FELINE INJECTION SITE SARCOMA AND ITS POSSIBLE THERAPEUTIC MODALITIES

By Victoria Stockman

The University of Veterinary Medicine Budapest

Thesis supervisor: Dr. Peter Vajdovich, head of the Department of Clinical Pathology and Oncology

The Department of Clinical Pathology and Oncology

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Abbreviations

FISS = Feline injection-site sarcomas

FeLV = Feline leukemia virus

VAFSTF = Vaccine-Associated Feline Sarcoma Task Force

FIV = Feline immunodeficiency virus

CBC = complete blood count

CT = computed tomography

MRI = magnetic resonance imaging

DFI = disease-free interval

C3 = the third cervical vertebrae

BCS = body condition score

MST = median survival time

SBRT = stereotactic body radiation therapy

IV = intravenous

LED = liposome-encapsulated doxorubicin

rFeIFN-ω = recombinant feline interferon-ω

MHC = major histocompatibility complex

ALVAC IL-2 = recombinant canarypox virus expressing feline interleukin 2

PDT = photodynamic therapy

HPD = hematoporphyrin derivative

ICG = indocyanine green

PHCT = photodynamic hyperthermal chemotherapy

HT = hyperthermia therapy

PHT = photodynamic hyperthermal therapy

ALA = aminolevulinic acid

PpIX = protoporphyrin IX

PDS = photodynamic surgery

RDT = radiodynamic therapy

OST = overall survival time

RFP = relapse free period

Feline Injection Site Sarcoma and its possible therapeutic modalities

Introduction

Feline injection-site sarcomas (FISS) are malignant tumours, and the treatment of these tumours poses a challenge for veterinary surgeons. The recommended therapeutic strategy is a multimodal approach and treatment options include radical surgery combined with radiotherapy and chemotherapy. Radiation therapy is not always available and other treatment options, such as immunotherapy and photodynamic therapy, should be looked into and will be discussed further.

FISSs are of mesenchymal origin and are locally aggressive tumours with a low to moderate risk of metastases. FISS is considered to be a rare form of cancer, but a tumour developing at the injection site can have devastating outcomes for the patient. They most commonly develop at the site of vaccination, but they can also appear following other injections, microchip implantations and surgical stitches. Several factors influence the development of a FISS, intrinsic factors, such as the genetics of the cat and the degree of inflammation, and extrinsic factors, such as how often the cat has been injected and the quality of the material injected. FISS is especially challenging because of the high recurrence rate. For the best possible outcome, an early diagnosis is of great importance. To obtain this, detailed keeping of vaccination history and taking an incisional biopsy once a mass is presented that fits into certain criteria, is crucial. The existing treatment options are often not effective. Even with radical surgery together with radiation therapy and chemotherapy, recurrence rates are high.

During the thesis preparation we perform a review of the therapeutic modalities which were used in the the past and has been recently used in Veterinary Hematology and Onology Centre, ÁHOK Ltd. to treat feline injection site sarcoma. Taking into account the generally poor prognosis of this cancer, the importance of prevention is emphasised. By following

vaccination recommendations and reacting to post-injection masses, some cases might be prevented.

What is FISSs?

A feline injection-site sarcoma is a malignant skin tumour that appears most frequently at the site of injections, primarily following vaccinations. This form of cancer is particularly seen in cats, but it also occurs in other species, like ferrets and rarely in dogs [1]. They are of mesenchymal origin, arises from connective tissue, in the area where the cat previously received an injection [2]. Initially, FISSs was associated with rabies and feline leukemia vaccines. Rabies and FeLV vaccinations remain the most commonly reported injections causing FISSs, but the use of several different substances has been reported to have been administered prior to the development of a sarcoma at the injection site. There have been reported cases after the administration of other pharmaceuticals such as long-acting antibiotics, steroidal- and non-steroidal anti-inflammatory drugs, and lufenuron. Sarcomas following microchip implantation, or a reaction to non-absorbable surgical sutures or surgical sponges, have also been reported [2, 3]. These tumours are primarily found in sites commonly used for vaccination, such as the interscapular region, the area of the hamstring muscles, the lumbar regions, the semimembranosus and semitendinosus muscles, and laterally on the thoracic or abdominal wall [1].

Feline injection-site sarcomas are considered the most serious among vaccine-related side effects in dogs and cats. It is regarded an uncommon form of cancer, but the consequences of the development of a malignant tumour at an injection-site, can have devastating consequences [4]. Injection-site sarcomas are aggressive tumours and untreated animals face a fatal outcome, but even after surgeries with radical excisions, recurrence rates are high. Metastatic potential is low, but have been reported, especially pulmonary metastasis.

Pathogenesis

The exact pathogenesis is still unknown despite considerable research. When FISSs were first reported, it was following the administration of rabies and feline leukemia vaccines. It is now known that these injection-site sarcomas may develop as the consequence of the administration of several different substances, and anything that causes a local inflammatory response has the potential to cause the development of these tumours [5]. On the background of this, the most accepted hypothesis states that it is the chronic, local inflammation following an injection that causes the tumour transformation and is supported by histological findings [2].

Exactly how the chronic inflammation causes tumour formation is unknown, but several factors might play a role. The chronic inflammatory reaction, together with a genetic predisposition, create an environment that increases the susceptibility to carcinogenesis. An increased level of growth factors and oncogene activation have been observed in patients with feline injection-site sarcomas. Growth factors can cause proliferation and induction of malignant transformation. The activation of oncogenes, and inactivation of tumour suppressor genes, promote a neoplastic transformation of fibroblasts. The production of free radicals can also be a consequence of chronic inflammation, and the free radicals cause damage to the DNA and mutations. These factors together contribute to initiate carcinogenic injuries[1].

Adjuvant vaccines have especially been associated with the development of FISSs. This is due to the more intense local inflammatory reaction caused by this type of vaccines [1]. In adjuvant vaccines there are substances added to an inactivated vaccine to enhance the immune response of the selected antigen at the site of injection. Adjuvants are necessary when applying inactivated vaccines, which contains killed virus or bacteria, to enhance the pro-inflammatory reaction [1, 4]. Adjuvants like aluminium have been found during analysis of FISS biopsy samples. Histological findings of vaccine adjuvants which contains aluminium, phagocytized by inflammatory cells which are found in a central area of necrosis in the tumour, support this hypothesis.

History of FISSs

This form of cancer was recognized in the 1980s and has been published since 1991 [6]. The discovery happened as rabies vaccination for pet cats was made mandatory in Pennsylvania in 1987, and cases of injection-site fibrosarcomas increased with 61% from 1987 to 1991 [6]. The suggestion that this significant increase in sarcomas at the injection-site was caused by rabies vaccination, was published in 1991. In 1992, the hypothesis stating that the inflammatory reaction to vaccines with aluminium based adjuvants was associated with the tumour formation at the injection-site, was developed, but still unproven. The administration of adjuvant feline leukemia virus-, and rabies virus vaccines was finally proven to be related to the development of tumours in 1993. The Vaccine-Associated Feline Sarcoma Task Force (VAFSTF) was formed in 1996 and is an organisation that was formed to plan research and to educate veterinarians about FISS [6].

Although there is still more investigation to be done, research has come a long way since FISS was first discovered [6]. When these tumours were first published, the only suspected causes were adjuvant rabies- and feline leukaemia vaccines. Today, it is known that any substance that causes an inflammatory reaction can in theory have the potential to initiate injection-site sarcomas as a consequence of malignant transformation [6].

Histology

FISSs are of mesenchymal origin. The most common histologic types of injection-site sarcomas are fibrosarcomas, rhabdomyosarcomas, osteosarcoma, chondrosarcoma, myxosarcoma, and histiocytic sarcomas. The most common, however, being fibrosarcomas making up around 80% of diagnosed FISSs [7]. According to the Hungarian oncology clinic, 94% of tumour lesions in cats examined and treated in the last 10 years are fibrosarcomas.

Typically described histological findings include a peripheral inflammatory infiltrate consisting of lymphocytes, macrophages, granulation tissue and multinucleated giant cells, and a central area of necrosis [7]. FISSs also show proliferation of atypical spindle cells [8, 9]. Compared to sarcomas not caused by injection, a high mitotic index and cell pleomorphism are more frequent findings in injection-site sarcomas [7]. In some cases of FISSs, it is demonstrated that the inflammatory cells, including the multinucleated giant cells,

have phagocytized the aluminium-based vaccine adjuvant. These neoplasms are highly aggressive and recurrence rates are high after surgical removal. The well-established histologic features of FISS give a good background for differential diagnosis with other sarcomas. FNA and biopsies are important diagnostic tools for the definite FISS diagnosis [7, 9].

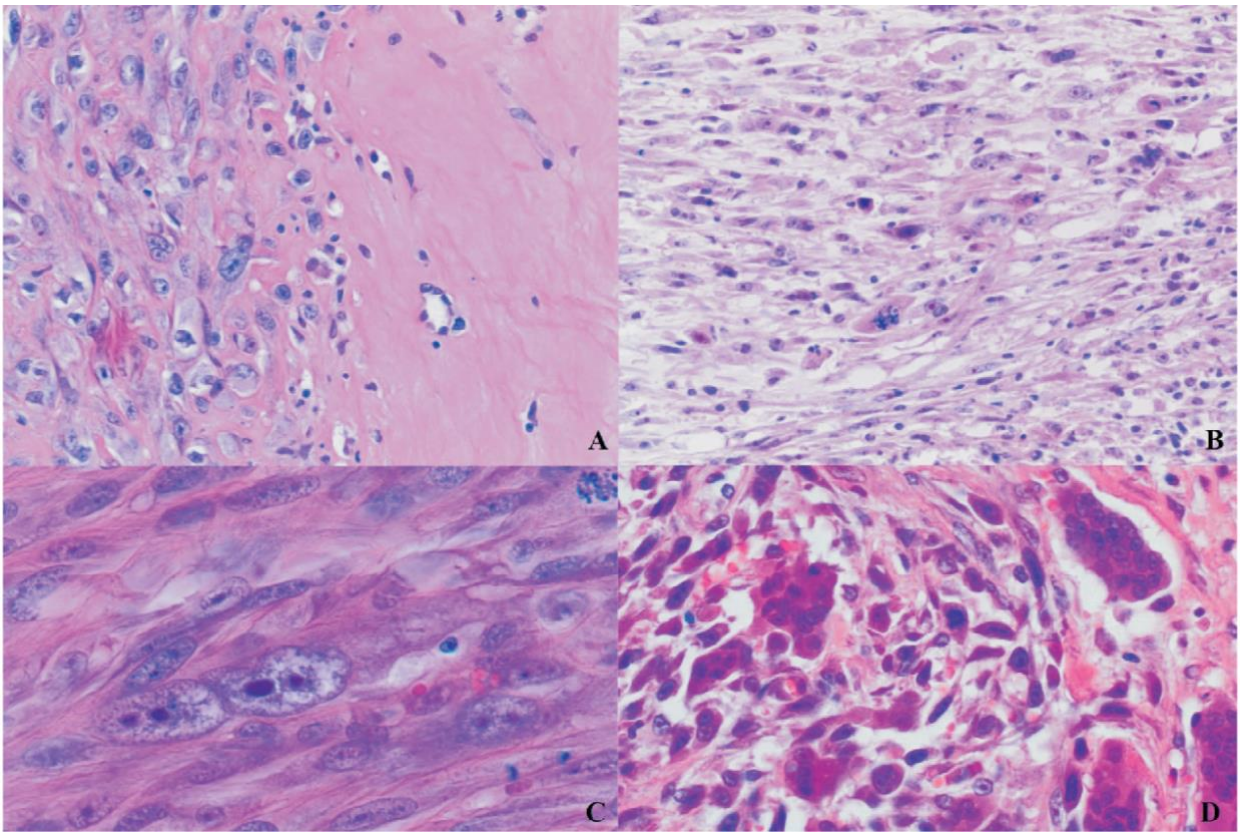

Figure 2: Histological findings in a sample taken from a FISS. A: intralesional necrosis can be seen on the right side. B: pleomorphism of neoplastic cells. C: macrokaryosis in neoplastic cells. D: multinucleated giant cells. [10]

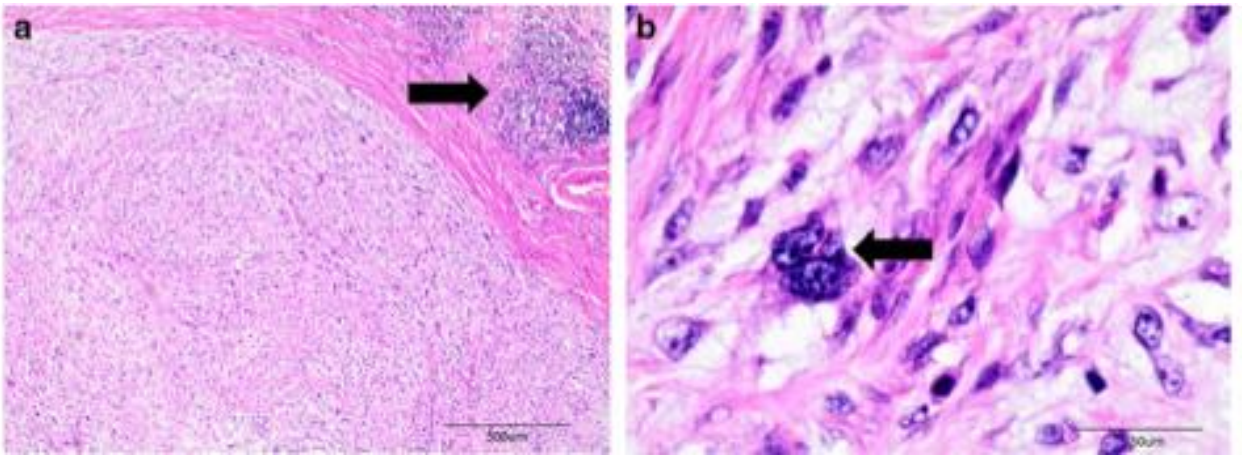

Figure 3: a: the arrow is showing the intralesional inflammatory reaction. b: arrow: multinucleated giant cell. These cells are often found in ISSs [2].

Occurrence of FISS

Feline injection-site sarcomas are aggressive tumours that can have devastating outcomes, but they are considered uncommon. One study shows that 1-10 out of 10 000 vaccinated cats develop FISSs [2]. Other studies report 1-3 out of 1000 cats develop the tumour [1, 6, 11]. The reported prevalence significantly depends on where the study was performed and if rabies vaccination is a routine procedure in the selected area. In the USA, a study estimated 1-4/10 000 vaccinated cats develop FISS. The incidence is estimated to be lower in the UK where FISSs was registered 1 in 16 000-50 000 cats by practices. Rabies is very rarely found in the UK and vaccination against it is not a routine procedure which will influence the result [1].

These tumours are known to develop anything from 3 months up to 10 years post-injection. Younger cats are often seen to develop FISSs compared to other forms of cancer. A peak in FISSs is seen in cats at 6-7 years of age. A second peak is seen at 10-11 years of age [11, 12, 13]]. It is suggested that a genetic predisposition plays a role in the number of cats that will develop ISSs. This is based on the low incidence of vaccinated cats that develop the tumour. Samples taken from injection-site sarcomas have shown mutations in gene p53, the tumour suppressor gene. It has therefore been suggested that the incidence of injection-site sarcomas is higher in siblings of cats with the diagnosis. Some cats are also observed to develop more than one FISS. One case-control study found a connection between cats developing FISS and polymorphisms in the genomic sequence of the p53 gene. The importance of this study is uncertain as another study was not able to find the same connection between the polymorphisms in the genomic sequence and a predisposition to FISS [1].

Clinical signs

Feline injection-site sarcomas are typically larger in size than other sarcomas. They generally develop in the subcutis, but can also occur intramuscularly [1, 11]. The majority of feline injection-site sarcomas are fibrosarcomas. They are usually discovered by the owner as firm, non-movable lumps in the subcutis. They are normally not painful and are sometimes discovered as an incidental finding on physical examination [1, 12].

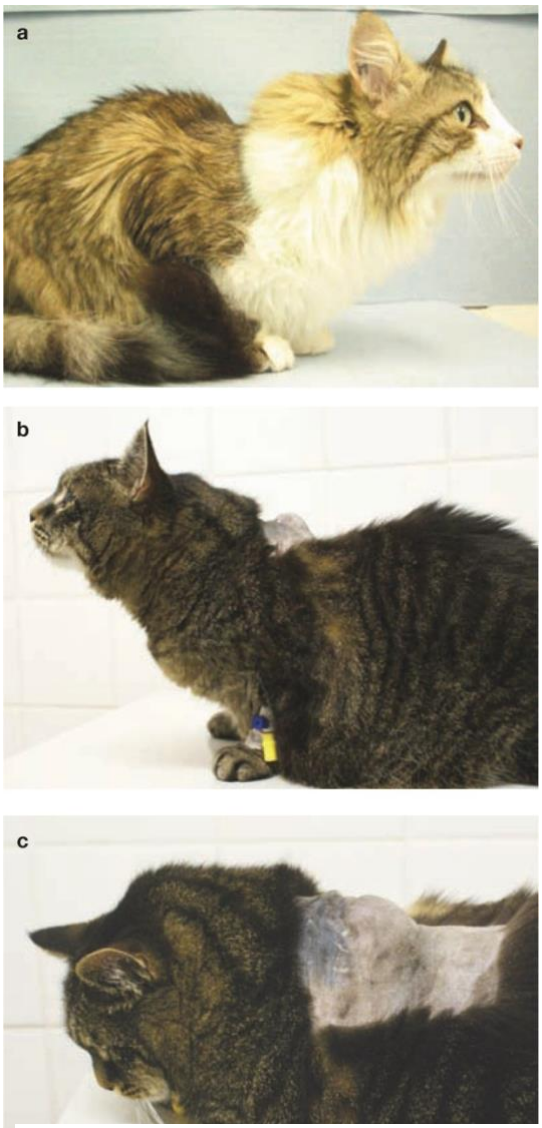

Figure 4*:* a-c: cats with FISSs in the interscapular area.

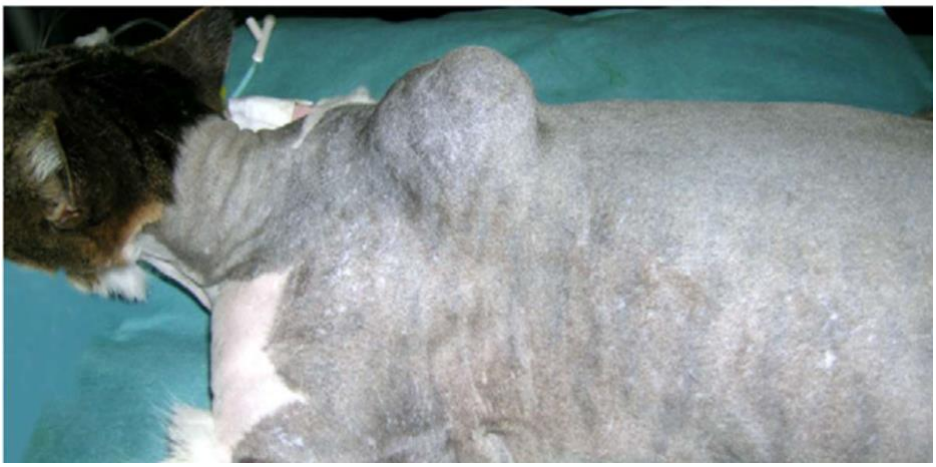

Figure 5: *Another example of a Feline injection-site sarcoma in the interscapular area [6].*

Signs of systemic disease can be present in case of metastasis. The rate of metastasis is low to moderate, but in case of metastasis, systemic signs may include lethargy, anorexia, vomiting, and increased respiratory rate. Metastasis is seen in 10-25% of cases [1, 6]. Pulmonary metastasis is seen most frequently and occurs in 21% of cats with grade 3 tumours [4]. Metastasis is also seen in other organs, such as the regional lymph nodes, spleen, kidney, intestine and liver [1]. Based on these statistics, thoracic radiography is recommended prior to treatment to exclude pulmonary metastasis [2].

Diagnosis

Patient history is important when a cat is presented with a subcutaneous lump as well as the patient's vaccination history and the location of the lump. Owners should be asked if there has been any change in the size of the lump, when the lump was first discovered, and the current size should be measured at the consultation [14]. A subcutaneous mass following vaccination is not uncommon. Most post-vaccination lumps resolve without treatment within 3 months, but because of the aggressive nature of FISSs, all masses that appear at an injection-site should be assumed malignant until the opposite is proven [6].

Recommendations to perform a biopsy of a mass at the injection-site is based on the 3-2-1 rule. An incisional biopsy is recommended if a mass meets at least one of the criteria:

3:	The mass was noticed more than 3 months ago.
2:	The mass is bigger than 2 cm in diameter.
1:	The mass has continued to grow in size 1-month post-injection.

If one or more of the criteria are met, a biopsy sample is required [4, 6, 14]. FNA with cytology is the least invasive form but cannot always provide a definitive diagnosis. It can, however, help exclude other differential diagnoses. Other forms of incisional biopsies, like needle core, punch, or wedge biopsies, can also be used. It is recommended to take multiple samples from the lesion as FISSs are heterogenous and this can be helpful to obtain a definitive diagnosis [14].

When obtaining a biopsy sample from a potential FISS, it is essential to minimize the risk of tumour seeding. It is therefore important to control bleeding and ensure that the biopsy tracts do not go through the sarcoma into healthy tissue. Preferably, the biopsy tracts should be within the planned surgical area [11, 14]. An incisional biopsy is always recommended over an excisional biopsy for the diagnosis of masses that occur in the area of an injection-site. An excisional biopsy is not recommended as the recurrence rate for FISSs is very high when a tumour is marginally excised. The tumour will most likely recur and the second treatment will become even more complicated [11, 14].

Further examinations are recommended when diagnosing FISSs. Haematology and biochemistry profiles should be taken, as well as urinalysis, and testing for FeLV and FIV. This is recommended to exclude concurrent diseases that possibly could affect the immune system and the patient's response to treatment. Following a diagnosed FISS, thoracic radiography is recommended. Thoracic radiographs are recommended on the basis that pulmonary metastasis is present in 10-25% of confirmed FISSs. Injection-site sarcomas are aggressive and invasive tumours and an early diagnosis is essential to obtain the best possible outcome of the treatment [6, 11, 14].

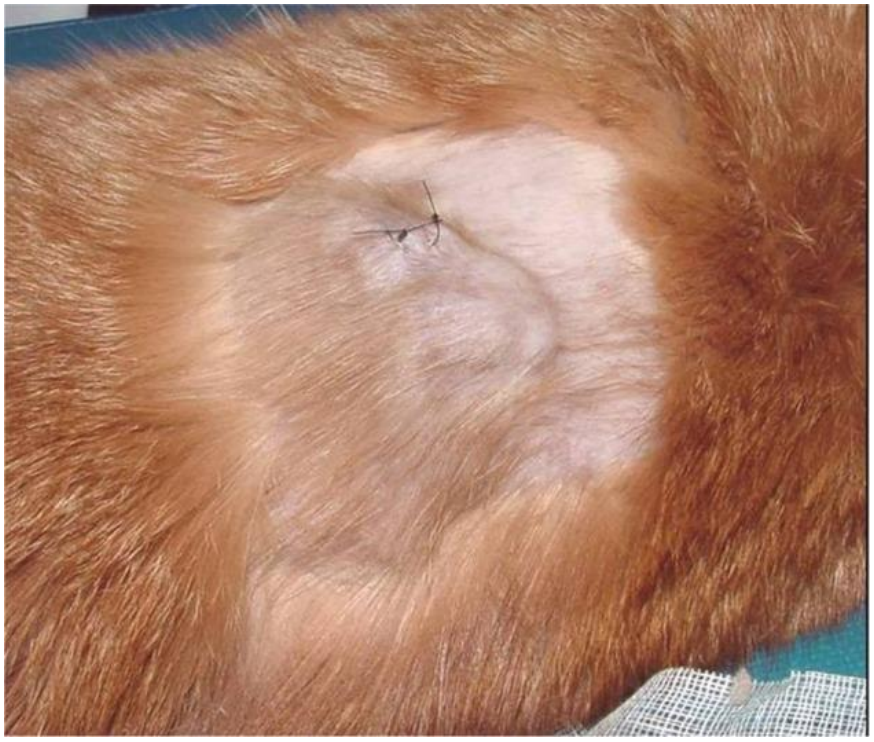

Figure 6: Incisional biopsy placed within planned treatment area [14].

Staging for choice of treatment plan

Following a confirmed case of an injection-site sarcoma, clinical staging is to be done prior to choosing a treatment plan and the clinical status of the patient needs to be considered. A complete blood count (CBC), biochemistry profiles, urinalysis, thoracic radiography, examination of lymph nodes, ultrasound of the abdomen and cytology are required for staging of the tumour [6]. These additional examinations are also recommended to exclude other diseases that can affect the patient's response to the chosen treatment, and if they are an appropriate candidate for anaesthesia and surgery. To measure the actual size of the tumour, CT (computed tomography) or MRI (magnetic resonance imaging) is recommended prior to surgery [14].

Radical surgery of the tumour, with a wide surgical margin, is advised in case of a confirmed FISS. The recommended therapeutic approach to these sarcomas is multimodal, but the choice of adjunctive therapies, like radiation therapy, chemotherapy, immunotherapy, depends on the patient and outcome of the clinical examinations [14]. Injection-site sarcomas

are locally aggressive tumours but have relatively low metastatic potential. Pulmonary metastases are, however, seen in some cases of confirmed FISSs. Because of this, 3-view thoracic x-rays are recommended before deciding on a treatment plan and possible aggressive, local treatment [7]. Vaccine-associated sarcomas in cats can be classified into different stages based on the size of the tumour [6]:

- Stage I: the diameter of the tumour is less than 2 cm.
- Stage II: the diameter of the tumour is between 2-3 cm.
- Stage III: the diameter of the tumour is above 3 cm.

There is no statistical significance between grading of the tumour and recurrence rate [15]. CT is a useful diagnostic tool to plan the margins for the surgery. The margins in a FISS surgery are wide and it is considered an aggressive and radical surgery. For patient follow-ups post-surgery, CT is also used to detect the area that potentially needs to be excised with a second surgery or should be in the radiation treatment field [2].

Treatment options

Feline injection-site sarcomas in cats are a serious health problem and lack of proper treatment in affected cats will have fatal outcomes. Aggressive treatment is required for these highly invasive tumours [7]. The recommended strategy for treating feline injection-site sarcomas, is a multimodal approach. The first-line treatment, and the most important component of a multimodal approach, is radical surgery. Because of the high recurrence rate of these tumours, surgery alone is not recommended as a treatment option. The choice of adjunctive therapies depends on the patient and what is available. Although metastases are rare, diagnostic imaging is important to rule out the spread of the neoplasms in the patient before initiating aggressive local treatment [9].

Surgical planning

For surgical planning, a CT or MRI is recommended prior to surgery. This is for a more accurate determination of the tumour size as some studies show that the tumour size can be more significant than first assumed by palpation [2]. According to the present-day guidance,

aggressive surgery with wide lateral margins of 5 cm and two fascial planes for deep margins, is recommended. This proves difficult in many regions on a cat's body and complete surgical excision cannot always be achieved. Even when wide margin surgery is performed, achieving completely clean margins is difficult, leading to recurrence rates that span from 30% to 70%. In cases where the FISS is located on a distal limb or tail, amputation is often necessary to avoid incomplete surgical margins [7, 9].

It is shown that complete surgical margins in the first surgery is of great significance for the outcome of the treatment. Patients with less surgeries often show increased survival rates. It is therefore suggested that survival rates can be improved if the surgery is performed by an experienced veterinary surgeon, preferably from a referral clinic [2, 6, 7]. One study shows that the average time to first recurrence was 274 days when the surgery was performed at a referral clinic, compared to 64 days when it was performed at a non-referral clinic [2]. Owners should be aware that not all FISSs can be completely removed by surgery. A patient with a tumour large in size or in a difficult position, will not always benefit from the aggressive surgery. It is recommended to run a CBC, serum biochemistry, and urinalaysis to determine the patient's clinical status prior to treatment. If the patient is in poor general condition, it is not a good candidate for the required surgery. Thoracic radiography should also be done prior to surgery to exclude pulmonary metastases [2].

Surgery

In the multimodal therapeutic strategy, the surgery is the most important component. For FISSs, wide excision surgery is necessary with at least 3 cm, preferably 5 cm, margins. For deep margins, two fascial planes are recommended [2, 7]. In cases where neighbouring bone structures are involved with the neoplasm, the bone structures have to be removed. This could include radical excisions such as vertebral process amputation, scapulectomy (partial or total), rib resection or limb- or tail amputation in cases where the tumour is located on the distal limb/tail.

Marginal surgeries are rarely curative and is contraindicated for FISSs. Local recurrence is common and can develop as soon as 6 months following a simple excision of the tumour. Histopathology of the surgical margins is recommended following removal of the tumour.

This is to assure the complete excision of the tumour as non-infiltrated margins are important for the success of the therapy. Study shows that the disease-free interval (DFI) in patients where wide excision surgery was performed, is significantly longer compared to when marginal excision surgery was performed. Tumours with infiltrated margins have recurrence at a rate approximately tenfold higher than those with non-infiltrated margins. Even though the recurrence rate is significantly lower when tumour margins appear to be free of neoplastic cells, tumour recurrence is still possible [2].

Surgical technique

The surgical technique depends on the location of the FISS. Generally, the hair is clipped in a wide area surrounding the tumour. The area depends on the location of the tumour, but typically extending from the occipital bone to the iliac wings and to the level of the sternum laterally. The clipped area is then prepared for surgery with an aseptic scrub. The margins of the tumour are marked with sterile marker and resection margins are drawn in a 3-5 cm circumference around the tumour [3]. The size of the tumour should be measured preoperatively using diagnostic imaging, such as CT or MRI, as it is shown that the actual size of the neoplasm could be twice that estimated by physical palpation [11].

The muscles defined by the diagnostic imaging before surgery, should be resected. There are two main ways to perform the muscular resection. For FISSs located on the midline, a trapezius resection is usually performed. For the trapezius resection, the trapezius muscle is elevated from the latissimus dorsi muscle underneath and excised at the insertion sites on the ventral border of the scapula and cranially on C3. The same procedure is performed on the contralateral side to the tumour. A vertebral process amputation is usually performed of the spinous process to ensure that the muscle is not penetrated. For tumours located more laterally on the body wall, a trapezius/latissimus dorsi resection is performed. In this case, the latissimus dorsi muscle is removed together with the trapezius muscle on the side of the tumour. On the contralateral side of the tumour, the surgery is performed as described above, where only the trapezius muscle is dissected. The muscles are cut from the spinous process the same way as for the trapezius resection [3]. The surgical wound can be closed in several techniques such as tension relieving sutures, axial pattern flaps or skin stretches. Complications with wound healing is influenced by several different factors. The most

common wound healing complications with this surgery, are seroma, infection, or dehiscence of the surgical wound. The most important factor for the development of complications with the surgical wound, is the duration of the surgery. Other factors can also play a smaller role, such as the body weight and the body condition score (BCS) of the animal, and the pattern of reconstruction used. The size of the tumour, deciding the extent of the surgery, also plays a role [3].

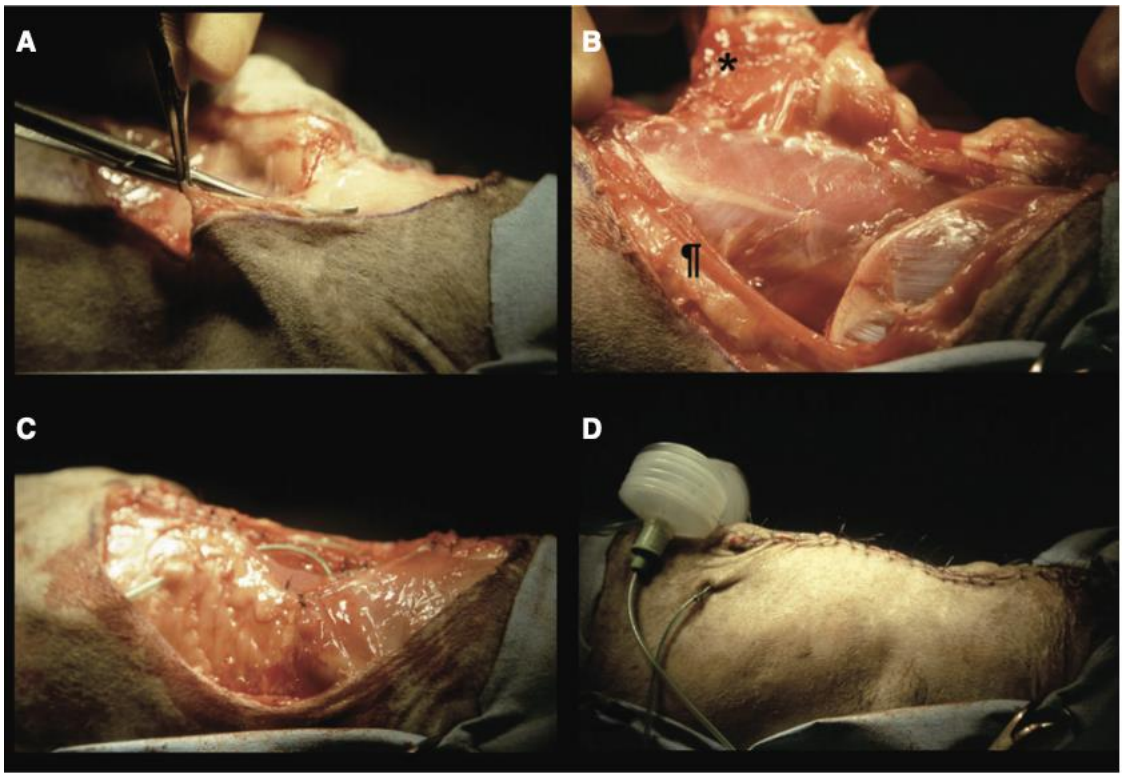

Figure 7: intraoperative pictures. A: surgical margins are marked with a sterile marker around the tumour, B: the trapezius muscle is elevated, C: the dorsal spinous processes are resected with the muscle to prevent penetration of the muscle, D: picture taking postoperatively after closing the surgical wound [3].

Radiation therapy

Even when wide-margin surgery is performed, margins are often infiltrated and recurrence rates are high. The highest success rate is therefore associated with a combination of radical surgery and adjunctive therapy, like radiation therapy which is shown to prolong the survival time when combined with surgery. Radiotherapy is frequently used pre- or postoperatively, or both. Different studies show varying results on the effect of the use preoperative radiation

compared to postoperatively [7]. A study by Mayer et al suggests that radiation therapy together with surgery gives the best success rate. The study was performed on 76 cats with confirmed FISS and the survival of patients following pre- and postoperative radiation was examined. The results showed that survival was longer in the cats obtaining this treatment [16].

Cronin et al. found no association in their study between the use of radiation therapy and longer survival. 33 cats with confirmed injection-site sarcomas participated in the study and were treated with postoperative radiation therapy. Their study shows that achieving a non-infiltrated tumour margin is the only factor that can significantly prolong the DFI. Cohen et al., on the other hand, analysed the median survival time (MST) and DFI of 50 cats that had surgery together with radiation therapy. As opposed to the study done by Cronin et al., the study shows that MST and DFI are prolonged when radiation therapy was used. Tumours that were smaller before the first surgery were associated with prolonged MST. The study also claims that the DFI and MST was increased for the cats that started radiotherapy earlier [17].

A study by Nolan et al. claims that stereotactic body radiation therapy (SBRT) used as palliative treatment prior to disease may be beneficial. The study is based on the medical records of 11 cats with confirmed FISS that had surgery together with SBRT. Stereotactic radiation therapy is high doses of ionizing radiation delivered to neoplasms in fewer treatments. The treatment is accurate and is supposed to deliver the intended dose to the tumour and less to the surrounding healthy tissue. The doses of radiation are lower than conventional curative-intent radiation therapy. The conventional therapy often exceeds 50 Gy, while the SBRT ranged from 24 to 35 Gy and was given in only 3 to 5 sessions. Unfortunately, the study has some limitations, for example the small number of patients [18].

The side effects of radiation therapy have to be taken into account when choosing the treatment strategy. Radiation therapy combined with surgery is an effective treatment for injection-site sarcomas, but the short- and long-term effects must be considered. Short-term side effects may include changes to the skin, like skin erythema, mucositis, etc., and gastrointestinal disorders. In case of prolonged exposure or too high doses, necrosis of the skin might happen and only experienced veterinarians should perform the radiation therapy. Because of the often short overall survival time of these cats and the shortage of studies performed, there is a lack of information on the long-term side effects of radiotherapy.

Anaemia is a common side effect in FISS patients getting treated with radiotherapy. Anaemia can be used as an indicator of the prognosis as it is suggested that anaemia in cats with FISS is associated with decreased survival time. In human medicine, long-term effects of radiation include atrophy, fibrosis, vascular- and neural damage, and endocrine disorders [2].

Chemotherapy

Chemotherapy is used in combined therapy as part of the multimodal approach that is recommended to treat FISSs. Chemotherapy can be used as palliative treatment or as neoadjuvant or adjuvant therapy [2]. Neoadjuvant chemotherapy before surgery is used to reduce the size of a tumour. Postoperative chemotherapy is given to eliminate residual cancer cells with the objective of lowering the risk of recurrence [19]. Drugs used in chemotherapy include doxorubicin, carboplatin, cyclophosphamide, ifosfamide, mitoxantrone and vincristine. The value and effectiveness of the use of chemotherapy is still under discussion and different studies show varying results [2].

A study was performed on the use of doxorubicin as part of the multimodal treatment on 71 cats following partial removal of the injection-site sarcoma together with the use of radiation therapy. The study showed a longer remission time in the cats that also received doxorubicin treatment. The cats received doxorubicin three to five times at 3-week intervals. The study showed a longer remission time in the cats given doxorubicin compared to the cats that did not receive it, but it was not shown to have prolonged the disease-free survival time [20].

In a study where 108 cats received doxorubicin or liposomal doxorubicin, the cats that received chemotherapy had a longer DFI compared to a historical control group that received surgery alone [21]. Another study performed on 12 cats with inoperable FISS, showed that doxorubicin combined with cyclophosphamide treatment gave results in 50% of the cats and prolonged survival time in the cats that responded to treatment [22]. This suggests that doxorubicin used in neoadjuvant chemotherapy together with surgery increases the DFI and the tumour-free survival time.

A big concern when the effectiveness of the use of chemotherapy has been evaluated, is that the studies are done on a small number of FISS patients. Some studies found no connection

between additional doxorubicin treatment together with surgery and radiotherapy, and disease-free survival time, overall survival time and tumour remission. However, the studies in question did not include a big number of participants and are not considered significant [2].

Doxorubicin administration also comes with challenges as the drug causes side effects because of its high toxicity and poor distribution in the body. It has a short half-life, and it is absorbed by the mononuclear phagocyte system quickly following IV administration, which will lead to a lower concentration in the neoplastic tissue. Doxorubicin is also known to cause anaemia, myelosuppression, and nephrotoxicity in cats and cats with diseases like chronic renal failure, haemolytic anaemia, autoimmune-mediated anaemia or bone marrow disorders are not good candidates for treatment with doxorubicin [2, 23]. A study was performed comparing doxorubicin treatment with liposome-encapsulated doxorubicin (LED) treatment on cats with confirmed FISS. The effectiveness of the two drugs were the same and both showed to have a good effect. However, LED showed more side effects compared to doxorubicin [21].

Immunotherapy

Immunochemistry is used to enhance the immune system's ability to recognise and destroy cancer cells. Cancer cells often evade detection by the immune system or suppress the immune response. Immunotherapy aims to reverse this by activating the immune system to target cancer cells specifically [24]. Interferons are types of cytokines that can stimulate the immune cells to attack the cancer cells. They have immune-modulatory, anti-viral, and anti-proliferative effects. IFN-ω have the same amino acid sequence in both cats and humans, and it is shown that IFN-ω in humans have a good anti-cancer efficacy [25]. Inflammation is a defining characteristic of feline injection-site sarcomas. Macrophages, neutrophils, lymphoplasmacytic infiltrates, and aggregates of macrophages are present in the tumours. A study done in a mouse xenograft model of rhabdomyosarcoma in a human, showed that giving antihistone antibody-conjugated IL-12 resulted in remission long-term and improved survival in the cats. This result suggests that administering IL-12 immunotherapy is also advantageous in the treatment of FISS [25].

A study was performed on 20 cats with fibrosarcoma, where recombinant feline interferon-ω (rFeIFN-ω) was assessed as treatment to measure safety and feasibility. rFeIFN-ω was injected 12 times over a 5-week period. The 1st through 4th injections were given into the tumour, while the 5th through 12th injections were given subcutaneously at the injection-site. Between the 4th and 5th injection, surgery was performed. In 45% of the cases, the neoplasm recurred, but at a different location from the first tumour. Flow cytometry examining the biological effect of rFeIFN-ω on fibrosarcoma cells, was performed in an in vitro study and showed an increase in the expression of major histocompatibility complex (MHC) class I. The same study showed that the expression of MHC class II molecules was not considerably altered. MHC class I molecules present tumour antigens on the surface of malignant cells. The increased expression of tumour antigens increases the susceptibility of the cancer cells to be attacked by macrophages, antibodies, cytotoxic T cells, and natural killer cells. MHC class II molecules present tumour antigens on antigen presenting cells like macrophages and dendritic cells [26].

Side effects such as changes in blood cell count, increased serum aspartate-amino-transferase, serum creatinine, serum electrolyte concentrations and serum bilirubin, anorexia and weight loss, increase in body temperature and reduced general condition were discovered. The side effects were mostly of minor importance and self-limiting and rFeIFN-ω used as treatment against feline fibrosarcoma is considered safe, well tolerated and practically doable [26].

71 cats participated in a study to assess the safety and efficacy of a recombinant canarypox virus expressing feline interleukin 2 (ALVAC IL-2). The efficacy was assessed at low and high doses of ALVAC IL-2, and the safety was measured when the highest dose was used. It was administered in combination with surgery and radiation therapy. The participating cats were put into three different groups that would receive different treatments, one reference treatment group, one group received a low dose of ALVAC IL-2, and one group received a high dose of ALVAC IL-2. IL-2 injected into the tumour stimulates antitumour immunity while reducing the toxicity that comes with systemic treatment and no systemic reaction associated with the cytokine's toxic effects was discovered, the biochemical and haematological parameters stayed the same. The results of the study showed that the ALVAC IL-2 treatment was well tolerated, even at the highest dose tested. Side effects were confined to mild, local reactions. The side effects experienced by some of the cats, could also have

been caused by the brachytherapy and not necessarily the immunotherapy. The cats treated with ALVAC IL-2 experienced a longer median time to relapse compared to the reference group. A considerable decrease in recurrence was also observed. Relapse was reduced by 56% at one year and 65% at two years [25].

Photodynamic therapy

Photodynamic therapy (PDT) is a treatment in two stages that uses light energy and a photosensitizer together to destroy cancer cells. Photosensitizers are activated by light at a specific wavelength. The drug itself is not toxic, until it is activated by light and it becomes toxic to targeted tissues, like tumour cells [27]. The use of light therapy is not a new concept and was already practiced in ancient Greece, Egypt, and India. What separates the phototherapy used by ancient physicians from the method we refer to as photodynamic therapy today, is the use of oxygen in light reactions for an improved response when used in therapy. The photoactive drug used will accumulate within the neoplasm and is activated by light of a specific wavelength. This combination, of a photoactive drug, light, and oxygen, leads to the photochemical reaction which results in the damage of tumour cells and impairment of tumour vasculature [28]. The photosensitising agent accumulates in the cancerous cells and a local irradiation reaction happens when combined with light. PDT is considered advantageous over other cancer treatments as it is highly selective [29].

Today, PDT is a well-recognised therapy for a number of indications in human medicine. In the past, PDT was rarely used in veterinary medicine, but PDT with hematoporphyrin derivative (HPD) became, with time, more commonly utilised as a treatment option for several tumours in dogs and cats. Neoplasms that were the main indication for use of PDT included mast cell tumours, malignant melanoma, metastatic prostatic carcinoma, sebaceous gland tumours, osteosarcoma and fibrosarcoma [30]. Porphyrins have also been used to localise tumours as it has been noted by several authors that after the administration of hematoporphyrin, embryonic- and neoplastic cells showed a brick-red fluorescence [29].

Mechanism of action of photodynamic therapy

PDT is a relatively new form of therapy. It is non-invasive and is used in the treatment of several different cancer types. The mechanism of action is based on the administration of a photosensitive compound, the photosensitiser, which accumulates in the tumour. The photosensitiser takes up light at a specific wavelength which initiates the processes leading to selective eradication of pathological cells. The photosensitiser is only toxic within the pathological tissues. PDT is well-tolerated because of the highly selective action of the drug and because it is a painless procedure [31].

The photosensitiser is also taken up by normal tissue, but it is retained for a longer time in pathological tissues, such as in tumours, compared to in healthy tissue. This mechanism is not completely understood. It is believed that the poor lymphatic drainage and the high amount of blood vessels with enhanced permeability in the tumour is a part of the explanation of the retained photosensitising agent in the tumour. The low pH of the interstitial fluid in tumours might also play a role as this increases the lipophilicity of the drug which will increase their uptake by the tumour cells [29]. The main limitation of PDT is the light's ability to penetrate the tissue. The wavelength of the light is what decides the depth of penetration into the tissue. A wavelength between 600 and 1200 nm can be used in PDT, as light with a wavelength shorter than 600 nm gets taken up by haemoglobin, while light with a wavelength longer than 1200 nm gets taken up by water in the tissues. The timing of light application to the neoplastic tissue area primarily relies on the choice of photosensitising agent and the specific duration required for the drug to accumulate sufficiently within the tumour tissue after administration [29].

Which photosensitising agent is used, is an important factor in the efficacy of the PDT. The optimal photosensitising agent has a short time period for the agent to accumulate maximally within the neoplastic cells following administration, a short half-life and rapid clearance. The characteristics of the photosensitiser that are important when selecting which agent to use, are the ability to selectively target neoplastic cells, chemical purity and specific composition, and that it is activated at an optimal wavelength to the bodily tissues. The photosensitising agents can be separated into porphyrin derivatives and non-porphyrin-base agents. Porphyrins can

further be divided into hematoporphyrin derivatives, porphyrin derivatives without some limitations of hematoporphyrins, and photosensitisers conjugates with antibodies [30, 32].

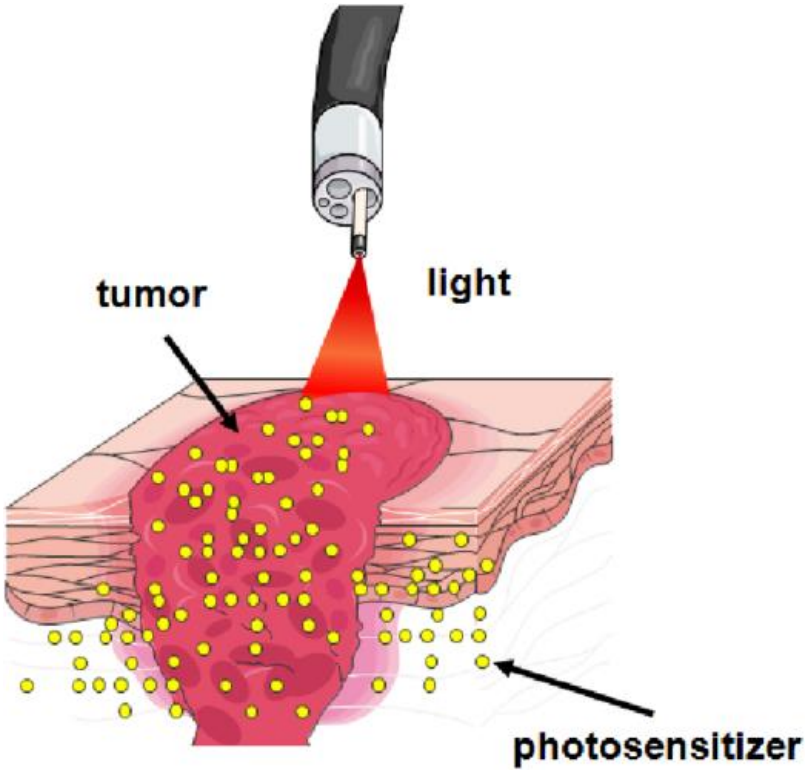

Figure 8: Overview of PDT. A photosensitizing agent is administered, distributes in the body, selectively target tumour cells, and accumulates. By illumination of a specific wavelength and the presence of oxygen, the photosensitiser is activated [33].

Porphyrins

The most commonly used photosensitising agents in PDT are hematoporphyrin derivatives (HPD). They accumulate in the Golgi apparatus and in the plasma membranes. HPD are shown to be a pain free treatment option when used in photodynamic therapy, but they have their limitations. Studies have indicated that HPD exhibit a greater affinity for uptake by normal cells compared to neoplastic cells. Furthermore, derivatives of hematoporphyrin are characterised by a slow clearance rate, limited penetration into large and deep tumours, and an extended period of photosensitivity lasting approximately three months [30].

Aminolevulinic acid

Aminolevulinic acid (ALA) is the first compound in the porphyrin synthesis pathway and is the precursor to protoporphyrin IX (PpIX) which is responsible for heme production, and it has photosensitizing properties. Conjugated ALA molecules form PpIX is converted to heme by an enzyme called ferrochelatase. ALA can be given systemically or topically. Exogenous administration causes accumulation of PpIX in the epithelial and neoplastic tissues as the produced PpIX is not turned into heme fast enough. ALA is very selective to neoplastic cells and therefore has few side effects. The high selectivity can be explained by the difference in the heme synthesis between neoplastic cells and normal cells. There is a lower ferrochelatase activity and lower iron availability in neoplastic cells, which results in an increased accumulation of PpIX in tumour cells. This increased accumulation of PpIX can be used in photodynamic therapy by exposing it to light 4-6 hours after ALA administration, when enough PpIX has been synthesized, which leads to oxygen production which has cytotoxic effects on the cells. PpIX induced by ALA administration has a rapid clearance and is cleared from the body in 24 hours. This property makes it possible to repeat the treatment regularly as often as every 48 hours without risking cumulative effect. [29, 34].

Aminolevulinic acid has showed some limitations due to its hydrophilic character. It is unable to go through skin and cell membranes and after topical treatment with ALA, PpIX will only be produced at 2-3 mm surface of the skin. Other factors that influence the accumulation of PpIX, like other photosensitizing agents, are oxygen availability, illumination, and pH. Unlike other photosensitisers, ALA can be given orally, but the bioavailability will be decreased when given orally as there is a high presystemic elimination. Some modified ALA substances exists that are more lipophilic and are able to penetrate membranes, but these substances have been shown to accumulate in the stratum corneum which causes a worse biological activity [29].

Indocyanine green

Photodynamic hyperthermal chemotherapy (PHCT) is a combination of indocyanine green photodynamic hyperthermal therapy and local chemotherapy and has proven effective against cancer. Indocyanine green (ICG) can be used as a photosensitising agent with a broadband light source, instead of a diode laser, in PDT together with hyperthermia therapy (HT) which is called photodynamic hyperthermal therapy (PHT). It was discovered that the anticancer effects of chemotherapy were improved by HT and the method where local chemotherapy and PHT was combined was developed and called photodynamic hyperthermal chemotherapy [35].

If stimulated by a light source with a wavelength of 808 nm, indocyanine green produces heat. A light source at wavelengths of 600-800 nm induces the production of oxygen radicals. A study was performed on six cats with confirmed FISS receiving photodynamic hyperthermal chemotherapy following surgical excision of the tumour. The cats were under general anaesthesia during the procedure, indocyanide green was used as the photosensitising agent and carboplastin with paclitaxel as chemotherapy drugs. 10 cm from the 2-3 cm margin, irradiation was applied for 20 minutes after injection of ICG every 2-4 weeks. In half of the participating cats, there was no recurrence. In the other half of the cats, recurrence was observed, but this result was associated with how many surgeries the cats had undergone previously. The tumours that recurred had already been surgically removed over three times before PHCT was used. In cats where no recurrence was observed, the cats had undergone no or one FISS surgery [35].

Acridine orange

Feline injection-site sarcomas are good models for human musculoskeletal sarcomas as they are both of mesenchymal origin and are aggressive neoplasms with a high recurrence rate. As musculoskeletal sarcomas are rare in humans, FISSs are being used as models to study the possible treatment of human musculoskeletal sarcomas. Human musculoskeletal sarcomas are rare, usually occur in children and young adults, and have a high mortality rate. Like with FISS, the gold standard therapy is radical surgery together with chemotherapy and radiotherapy, but morbidity and mortality are still high and it has a high recurrence rate. Wide

resection surgery and limb reconstruction is a well-established technique, but this might prove difficult in certain anatomical locations with structures that could lead to limb disabilities. Because of this, an innovative technique with acridine orange together with photodynamic surgery (PDS), photodynamic therapy and radiodynamic therapy (RDT), has been proposed with positive results. This method allows minimally invasive surgical margins which leads to less complications [23]. Acridine orange is a fluorescent cationic dye specific for DNA and RNA. It is also a pH indicator, a photosensitizing- and anticancer agent. Acridine orange has a low molecular weight that allows it to penetrate interstitial tissues and cytoplasm of cells. Acridine orange accumulates into intracellular acid vesicles due to protonation, and this leads to monomeric, dimeric, or oligomeric aggregates that are impermeable. Because of an altered glycolytic metabolism in malignant tumours, they are more acidic than healthy tissue. The increased acidification could also be explained by oxidative cancer cells. Sarcoma cells are especially acidic as they have highly acidic lysosomes that acidifies the extracellular microenvironment. Acridine orange is highly selective for acidifying tumour cells and become cytotoxic by illumination by a specific wavelength. The fatty acids of the lysosomal membranes are oxidized which leads to the outflow of lysosomal enzymes followed by cell death [23].

Musculoskeletal sarcomas have successfully been treated with PDT and RDT and acridine orange in the context of preventing tumour recurrence and maintaining limb functionality following intra- or marginal tumour removal. These tumours are infrequent in humans and it is therefore challenging to demonstrate the efficacy of this treatment due to the lack of incidences. Feline injection-site sarcomas in cats are therefore used as a model for musculoskeletal sarcomas in humans, although also rare, they are more frequent and have several similarities. Both types of tumours have fibroblast-like histology, are considered aggressive, invasive tumours, and recurrence rates are high even when wide surgical excision and radiotherapy are performed. The cat’s shorter lifetime is also useful to achieve rapid trial conclusions and data [23].

Side effects of photodynamic therapy

PDT is considered a safe treatment because of its high selectivity, but like any medical procedure, there is always a risk of side effects. Photosensitising agents also affect healthy cells and the treatment can increase sensitivity to light even after the treatment is finished. Other side effects that have been observed include swelling in the area of the treated skin, discoloration of skin, itching, stinging or burning, vomiting, allergic reactions, like oedema, and tumour necrosis. It takes approximately 15-25 days for re-epithelialisation of the treated area [29, 36].

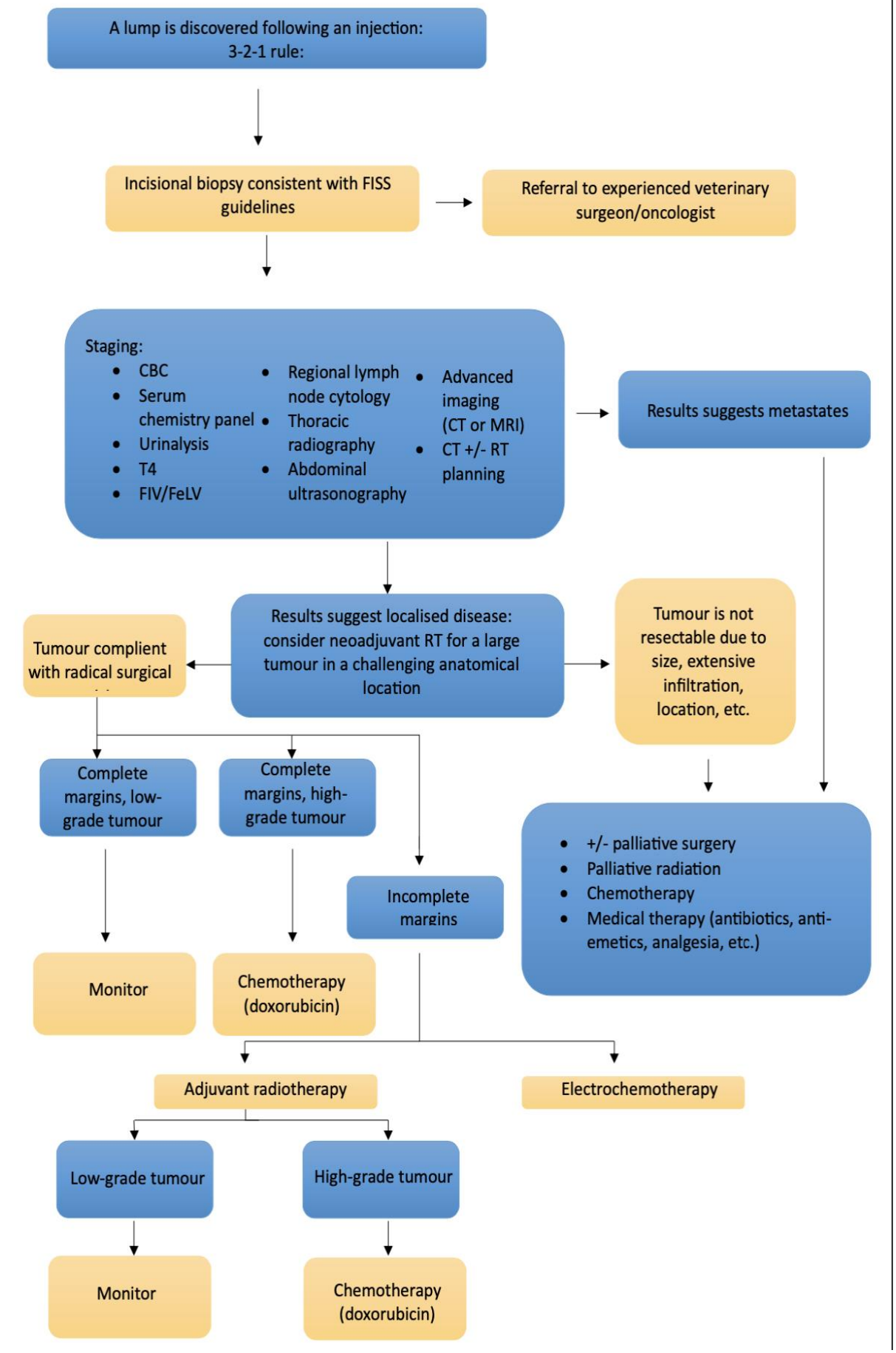
A lump is discovered following an injection: 3-2-1 rule:
Incisional biopsy consistent with FISS guidelines
Referral to experienced veterinary surgeon/oncologist
Staging:
CBC
Serum chemistry panel
Urinalysis
T4
FIV/FeLV
Regional lymph node cytology
Thoracic radiography
Abdominal ultrasonography
Advanced imaging (CT or MRI)
CT +/- RT planning
Results suggests metastates
Tumour complient with radical surgical
Results suggest localised disease: consider neoadjuvant RT for a large tumour in a challenging anatomical location
Tumour is not resectable due to size, extensive infiltration, location, etc.
Complete margins, low-grade tumour
Complete margins, high-grade tumour
Incomplete margins
+/- palliative surgery
Palliative radiation
Chemotherapy
Medical therapy (antibiotics, anti-emetics, analgesia, etc.)
Monitor
Chemotherapy (doxorubicin)
Adjuvant radiotherapy
Electrochemotherapy
Low-grade tumour
High-grade tumour
Monitor
Chemotherapy (doxorubicin)

Figure 9: A summary of treatment options for cats with feline injection-site sarcoma [37].

Prevention

Taking into account the generally poor prognosis of this cancer, prevention is especially important. Vaccines should only be administered when there is a reasonable risk that the cat could be exposed of the pathogen. Vaccines should be administered subcutaneously as intramuscular administration does not lower the chance of a tumour developing. On the other hand, should a tumour develop following an intramuscular injection, the tumour would likely be discovered later as it goes deeper. Limiting the number of injections given to one cat over time may decrease the number of injection-site sarcomas and veterinarians worldwide are recommended to follow the vaccination guidelines that exist for cats. Current guidelines state that rabies vaccines should be administered on the right rear, while FeLV on the left rear. Vaccines are typically administered at sites above the stifle and over the right shoulder, while it is recommended to vaccinate below both the stifle and the elbow joint. The interscapular space is a common vaccination site, but is not recommended by current guidelines. Distal limb and tail injections are recommended to facilitate amputation if necessary for complete removal of the tumour with 5 cm margins, and to decrease the risk of local recurrence. Ventral abdominal injections have lately become more common because of the perception that tumour removal is easier from this site while avoiding amputation, but following current guidelines, by removing two fascial planes deep and 5 cm margins around the tumour, it would still require extensive tissue excision from the abdomen and abdominal cavity.

Another important factor in the prevention and early diagnosis of FISS, is to educate the owners about lumps following vaccinations and when they need to contact a veterinarian. Owners should observe and palpate their animals to look for lumps following vaccinations or other injections. In case of a postvaccination lump that increases in size or is palpable for more than one month, the owner must contact a veterinarian. Most lumps following vaccination are benign, but it is important to treat postinjection lumps according to the 3-2-1 rule. In case of a confirmed case of FISS, routine thoracic radiographs should be performed to exclude pulmonary metastases before deciding on a definitive treatment option [4, 38].

Adjuvant vaccines should be avoided whenever possible. The adjuvant is either a microbial constituent, a chemical, or a mammalian protein that is added to an inactivated vaccine. The function of adjuvants is to increase the immune response against a selected pathogen. Adjuvants can cause granulomas and inflammation and the connection between chronic inflammation caused by adjuvants and the formation of an injection-site sarcoma, has been suggested by several studies since the 1990's. A few studies claim that there was no difference in the occurrence of FISS when using adjuvant vaccines compared to non-adjuvanted vaccines. Despite the ongoing discussion regarding the safety of the use of adjuvant vaccines, it is still recommended to avoid it whenever possible due to the fact that the number of diagnosed sarcomas in cats started to increase at the same adjuvant vaccines became prevalent [4, 38].

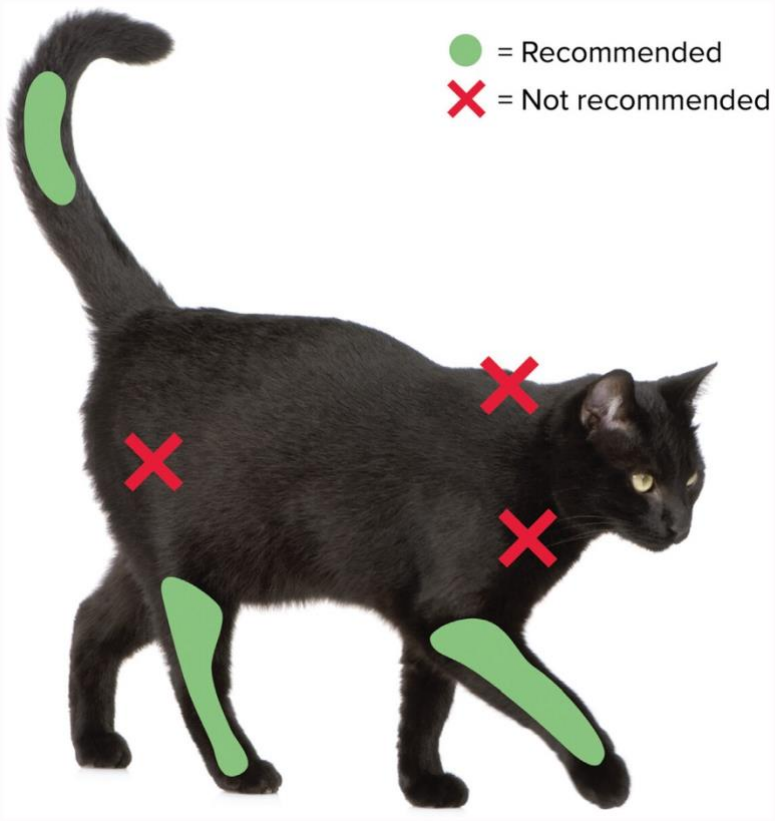

Figure 10: Recommended injection-sites following current guidelines [38].

The study

To provide real facts about the basic findings about feline sarcoma experienced at a Hungarian clinic, we add some frequency analysis. There were 297 cat cases examined during the last 10 years from 2013 until 2023 at the Veterinary Hematology and Oncology Center, Á.H.O.K Ltd. (1148, Bolgárkertész str. 31., Budapest, Hungary). The summarizing tables 1, 2, 3, 4 and 5 contain all the major information about these cats.

Materials and methods

Table 1: Summarising basic data of the cats examined and treated at a Hungarian oncology clinic during the last 10 years.

	Mean	No	Percentage (%)
Age (mean)	9.96		
Male		124.0	41.75
Female		173.0	58.25
Neutralized		266.0	89.56
Time of death was recorded		38.0	12.79
Operated		121.0	40.74
Relapsed		77.0	25.93
Largest tumour diameter (mean)		3.8	
Staging		178.0	59.93
No of Visits (mean)	5.5		
At least two visits		190.0	63.97
No of Reports where relapse is mentioned		125.0	42.09
Has been already relapsed at 1st visit		63.0	21.21
Body weight (mean)	4.2		
MRI/CT scan	41.0	41.0	13.80

Tumour location was typically more frequent at the injection site area (thoracic wall, interscapular area, flank and dorsal lumbar area. Although, some other site were also affected (Figure 11., 12., Table 2.)

Figure 11: Location of tumour in cats expressed in chart (number of lesions at specific areas indicated on the chart).

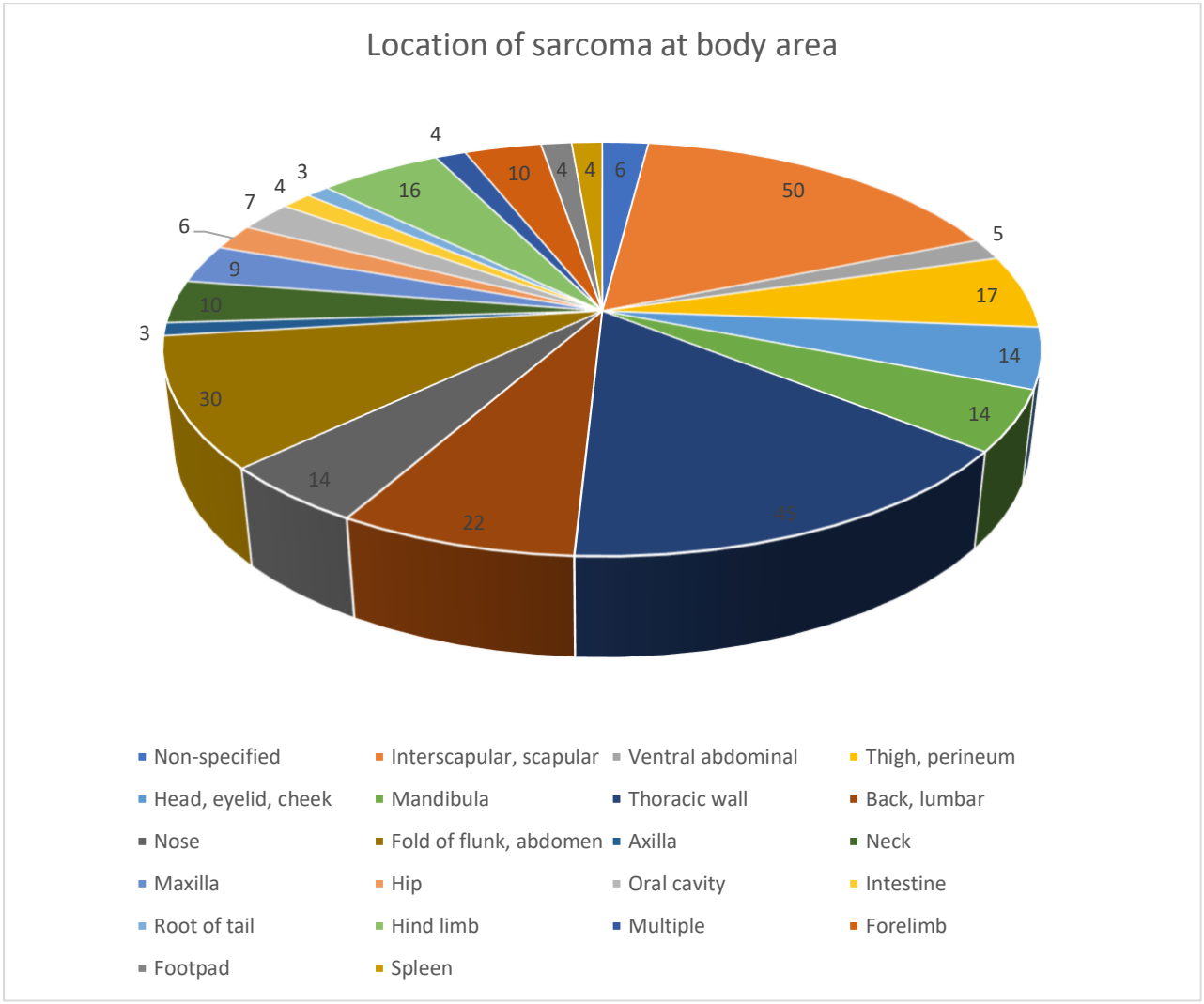

Figure 12: Location of tumour in cats expressed in a picture (number of lesions at specific areas indicated on the drawing).

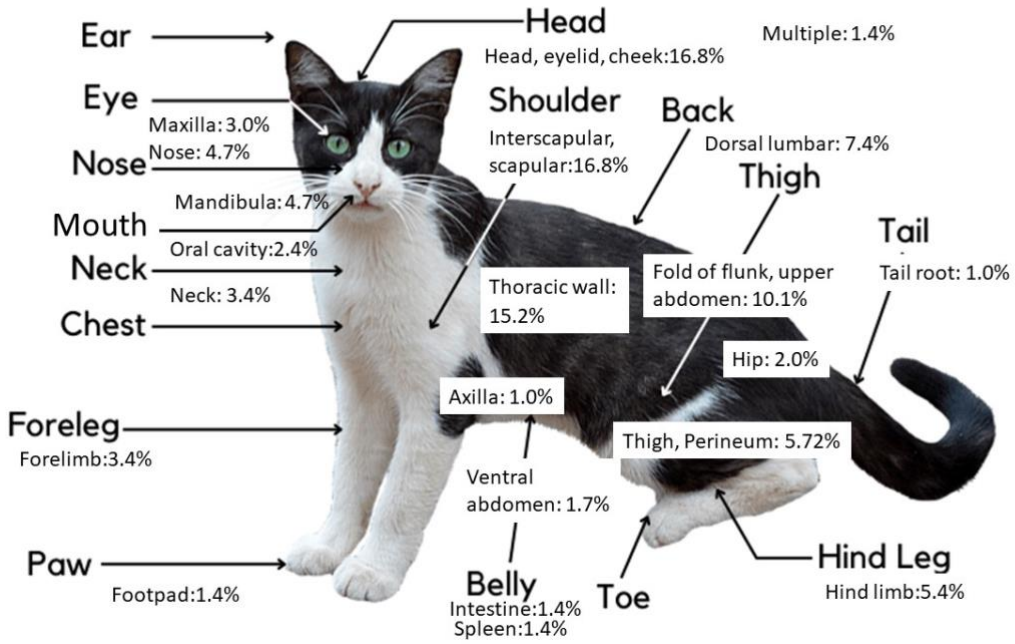

Table 2: Location of tumour lesions in cats examined and treated at a Hungarian oncology clinic during the last 10 years (number and % is indicated)

Body area	No	Percentage (%)
Non-specified	6	2.02
Interscapular. scapular	50	16.84
Ventral abdominal	5	1.68
Thigh. perineum	17	5.72
Head. eyelid. cheek	14	4.71
Mandibula	14	4.71
Thoracic wall	45	15.15
Back. lumbar	22	7.41
Nose	14	4.71
Fold of flunk. abdomen	30	10.10
Axilla	3	1.01
Neck	10	3.37
Maxilla	9	3.03
Hip	6	2.02
Oral cavity	7	2.36
Intestine	4	1.35
Root of tail	3	1.01
Hind limb	16	5.39
Multiple	4	1.35
Forelimb	10	3.37
Footpad	4	1.35
Spleen	4	1.35

Histopathology types were variable with mostly fibrosarcoma. In fact, there were many otherwise non-specified sarcoma, due to the high number of cases that had been already operated elsewhere and the histopathology results issued by various labs were not detailed, enough (Figure 13, Table 3).

Figure 13: Histopathology types of tumour lesions in cats examined and treated at a Hungarian oncology clinic during the last 10 years (number of histopathology types at specific areas indicated on the chart).

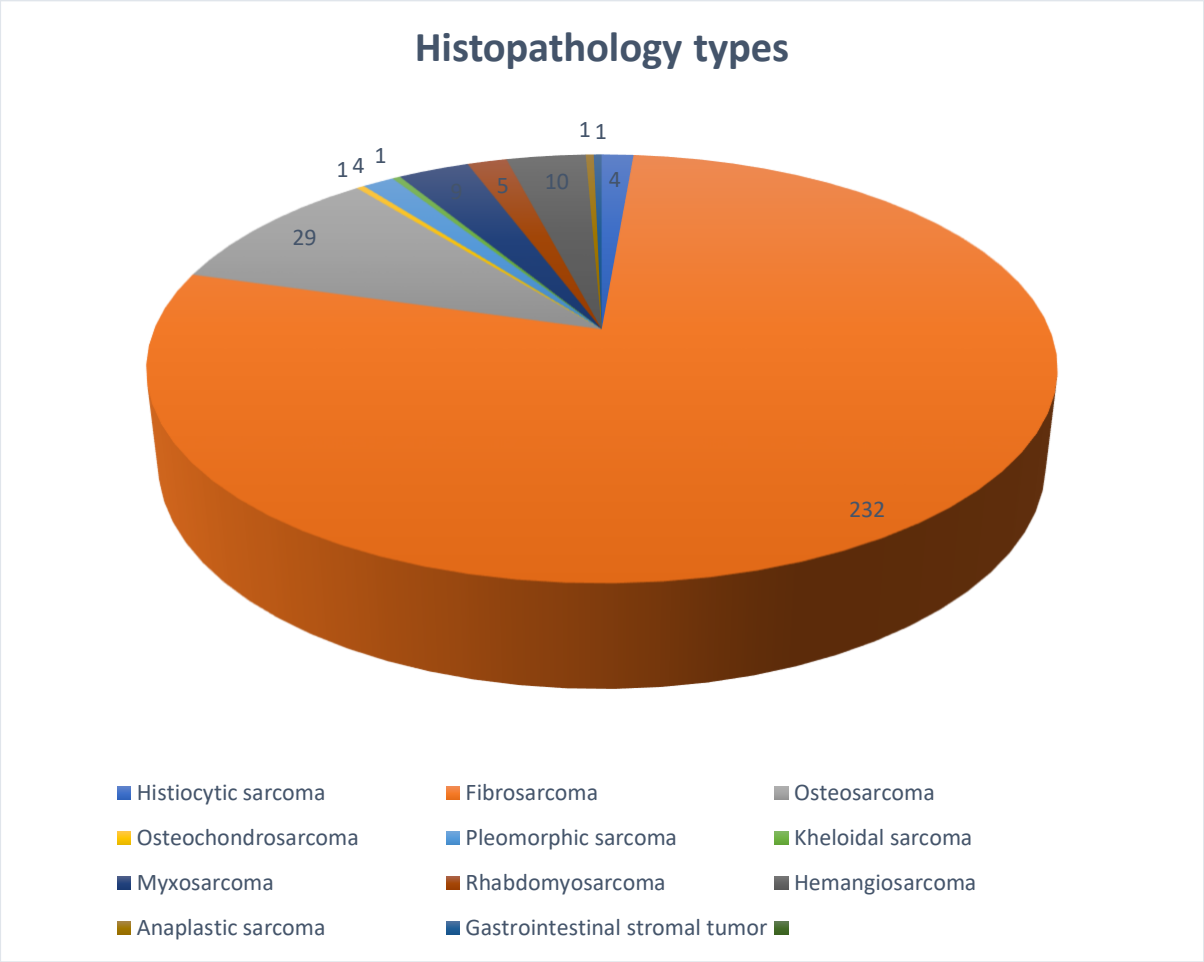

Table 3: Histopathology types in number and percentage of tumour lesions in cats examined and treated at a Hungarian oncology clinic during the last 10 years.

Histopathology type	No	Percentage (%)
Histiocytic sarcoma	4	1.35
Non-specified sarcoma	137	46.13
Fibrosarcoma	94	31.65
Osteosarcoma	29	9.76
Osteochondrosarcoma	1	0.34
Pleomorphic sarcoma	4	1.35
Kheloidal sarcoma	1	0.34
Myxosarcoma	9	3.03
Rhabdomyosarcoma	5	1.68
Myofibroblastic sarcoma	1	0.34
Hemangiosarcoma	10	3.37
Anaplastic sarcoma	1	0.34
Gastrointestinal stromal tumor	1	0.34

The animals were treated by various methods (Figure 4), such as non-well documented or marginal surgery, wide surgery done by wide surgical margin (> 4 cm), some cases received chemotherapy basically anthracycline antibiotics, such as doxorubicin, liposome encapsulated doxorubicin (Caelyx, Doxil) and epirubicin). The number of cycles were varied between 1 to 8 cycles with 3 to 4 weeks intervals. Some cases received chemotherapy before and after surgical intervention as neo-adjuvant and post-adjuvant treatment (n=25, 8.4%), and some received chemotherapy after surgery, only. Some cases were sent by veterinarians right after surgical interventions, more patients were sent when they experienced one or two relapses after their surgical procedures at (n=63, 21.2%) (Table 1). Some patients received photodynamic therapy. The surgical procedures and photodynamic therapy were done by the following way.

Anesthesia and preparation for the photodynamic therapy

Surgery and the photodynamic therapy was performed under general anaesthesia. (*Figure 14 – (a)*). *5-Aminolevulinic acid hydrochloride* (Beilstein Registry Number: 3690651, EC Number: 226-679-5, MDL number: MFCD00012869, Sigma-Aldrich) was given IV at least 1.5-2 hours

before induction of anesthesia. It was mixed with physiological saline (1: 100 solution) in 60 mg/kg body weigh dose, together with CRI saline and administered by slow bolus. Premedication of midazolam (0.5 mg/kg IV) (Dormicum inj. 5 mg/ml, Egis Pharmaceuticals PLC, Hungary) and Propofol 0.5 to 2.0 mg/kg IV was administered prior to surgery (Propofol 1% MCT/LCT Fresenius emulsion, Fresenius Kabi Deutschland). A loading bolus of fentanyl at 0.05-0.1 mg/kg IV was given followed by a constant rate infusion (CRI) of 0.025-0.2 mg/kg/hr. Inhalation narcosis was maintained by Isoflurane (Forane solution, AbbVie Ltd., Hugary) in 1 to 5 % with supplemental oxygen inhalation.

Surgery

The surgery was performed with a wide excision of 2 to 3 cm or with wide (> 4 cm) margins around the lesions including one or two muscular fasciae (*Figure 14 - (b)).* Operations were performed in Állatorvosi Hematológiai és Onkológiai Központ, Állatpatika (Á.H.O.K.)..

(a) **(b)**

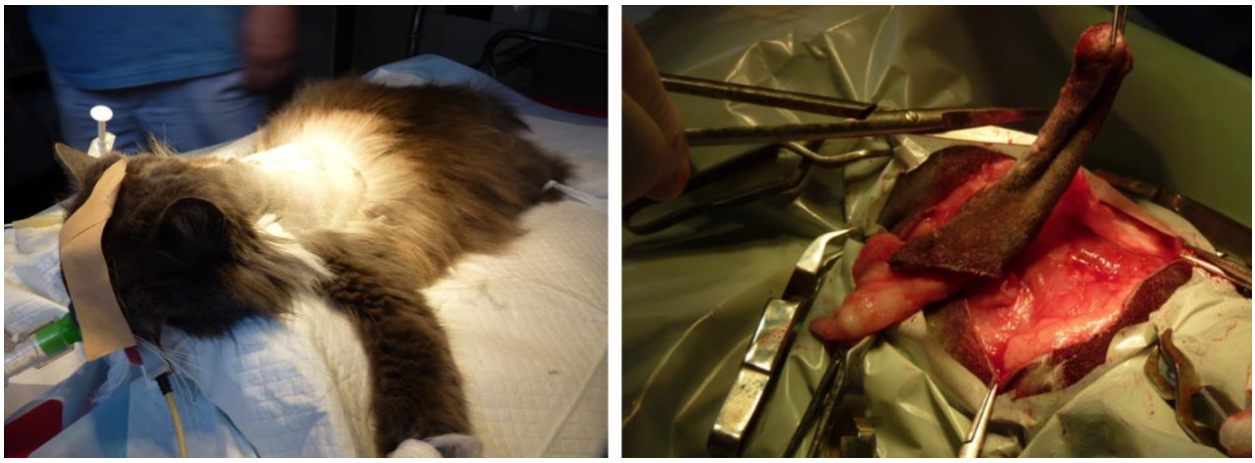

Figure 14: One of our cases with FISS in the interscapular region – under general anaesthesia. a): Prior to surgical excision. b): Wide surgical excision of the tumour.

Photodynamic therapy (PDT)

Photodynamic therapy was performed on the open wound after tumor removal and wound washing. The surgical site was illuminated by light for 10 minutes, at least 1-3 times at the original site of the tumors and around the wound edges (*Figure 15*). The orange coloured light was 660n wavelength with a high-energy rate (100 J/cm2). It was applied for 5 minutes at surface intervals of $3cm^2$ as previously described by Schmidt et al., 1996 [39]. Photodynamic therapy was performed on 27 cases after proper staging during surgery. The excised tumor

tissues were sent for histopathology examinations (Department of Pathology, University of Veterinary Medicine and Matrix Ltd., Budapest, Hungary).

PDT was performed in 27 cases. In total number of PDT interventions was 34. Ninteen cases received PDT once, 3 cases received it twice, 3 patients 3 times, and one 4 times.

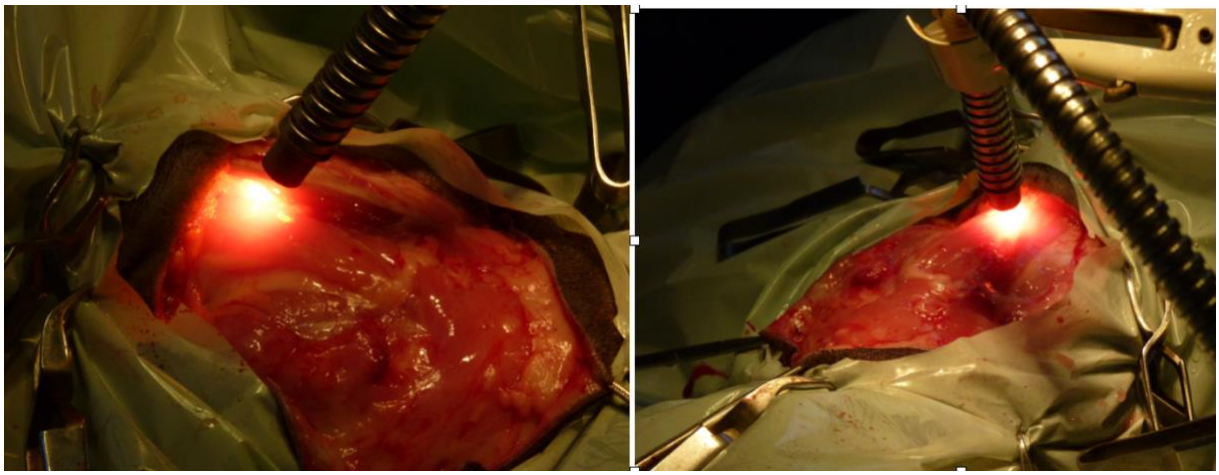

Figure 15: PDT illumination of the edges of the wound for 10 minutes at each site.

Figure 4: Therapeutic interventions of the cats examined and treated at a Hungarian oncology clinic during the last 10 years.

	Mean	No	Percentage (%)
Operated		171.0	57.58
Number of operations (mean)	1.5		
Operation with wide (> 4 cm) tumour margin		72.0	24.24
More than 1 operation		52.0	17.51
Photodinamic therapy		27.0	9.09
Neo-adjuvant chemotherapy (etoposide. doxorubicin 3 to 4 occasions before surgery q 21 days)		25.0	8.42
Chemotherapy at all		138.0	46.46
Pegylated doxorubicin 1 mg/ kg bw		26.0	8.75
Epirubicin 25 mg/m2 q 21 days		94.0	31.65
Doxorubicin 25 mg/m2 q 21 days		36.0	12.12
Cyclophopshamide 50 mg/m2 po q 7 days		12.0	4.04
Vincristine 0.5 mg/m2 q 7 days		7.0	2.36

Carboplatin 250 mg/m2 q 21 days		5.0	1.68
Toceranib 2.5 mg/kg bw po. every 2nd day		5.0	1.68
Lomustin 40 mg/m2 po. q 21 days		2.0	0.67
Ifosphamide 1.2 g/m2 iv. over at least 30 minutes daily for 5 consecutive days; repeat every 3 weeks		1.0	0.34
Etoposide 375–500 mg/m iv. over three to five days days		2.0	0.67
Chlorambucil 20 mg/m2 po q 14 days		7.0	2.36
Mastinib 2.5 mg/kg bw po SID		1.0	0.34

Results

Outcome of therapy

The median overall survival time (OST) of the cats was 1995 (mean: 1239.8) days. whereas the median relapse free period (RFP) was 113 (mean: 174.9) days. Among the examined cats we could include 199 cats to calculate overall survival time and 70 to calculate relapse free period (Table 5, 6, 7, 8, 9, Figure 17, 18, 19, 20, 21, 22).

The stage of cats was based upon the largest diameter of the tumors (Stage I: < 2 cm. Stage II: 2-3 cm; Stage III < 3 cm.

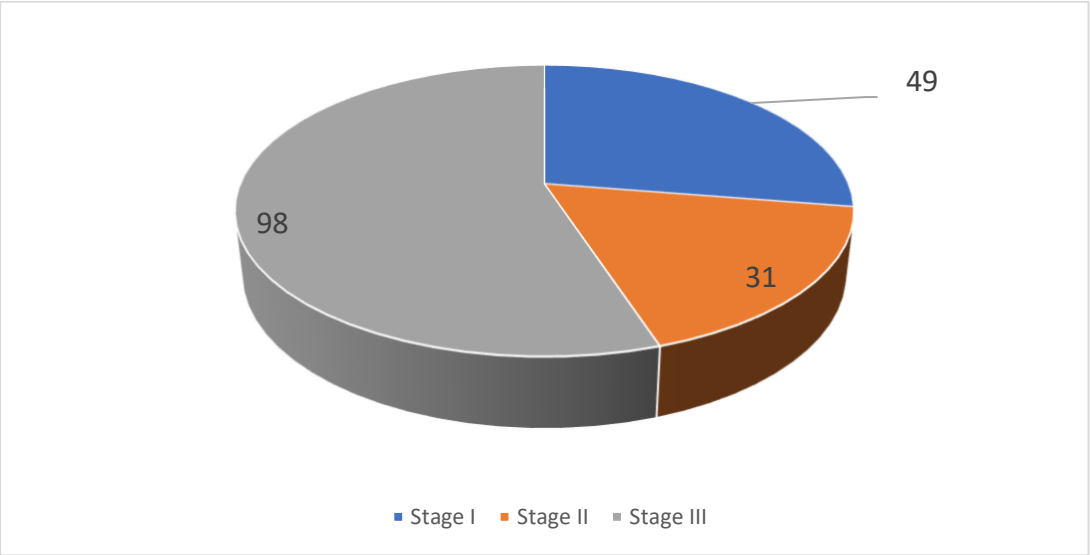

Figure 16: Stage distribution of cats with sarcoma.

		Stage I (days)	Stage II (days)	Stage III (days)	Not measured (days)
OST	Median	can not estimate	1995	can not estimate	335
	Mean	1535	1581	1137	776
Chi-square for equivalence of death rates = 9.012406 P = 0.0291; Chi-square for trend = 5.207331 P = 0.0225					

Table 5: Overall survival time of sarcoma treated cats with different stages.

		Stage I (days)	Stage II (days)	Stage III (days)	Not measured (days)
RFP	Median	132	183	117	78
	Mean	180.2	418.4	135	95.8
Chi-square for equivalence of death rates = 8.422251 P = 0.038; Chi-square for trend = 7.921812 P = 0.0049					

Table 6: Relapse free period of sarcoma treated cats with different stages.

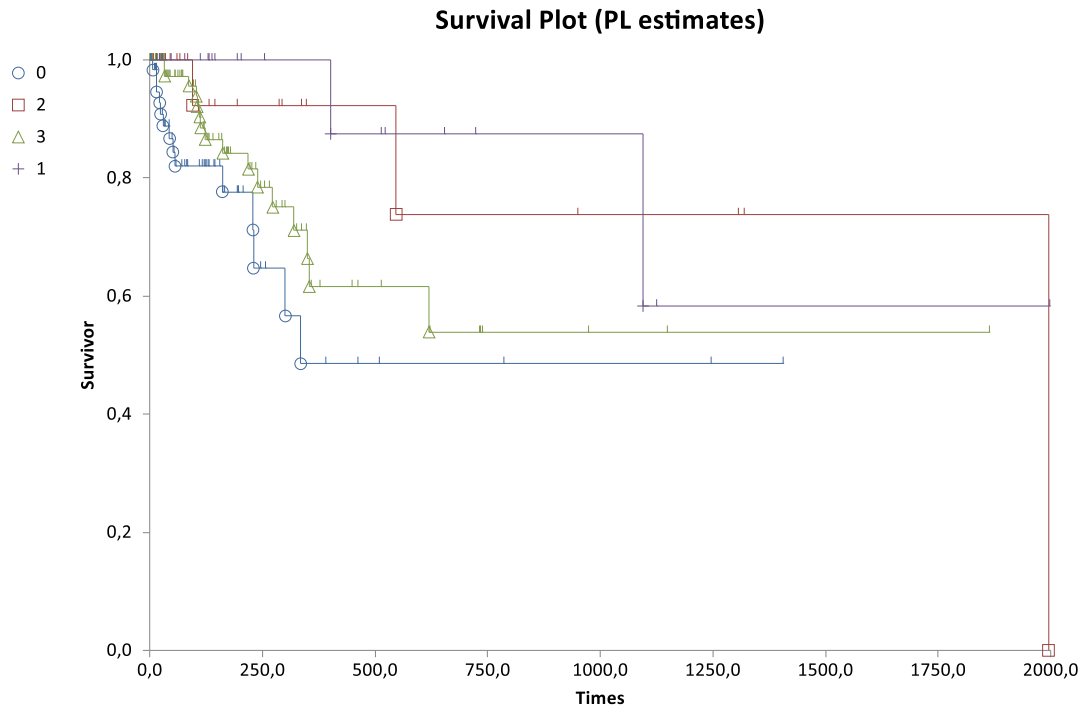

Figure 17: Overall survival times in cats distributed into different stages with sarcoma expressed by Kaplan-Meier curves.

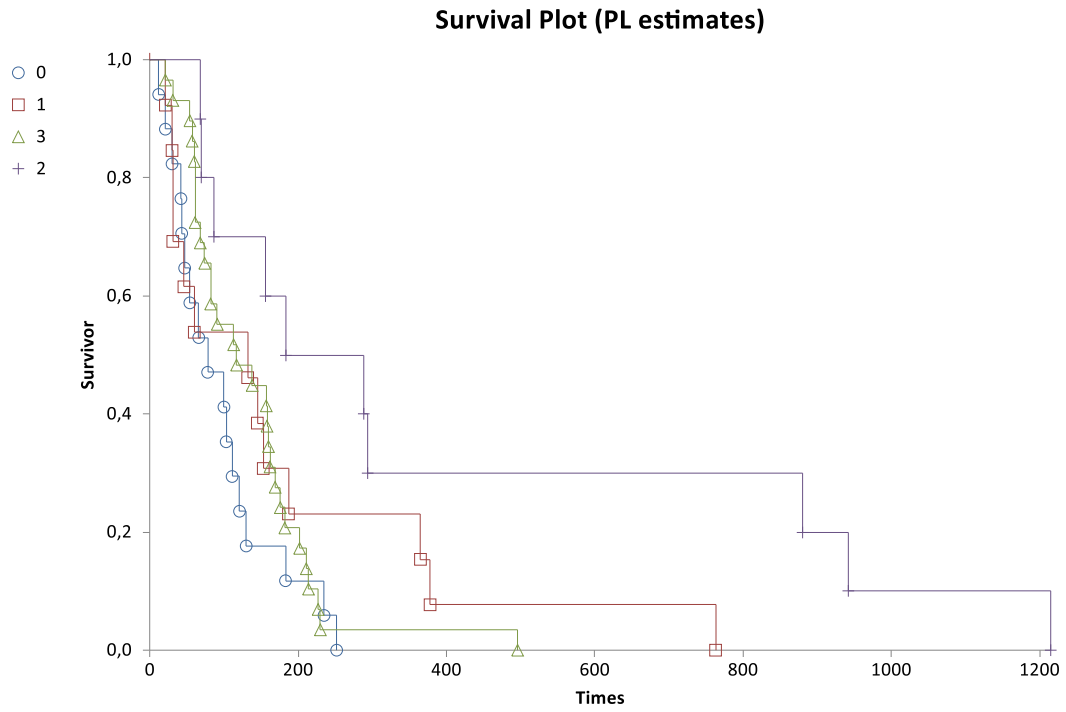

Figure 18: Relapse free period in cats distributed into different stages with sarcoma expressed by Kaplan-Meier curves.

The median overall survival time of cats that were already relapsed during the first examination at referral clinic (ÁHOK Ltd.) was 620 days (mean: 624.3 days). Whereas the cats that had primary tumour when appeared in the referral clinic was 1995 days (mean: 1374.6 days). This difference was non-significant. but close: Chi-square for equivalence of death rates = 3.363238 P = 0.0667.

The median relapse free period of cats that were already relapsed during the first examination at referral clinic (ÁHOK Ltd.) was 100 days (mean: 132.6 days). Whereas the cats that had primary tumour when appeared in the referral clinic was 160 days (mean: 236.8 days). This difference was significant: Chi-square for equivalence of death rates = 4.145456 P = 0.0417

The median overall survival time of cats without neoadjuvant chemotherapy (chemotherapy before surgery) was 1096 days (mean: 1 127 days). Whereas the cats receiving neoadjuvant chemotherapy before surgery the median survival time could not be calculated. although the mean was 1660.8 days. This difference was non-significant. but close: Chi-square for equivalence of death rates = 2.186179 P = 0.1393.

The median relapse time of cats without neoadjuvant chemotherapy (chemotherapy before surgery) was 91 days (mean: 138 days). Whereas the cats receiving neoadjuvant chemotherapy before surgery. the median survival time was 183 days (mean: 433 days). This difference was significant: Chi-square for equivalence of death rates = 7.116281 P = 0.0076.

The overall survival time and relapse free period of cats with different histopathology result could be evaluated. The changes were significant: Chi-square for equivalence of death rates = 19.609497 P = 0.051; Chi-square for trend = 8.89287 P = 0.0029

Histopathology type	**RFP**			**OST**		
	No	**Median**	**Mean**	**No**	**Median**	**Mean**
Histiocytic sarcoma	1	87	87	2	29	44.5
Non-specified sarcoma	28	132	163.4	82	can not estimate	1524.8
Fibrosarcoma	30	117	172.6	67	1096	1 087.8
Osteosarcoma	2	21	49.5	14	547	475.1
Osteochondrosarcoma	1	82	82	7	620	533.6
Pleomorphic sarcoma	0	NS	NS	4	can not estimate	134.2
Kheloidal sarcoma	0	NS	NS	1	can not estimate	14
Myxosarcoma	3	230	499.7	7	can not estimate	778.2
Rhabdomyosarcoma	2	54	140.5	4	320	427.1
Hemangiosarcoma	3	182	143.3	8	219	299.7
Anaplastic sarcoma	0	NS	NS	1	can not estimate	130
Gastrointestinal stromal tumor	0	NS	NS	2	can not estimate	22
Sum	70			199		

Table 7: Overall survival time and relapse free period of cats with different histopathology result.

The efficacy of therapy was calculated. The differences were not significant.

Overal Survival Time	No	Median	Mean
Wide surgery. Chemo. PDT	10	1096	1151
Wide surgery. Chemo	36	can not estimate	1559
Wide surgery. PDT	7	can not estimate	279
Wide surgery	12	can not estimate	312
Marginal surgery. Chemo. PDT	7	401	418
Marginal surgery. Chemo	35	can not estimate	238
Marginal surgery. PDT	2	1995	1995
Marginal surgery. only	17	can not estimate	944
Chemo. only	45	547	616
No recorded therapy	28	335	564
Chi-square for equivalence of death rates = 10.213874 P = 0.3335			
Chi-square for trend = 3.269884 P = 0.0706			

Table 8: Overall survival time of different therapy options in cats with sarcoma.

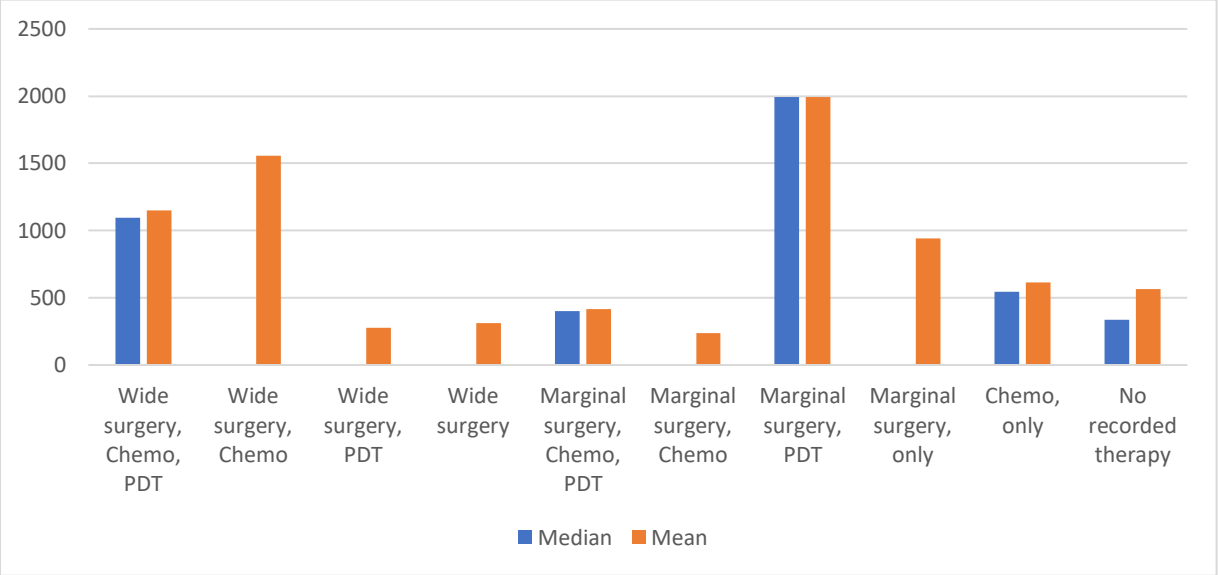

Figure 19: Overall survival time of different options in cats with sarcoma.

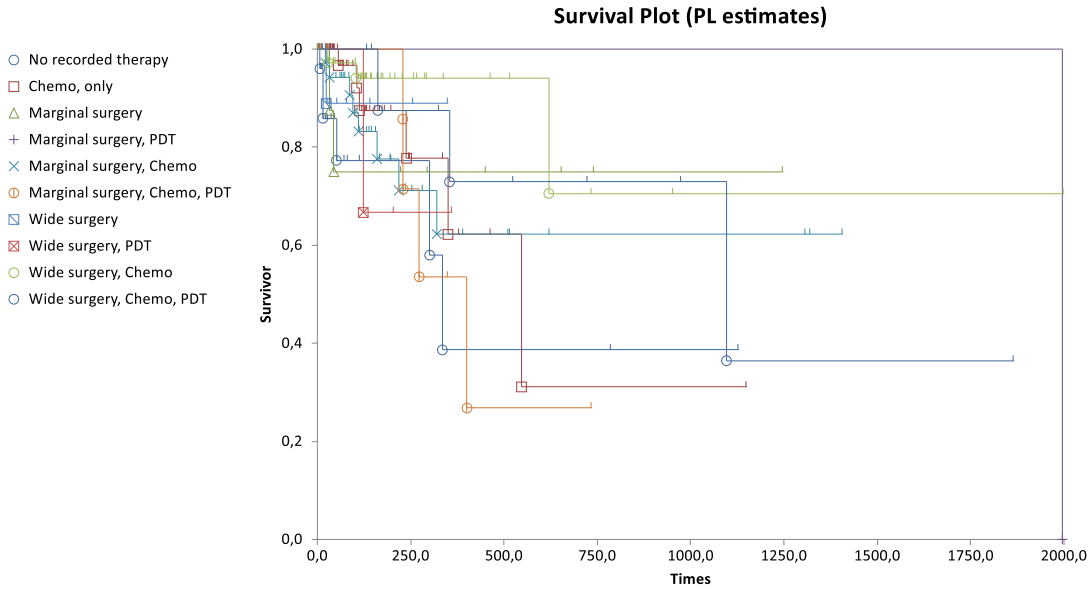

Figure 20: Overall survival time of different treatment options in cats with sarcoma expressed in Kapplan-Meier curves.

Relapse Free Period	No	Median	Mean
Wide surgery. Chemo. PDT	1	31	125
Wide surgery. Chemo	15	160	236
Wide surgery. PDT	1	74	74
Wide surgery	2	153	191
Marginal surgery. Chemo. PDT	4	82	252
Marginal surgery. Chemo	21	91	188
Marginal surgery. PDT	2	70	113
Marginal surgery. only	18	103	130
Chemo. only	1	82	82
No recorded therapy	1	47	47
Chi-square for equivalence of death rates = 6.964648 P = 0.6408			
Chi-square for trend = 0.995181 P = 0.3185			

Table 9: Relapse free period of different therapy options in cats with sarcoma.

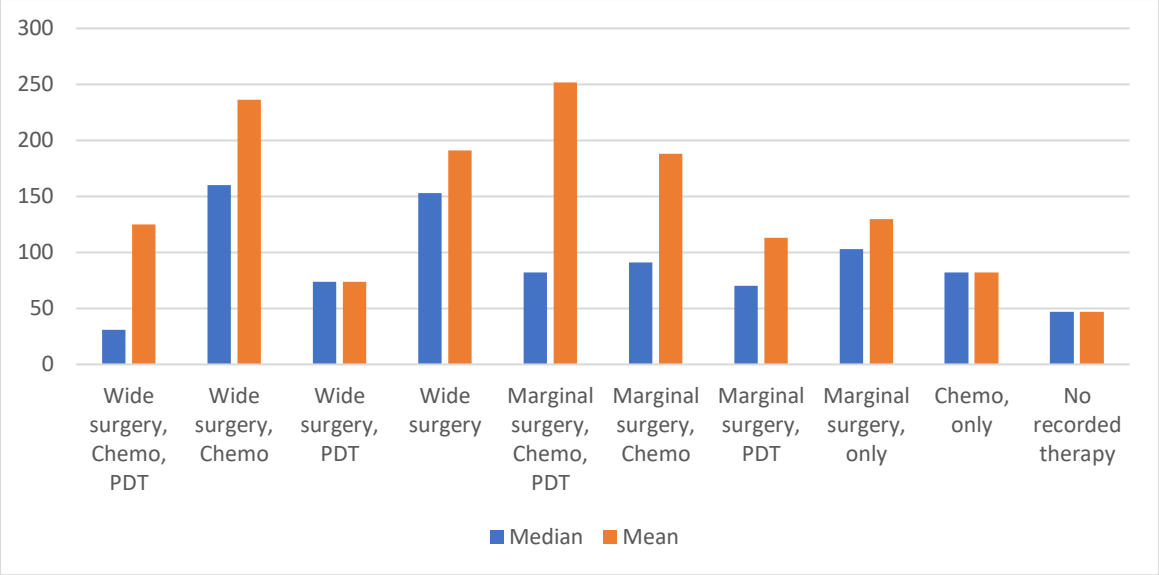

Figure 21: Relapse free period of different therapy options in cats with sarcoma.

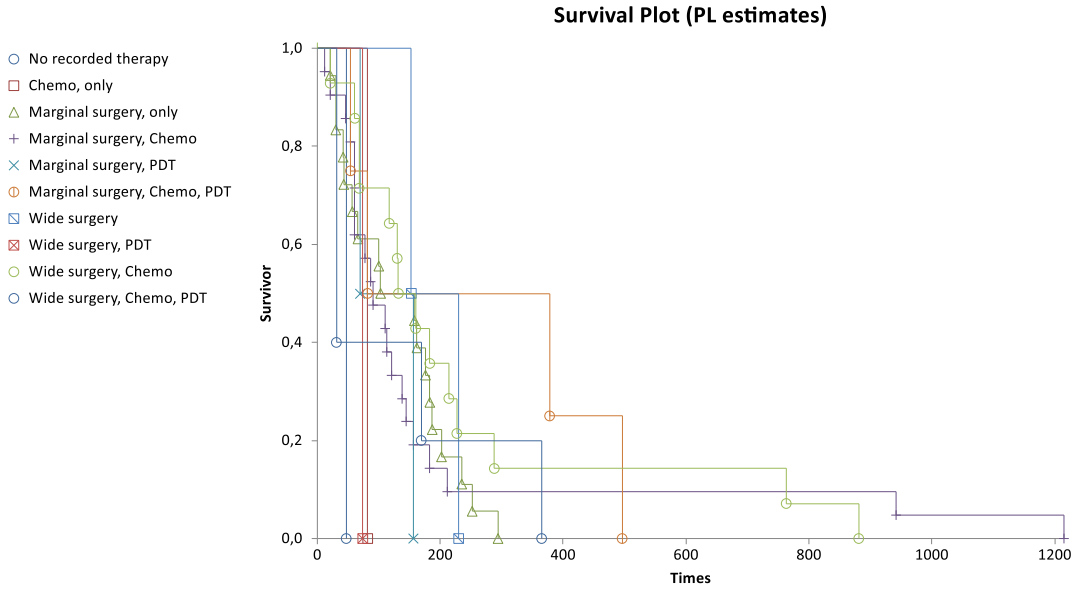

Figure 22: Relapse free period of different therapy options in cats with sarcoma expressed in Kapplan-Meier curves.

Table 10*:* Side effects and adverse drug reactions of the cats examined and treated at a Hungarian oncology clinic during the last 10 years.

	No	Percentage (%)
Nephropathy	21.0	7.07
Enteritis	5.0	1.68
Anorexia	2.0	0.67

Discussion

The demographic data of the cats were not different. and remarkable. Although, the mean age of the cats was 9.96 years (2 months to 21 years) which corresponds with the report as age at the time of diagnosis varied from 4 to 16 years (mean: 10.4) [15]. Gender distribution is not remarkable, although females were slightly more in number. The mean of largest tumour diameter was 3.8 cm (0.3 to 9 cm), whereas according to reports it was ranging from 1 to 15 cm (mean: 4.24 cm) [15]. The locations of the tumours were interscapular (16.84 %), thoracic wall (15.15 %) and the flank (10.1 %), and the lumbar region (7.41 %). According to reports the tumours localised at the sites of injections as in our cases: interscapular (22/48), thoracic (14/48), flank (9/48) and lumbar (3/48) regions [15].

According to stages, we found that there was marked difference in overall survival time and relapse free period according to stages, although, some median survival times could not be estimated properly due to the large numbers of censored data. It was marked that with stage I tumours (largest diameter < than 2 cm) showed as a mean of 1535 days, whereas stage III tumours (largest diameter > than 3 cm) showed a mean of 1137 days, which is approximately 1 year difference. The relapse time showed marked differences, too. A study reports that they evaluated a proper cut off value for tumour diameter and they found that the optimal cutoff for predicting recurrence was 3.75 cm (sensitivity = 88%, specificity = 85%, accuracy = 86%). They reported that a lower median survival time in cats with tumours ≥ 3.75 cm than in cats with tumours < 3.75 cm [9].

Histopathology types represented a large scale, but mainly fibrosarcoma (31.65 %) was found or non-specified sarcomas (46.13 %) due to the fact that some cases appeared to our

referral clinic with not detailed histopathology results. We had pleomorphic and anaplastic sarcomas and, while histiocytic sarcomas were not found in 5-10 % as it was reported, but 1.35 %, only. Our results mainly corresponded with most of the reports [15] as fibrosarcoma (30/48), pleomorphic sarcoma, (14/48) and malignant fibrous histiocytoma (4/48) was recorded by authors. The treatment options varied. Although, we found some similarities with other findings. Wide surgery (> 4 cm surgical margin) considered a good option and the overall survival time varied between 312 and 1151 days, according to adjuvant therapy. While it was reported that completeness of surgical margins was considered as one of the most important predictive factors for FISS treatment. According to a report where 5 cats with tumour cells in the margins of the resected tissue, showed a median disease-free interval (DFI) of 170 days, while 26 cats with negative tumour margins showed a median DFI of 700 days ($P < 0.0001$) [40].

We found that our cases treated with surgery and chemotherapy, that median survival time was with wide surgery 1559 days, and with marginal surgery as a mean of 238. While with wide surgery, only the mean survival was 312 days, and interestingly the marginal surgery mean was 944 days. We could not calculate with the histopathological margins, so, maybe the marginal surgery was sufficient in some cases.
Concerning chemotherapy, a report states that, when comparing the results of a multicentre study including 108 cats with sarcoma treated with either doxorubicin or liposomal doxorubicin (Caelyx) to a historical control group of cats with FISS treated with surgery alone, the cats receiving chemotherapy showed prolonged relapse free period (RFP) (388 versus 93 days) [21]. We also used Caelyx and doxorubicin, moreover epirubicin, too. For RFP, our result were not as good as in the mentioned article, as for Liposomal doxorubicin (Caelyx), Epirubicin, and Doxorubicin the median RFPs were 112, 111, 157 days, respectively. The number of the patients that were eligible for RFP calculation was 14, 31, 15, respectively. The overall survival time (OST) data were more reliable as the number of cases which were eligible for OST calculation was much more with patients treated by either Liposomal doxorubicin (Caelyx), Epirubicin, and Doxorubicin. The number of cases were 26, 92, 35, respectively, whereas the median OST data were 120, 132, 229.

Our results about the usefulness of neo-adjuvant chemotherapy then wide surgery and post-adjuvant therapy showed remarkable results which were corresponding to some other findings [3]. The median overall survival time of cats without neoadjuvant chemotherapy

(chemotherapy before surgery) was 1096 days (mean: 1127 days). Whereas the cats receiving neoadjuvant chemotherapy before surgery, the median survival time could not be calculated, although the mean was 1660.8 days. Moreover, the relapse free periods were also better with neo-adjuvant therapy vs. post-adjuvant therapy, only (median: 183 and 91 days, only).

We could not afford radiation therapy for cats, so we aimed to find an alternative method, the photodynamic therapy (PDT) to enhance the treatment efficacy by reducing the remaining tumour cells in tissues after surgical interventions. We found that there was a marked difference between the median survival times and mean between marginal surgery with and without PDT (mean: 1995 and 944 days, respectively). This finding is supported by previous studies where PDT was shown to prolong the survival time of animals [35]. PDT was evaluated with different conditions such as pancreatic cancer in Syrian golden hamsters and urothelial carcinoma in fisher rats [41]. The main indication for the use of PDT in veterinary medicine is the treatment of cutaneous squamous cell carcinoma in cats [42]. Our study showed a significant increase in both survival time and relapse free time; therefore, these findings suggest that PDT can be effectively used in feline injection site sarcoma as part of a multimodal treatment or best management when radiotherapy is not present.

Concerning side and adverse effects, the main threat in these therapies is the development of kidney failure. This was the found in 21 cases (7 %). The disease was sometimes fatal in 11 cases. Although, the cats were old even at the start of the therapies, so it is not known whether they could have limited renal function in the beginning which showed worsening during different therapeutic interventions. The frequency of adverse reactions in our cases are corresponding with other reports [2, 9].

Summary

Feline injection-site sarcomas were first recognised in the early 1990s. Initially, they were associated with rabies and feline leukaemia virus vaccinations, but further research has shown that these sarcomas primarily result from an abnormal tissue reaction to chronic inflammation and can be caused by anything that creates a local inflammatory reaction. Treatment of feline injection-site sarcomas is challenging due to the high local malignancy of these tumours and high recurrence rates. While no effective treatment options have been

found yet, a multifactorial management is the current recommendation. Radical surgery with clean surgical margins is believed to be the most important prognostic indicator, but in a multimodal therapeutic approach, neoadjunctive and adjunctive radiotherapy and chemotherapy together with surgery have shown promising results. Radiation therapy is not always available, and other treatment methods like immunotherapy and photodynamic therapy have shown positive results when used as alternatives.

In general, we can state that the demographic and tumour type frequency data moreover the survival results of multimodal therapy found in our cases, from the Hungarian Veterinary Hematology and Oncology Center, were similar to the findings reported by other authors. Nevertheless, we could not provide results of radiation therapy as we could not get an excess to this valuable tool.

Summary in Hungarian

A macska injekció beadásának helyén kialakuló szarkómákat először az 1990-es évek elején ismerték fel. Kezdetben a veszettség és a macskaleukémia vírus elleni oltással hozták összefüggésbe, de a további kutatások kimutatták, hogy ezek a szarkómák elsősorban a krónikus gyulladásra adott abnormális szöveti reakcióból származnak, és bármi okozhatja, ami helyi gyulladásos reakciót vált ki. A macskainjekció helyén fellépő szarkómák kezelése kihívást jelent e daganatok magas lokális rosszindulatúsága és magas kiújulási aránya miatt. Bár még nem találtak hatékony kezelési lehetőségeket, a jelenlegi ajánlás a többtényezős kezelés. A legfontosabb prognosztikai indikátornak a tiszta sebészeti határokkal rendelkező radikális műtétet tartják, de a multimodális terápiás megközelítésben a neoadjunctív és kiegészítő sugárkezelés, valamint a kemoterápia a műtéttel együtt ígéretes eredményeket mutatott. A sugárterápia nem mindig elérhető, és más kezelési módszerek, például az immunterápia és a fotodinamikus terápia pozitív eredményeket mutattak, ha alternatívaként alkalmazták őket.

Összességében megállapítható, hogy az eseteinkben a Magyar Állatorvosi Hematológiai és Onkológiai Centrumból a demográfiai és daganattípus gyakorisági adatai szmpontjából, valamint a multimodális terápia túlélési eredményei szempontjából is hasonlóak voltak más szerzők által közölt eredményekhez. Sajnos, azonban nem tudtunk eredményt adni a sugárkezelésről, mivel nem volt alkalmunk ehhez az értékes eszközhöz jutni, és azt alkalmazni.

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