy using controlled acoustic cavitation to mechanically disintegrate tissue, was used three to six days before surgery and was well tolerated (Ruger et al., 2023); this might be an interesting additional treatment option in the future.

On the other hand, chemotherapy in addition to surgery is less effective than radiation therapy in addition to surgery. However, two studies showed some benefits of chemotherapy. In one study, some efficacy of three epirubicin doses before and after surgery was demonstrated, compared to the outcomes of historical controls (Bray and Polton, 2016). Doxorubicin has also been suggested as adjuvant treatment (Petznek et al., 2014). In a randomized multicentre 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). Newer approaches, such as combining DOX-loaded phosphatidylglycerol-based thermosensitive liposomes with local hyperthermia, also hold some promise (Zimmermann et al., 2016). One study using both bleomycin and cisplatin as an adjuvant electrochemotherapy (ECT) protocol demonstrated superior rates of tumour-free survival and disease-free interval (Spugnini et al., 2020). In contrast to these approaches, the tyrosine kinase inhibitor toceranib, which is licensed for the treatment of canine mast cell tumours, did not lead to a positive clinical response in cats with FISS (Holtermann et al., 2017). Some in vitro studies looked into the efficacy of other chemotherapeutic drugs to inhibit growth of FISS cell lines, such as the proteasome inhibitor bortezomib (Laver et al., 2022), but in vivo studies are missing. Chemosensitivity can be determined by FISS tissue yH2AX immunohistochemistry that can indicate DNA damage, as yH2AX expression might be useful to determine chemosensitivity (Kang et al., 2017), but further studies are needed. Thus, in general, chemotherapy mainly remains an option for palliative treatment in cats with non-resectable FISS, when radiation therapy is not available (Zabieliska-Koczynska et al., 2017).

Additional immunotherapy appears to be a promising option (Jahneke 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 immunotherapy 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 (Jahneke 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 (and instead focus on limiting the risk of infections) or any other avoidable injections.

**Prognosis**

The prognosis of FISS is most importantly influenced by the size of the tumour (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; Scher et al., 2013). Therefore, preoperative 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 resection, further therapeutic options such as radiotherapy (Cronin et
necessitate aggressive tissue removal from the abdomen and abdominal cavity (Stone et al., 2020). Tail vaccination has also been used to facilitate amputation with 5 cm margins in two fascial planes in FISS cases (Stone et al., 2020). Ventral abdominal subcutaneous injections also have been used because of the perceived relative ease of tumour removal without the need for amputation. However, the need to remove two fascial planes and 5 cm margins would still necessitate aggressive tissue removal from the abdomen and abdominal cavity (Stone et al., 2020); thus, the technique is of limited prognostic value when a FISS has already been diagnosed.

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 triennial vaccination for FHV and FCV in cats with low risk of exposure, avoiding FeLV vaccination in already FeLV-infected cats and avoiding FPV vaccination in cats with pre-existing antibodies against FPV).

In general, injecting distally in the limb aids in the treatment of subsequent FISS (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) recommendations that include not injecting vaccines interscapularly (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%), in 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 hindlimbs. 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 of the LMU Small Animal Clinic in Munich, Germany, 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 35% of the tumours were located on the thoracic wall, 29% in the flank, 21% in the interscapular region, and 15% in the limbs (Cecco et al., 2019).

A cross-sectional study in the UK evaluated cat owners’ attitudes towards surgical treatments of different anatomical region: less than half of the owners (39%) would pursue surgery regardless of tumour site; 1% 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. 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 a study 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 distal limb injection to facilitate amputation with 5 cm margins in two fascial planes in FISS cases (Stone et al., 2020). Tail vaccination has also been
reported as being well tolerated and elicited acceptable antibody responses when compared to vaccination in the distal limbs (Hendricks et al., 2014). However, to facilitate 5 cm margins in case of FISS removal, vaccinations must be administered in the distal tail, something that might not be practical for most clinicians (Stone et al., 2020). Further studies should be performed to confirm that this would be an alternative option leading to similar vaccine-induced protection rates.

In addition to considering appropriate vaccination sites, post vaccination monitoring plays an important role. Vaccination sites should be noted in clinical records (Day et al., 2016; Stone et al., 2020), and veterinarians should instruct their clients to monitor vaccination (or injection) sites for swelling or lumps so that potential FISS can be detected early, and thus 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 2 cm in diameter or if the mass is increasing in size one month after vaccination. Fine-needle aspirates might not provide diagnostic cellular tissue, whereas excisional biopsies rarely meet the appropriate margins (5 cm in two fascial planes) as required in the case of FISS, thus increasing the morbidity and mortality risks associated with sarcoma invasion (Stone et al., 2020). In general, diagnostic investigation is warranted when any cutaneous mass is noted in a cat.

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 and be brought to room temperature before injection, e.g., by keeping the vial in the palm of the hand for a short time but should be kept only briefly out of the refrigerator to avoid reduction in vaccinal efficacy. For lyophilized vaccines, the diluent can be kept out of the refrigerator for a longer period. In addition, multi-dose vaccine vials, i.e., ten 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 if similar efficacy has been demonstrated. However, in most countries, only injectable vaccines are available. Therefore, the vaccines that are preferred are those that cause the least subcutaneous inflammatory reaction. Considering injectable vaccines, there has been much discussion of whether non-adjuvanted vaccines should be generally preferred over those containing adjuvant, if available and with proven similar efficacy. However, currently, there is insufficient information to make definitive recommendations on the vaccine type (Kass, 2018).

The current knowledge is as follows:

- It is a fact that every injectable vaccine can cause FISS (Kass et al., 2003; Srivastav et al., 2012).
- It is a fact that vaccines containing adjuvant cause more inflammation than vaccines without adjuvants (Day et al., 2007).
- It has been reported that inflammation might contribute to FISS development (Mikiewicz et al., 2023).
- There are conflicting data whether vaccine adjuvants truly are associated with increased risk of FISS development (Kass et al., 2003; Srivastav et al., 2012).
- It is currently difficult to truly compare vaccine efficacy studies because of differences in study designs.

Therefore, there is insufficient information to make definitive recommendations on the vaccine type and it is important to consider efficacy data. Where efficacy studies have shown higher protection rates in cats vaccinated with adjuvanted vaccines versus non-adjuvanted vaccines, the former are preferable in cats at high risk of infection. Otherwise, given the current state of knowledge, if vaccines without adjuvants have been proven to be equally effective as adjuvant-containing ones, it is reasonable to suggest that vaccines without adjuvants should be preferred, at least until the pathogenesis of FISS is better understood.

Finally, to prevent the development of FISS, cats should only be vaccinated as frequently as necessary, and long vaccination intervals.
should be applied in adult animals where possible. Although all vaccines are licensed with specific vaccination intervals, ABCD guidelines (Hosie et al., 2015) sometimes suggest longer intervals depending on the cats’ lifestyle. No non-core vaccines, such as FeLV, should be administered to cats without an exposure risk. In addition, immune cats should not be vaccinated (e.g., with a FPV vaccine if the presence of FPV antibodies is demonstrated as FPV antibodies are correlated with protection) (Bergmann et al., 2018; Egberink et al., 2022). This clearly demonstrates the necessity of individual vaccination schedules (Hosie et al., 2013; Stone et al., 2020).

Table 1: Important considerations to avoid feline injection-site sarcoma (FISS) development

<table>
<thead>
<tr>
<th>Consideration</th>
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<tbody>
<tr>
<td>Vaccination of cats provides essential protection and should not be stopped because of the risk of FISS. It is important to realize that vaccines are not the only injectable medical products associated with FISS.</td>
</tr>
<tr>
<td>An individually tailored vaccination schedule is important for each cat. Cats should be vaccinated only as often as necessary in accordance with current guidelines.</td>
</tr>
<tr>
<td>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.</td>
</tr>
<tr>
<td>Vaccines should be brought to room temperature prior to administration but should not be kept unrefrigerated for hours.</td>
</tr>
<tr>
<td>Multi-dose vaccine vials should not be used in cats.</td>
</tr>
<tr>
<td>Mucosal intranasal vaccines are preferred over injectable vaccines, if available.</td>
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<tr>
<td>Subcutaneous injection is preferred to intramuscular injection.</td>
</tr>
<tr>
<td>Among injectable vaccines, there is insufficient information to make definitive recommendations on the preferred vaccine type.</td>
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<tr>
<td>Vaccines with a long duration of immunity are preferred over those with a short duration of immunity.</td>
</tr>
<tr>
<td>Generally, any skin or subcutaneous mass in a cat requires further diagnostics. Specifically thorough post-vaccination monitoring should be performed (the “3-2-1”-rule): Any lump at the site of injection that is still present three months after vaccination, or that is larger than 2 cm in diameter, or that is increasing in size one month after vaccination, should be surgically removed and investigated through histopathology.</td>
</tr>
<tr>
<td>ABCD recommends that veterinarians carry out a risk-benefit analysis for each vaccine in order to avoid unnecessary vaccination of cats, monitor the injection site, and report all VAAEs to the manufacturer and/or to their competent authority.</td>
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