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Summary of **'Feline Injection-Site Sarcoma: Matrix Remodelling and Prognosis -**

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Abstract

Feline injection-site sarcoma (FISS) represents a highly aggressive neoplasm that is thought to originate from the uncontrolled proliferation of fibroblasts and myofibroblasts within regions of persistent inflammation, most commonly corresponding to sites of previous injections. Surgical removal is frequently followed by local tumour regrowth. Gelatinases, specifically matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9), together with their endogenous inhibitor tissue inhibitor of metalloproteinases-2 (TIMP-2), are endopeptidases that play a central role in extracellular matrix remodelling and are therefore implicated in tumour invasiveness. The objective of the present study was to evaluate the immunohistochemical expression of MMP-2, MMP-9, and TIMP-2 in cases of FISS and to determine whether these markers could serve as prognostic indicators. Additional variables assessed for prognostic relevance included tumour size, grading according to the soft tissue sarcoma (STS) grading system, depth of tissue infiltration, surgical margin status, and the Ki-67 proliferative index.

A total of twenty-four primary FISS cases were retrospectively analysed and categorised based on clinical follow-up as non-recurrent (NR; fourteen cases, fifty-eight point three percent) or recurrent (R; ten cases, forty-one point seven percent). Immunohistochemical evaluation demonstrated variable expression of MMP-2, MMP-9, and TIMP-2 across tumours, supporting their involvement in tumour invasiveness; however, no statistically significant differences in expression were identified between recurrent and non-recurrent groups. This lack of association may reflect differences in tumour stage at the time of sampling or the diverse biological functions of these enzymes beyond extracellular matrix degradation. When considered in isolation, immunohistochemical expression of these proteins did not appear to provide prognostic utility. Likewise, STS grade, depth of infiltration, surgical margin status, and Ki-67 index showed no association with tumour recurrence. In contrast, tumour size measured after formalin fixation, with an optimal cut-off value of three point seven five centimetres (accuracy eighty-six percent; $P < .05$), and mitotic count, with an optimal threshold of twenty mitoses per ten high-power fields (accuracy eighty percent; $P < .05$), emerged as potentially valuable prognostic indicators.

Keywords

cats, immunohistochemistry, matrix metalloproteinases, mitotic count, neoplasm, postvaccinal sarcoma, skin, vaccine-associated fibrosarcoma

Introduction

Feline injection-site sarcoma (FISS) is a malignant neoplasm arising primarily within the subcutaneous tissues and only infrequently involving the dermis. It most commonly develops at sites used for vaccination, which historically led to the designation “vaccine-associated fibrosarcoma” or “postvaccinal sarcoma.” More recent investigations have suggested that vaccination is not the sole inciting factor, as a range of stimuli—including injections of non-vaccine materials, traumatic injury, and microchip implantation—may induce chronic inflammatory responses within the subcutis or musculature, ultimately contributing to malignant transformation and the development of FISS.

Histologically, these tumours are characterised by proliferations of atypical spindle-shaped cells and frequently contain varying numbers of multinucleated giant cells. Although fibrosarcoma represents the most common histological subtype, additional variants have been documented, including rhabdomyosarcoma, myxosarcoma, chondrosarcoma, osteosarcoma, and histiocytic sarcoma. Lymphocytic aggregates are often present, particularly at the periphery of the tumour mass, and extensive necrosis is commonly observed. Clinically, FISSs are notable for their marked local aggressiveness and high rate of post-surgical recurrence, while distant metastasis remains relatively uncommon.

Tumour aggressiveness is fundamentally linked to the capacity of neoplastic cells to infiltrate adjacent healthy tissues and, in some cases, to disseminate to distant sites. Disruption of normal extracellular proteolytic processes can facilitate unregulated tumour expansion, tissue remodelling, inflammatory responses, local invasion, and metastatic progression. Under physiological conditions, extracellular matrix turnover is tightly controlled; however, dysregulation of this process contributes to numerous pathological states, including invasive malignancies.

Matrix metalloproteinases (MMPs) constitute a family of proteolytic enzymes that participate in extracellular matrix degradation and are integral to tumour invasion. Their ability to enable cancer cell migration has prompted extensive investigation into their potential roles as prognostic markers and therapeutic targets in both human and veterinary oncology, including studies focused on sarcomas. Gelatinases, particularly MMP-2 and MMP-9, represent a subgroup of MMPs capable of degrading gelatin, various collagen types, and other extracellular matrix components. The activity of these enzymes is regulated by tissue inhibitors of matrix metalloproteinases (TIMPs), among which TIMP-2 is notable for its inhibitory effects on both MMP-2 and MMP-9. By modulating extracellular matrix remodelling, TIMP-2 can restrict tumour cell invasion and migration.

Alterations in the expression of MMPs and TIMPs play important roles in numerous physiological and pathological processes and have been examined in a variety of feline diseases. In the context of FISS, previous work using reverse transcriptase-polymerase chain

reaction on fresh tumour biopsies identified an association between the expression of MMP-2 and membrane-type MMP-16 and overall survival time.

The soft tissue sarcoma (STS) grading system, originally developed for human tumours and subsequently adapted for canine neoplasms, incorporates three principal parameters: degree of differentiation, mitotic activity, and extent of tumour necrosis. This system has also been applied to FISS, though studies have reported inconsistent prognostic significance. Surgical margin status has likewise been proposed as a prognostic indicator; however, the occurrence of recurrence despite histologically clean margins suggests that even aggressive surgical approaches may not fully eliminate the risk of local tumour regrowth.

The present retrospective study was designed to evaluate the prognostic relevance of immunohistochemical expression of the gelatinases MMP-2 and MMP-9, along with their inhibitor TIMP-2, in feline injection-site sarcomas. In addition, the prognostic significance of tumour size, STS grade, depth of infiltration, surgical margin status, and Ki-67 expression was systematically assessed.

Materials and Methods

Case Selection

Formalin-fixed, paraffin-embedded specimens from twenty-four cats diagnosed with primary feline injection-site sarcoma were retrospectively retrieved from the archival material of the Department of Veterinary Medicine, University of Perugia, Italy

All cases were originally diagnosed between 2008 and 2013, and clinical follow-up data were collected through December 2015. Inclusion of cases was based on the following predefined criteria: localisation of the tumour in the interscapular or dorsal region; treatment consisting of curative-intent surgical excision; availability of follow-up information obtained through telephone interviews with the referring veterinarians; a minimum disease-free follow-up period of twenty-four months, in accordance with disease-free intervals reported in the literature; and a consensual diagnosis of feline injection-site sarcoma established on the basis of characteristic histological features.

Signalment data, including age, breed, sex, and neutering or spaying status, were obtained from the institutional database. Tumour size was defined as the major diameter and was measured after fixation in formalin for a period ranging from twenty-four to forty-eight hours, depending on specimen dimensions. Measurements were performed using a calliper at the time of tissue trimming. Clinical measurements obtained prior to fixation were unavailable for all cases and therefore were not considered.

Histologic Evaluation

All tumour specimens were sectioned at a thickness of five micrometres and routinely stained with haematoxylin and eosin. Histological assessment was conducted using light microscopy. Each case underwent blind evaluation by three independent observers, and any discrepancies were resolved through joint re-examination. The diagnosis of feline injection-site sarcoma was confirmed for all samples, and tumours were graded according to the soft tissue sarcoma grading system adapted for veterinary use.

The grading system incorporated three histological parameters: degree of tumour cell differentiation, mitotic activity, and extent of tumour necrosis. Mitotic count was assessed in a blinded manner by enumerating mitotic figures across ten consecutive high-power fields, beginning in areas exhibiting the highest mitotic activity while deliberately avoiding regions affected by necrosis or marked inflammation. The microscopic field corresponded to an ocular field number of twenty-two with a forty times objective, yielding a standardised area. The total number of mitoses was recorded for each observer, and the mean value across the three evaluations was calculated for subsequent analyses.

Tumour infiltration depth was documented and categorised according to anatomical involvement as dermal, subcutaneous, or muscular. Evaluation of surgical margins was feasible in nineteen of the twenty-four cases. Specimens were trimmed into slices approximately three millimetres thick using parallel sectioning at regular intervals. Sections were selected preferentially from regions where the tumour appeared macroscopically closest to the excisional margins. The number of tissue cassettes processed varied with tumour size and ranged from two to ten per case. Histological margin status was classified following published guidelines into infiltrated margins, defined by the presence of neoplastic cells in continuity with at least one surgical margin in any plane, and non-infiltrated margins, defined by the absence of tumour cells at the margins. Margins described as close, measuring less than three millimetres, as well as margins considered clean, with distances of three to five millimetres, were both included within the non-infiltrated category.

Immunohistochemistry

For immunohistochemical analysis, tissue sections five micrometres thick were mounted on positively charged slides, followed by dewaxing and rehydration. Immunostaining procedures were performed using established protocols and antibodies directed against matrix metalloproteinase-2, matrix metalloproteinase-9, tissue inhibitor of metalloproteinases-2, and Ki-67. After incubation with the primary antibodies, slides were treated using a ready-to-use avidin–biotin complex detection system in accordance with the manufacturer’s instructions. Immunoreactivity was visualised using 3-amino-9-ethylcarbazole as the chromogen, and Carazzi’s haematoxylin was applied as a counterstain.

Normal feline myocardium served as a positive control for matrix metalloproteinases and tissue inhibitor of metalloproteinases-2. Immunostaining for vimentin was carried out to confirm the mesenchymal origin of the tumours. Negative control sections were incubated with tris-buffered saline in place of the primary antibody.

Evaluation of immunohistochemical staining for matrix metalloproteinase-2, matrix metalloproteinase-9, and tissue inhibitor of metalloproteinases-2 was performed independently and blindly by three observers. The proportion of positively stained neoplastic cells was expressed as a percentage ranging from zero to one hundred percent of the total tumour cell population. Ki-67 immunoreactivity was assessed by counting positively stained nuclei within ten randomly selected high-power fields, excluding areas of necrosis and inflammation. Counts were recorded separately by each observer, and mean values were calculated for each tumour.

Statistical Analysis

Preliminary evaluation of data distributions and detection of outliers were conducted using diagnostic graphical methods and Levene’s test. At the conclusion of the follow-up period, cases were assigned to either the recurrent or non-recurrent group. Continuous variables, including age, tumour size, mitotic count, and the immunohistochemical expression of matrix metalloproteinase-2, matrix metalloproteinase-9, tissue inhibitor of metalloproteinases-2, and Ki-67, were initially compared between groups using the Mann–Whitney test. Associations between mitotic activity and Ki-67 expression were assessed using Spearman rank correlation coefficients.

Continuous data were summarised as mean values with standard deviations, as well as medians with interquartile ranges. Categorical variables, including breed, sex, neutering or spaying status, mitotic score, differentiation score, necrosis score, soft tissue sarcoma grade, surgical margin status, and depth of infiltration, were compared between recurrent and non-recurrent groups using Fisher’s exact test and reported as counts and percentages.

Survival probabilities were estimated using the life table method, while disease-free and overall survival times were calculated using Kaplan–Meier analysis. Differences in survival distributions between groups were assessed using the log-rank test. Animals lost to follow-up or those that died from causes unrelated to feline injection-site sarcoma were censored and included in survival analyses. The prognostic significance of individual variables for recurrence and survival was further examined using univariate Cox proportional hazards regression models, with results expressed as hazard ratios accompanied by ninety-five percent confidence intervals and P values derived from Wald statistics.

Receiver operating characteristic curve analysis was employed to evaluate the diagnostic accuracy of selected parameters for predicting tumour recurrence. Based on the area under

the curve statistic, diagnostic performance was classified as non-informative, less accurate, moderately accurate, highly accurate, or perfect. Optimal cut-off values were identified as points closest to the coordinate zero, one, and by calculation of the Youden index. Comparisons between receiver operating characteristic curves were conducted using a critical ratio z-test. All statistical analyses were performed using SPSS version twenty point zero, and a P value of less than zero point zero five was considered statistically significant.

Results

Clinical Data

The age of the cats at the time of diagnosis ranged from six to sixteen years, with a mean age of eleven years plus or minus three years and a median age of eleven years with an interquartile range of ten to thirteen years

The majority of animals were domestic shorthair cats, accounting for twenty-two of the twenty-four cases, while the remaining two cats were Siamese. Of the total study population, ten cats were male and thirteen were female; sex was not recorded for one individual. Among male cats, six of ten were neutered, whereas seven of thirteen females had been spayed.

Comparative analysis between recurrent and non-recurrent groups revealed no statistically significant differences with respect to age, breed, sex, or neutering or spaying status. The duration of clinical follow-up extended from seven hundred and thirty-nine to two thousand four hundred and sixty-seven days, with a median follow-up period of one thousand two hundred and twenty-two days. More than seventy percent of cats were monitored for longer than three years, and approximately thirty percent were followed for periods exceeding four years.

Estimated probabilities of remaining free from tumour recurrence following primary feline injection-site sarcoma excision were seventy-nine percent plus or minus eight percent at six months, sixty-seven percent plus or minus ten percent at one year, and sixty-two percent plus or minus ten percent at two years. Corresponding overall survival probabilities were ninety-six percent plus or minus four percent at six months and seventy-five percent plus or minus nine percent at both one and two years.

At a minimum of twenty-four months following surgery, tumour recurrence was documented in ten of the twenty-four cases, representing forty-one point seven percent of the study population. No metastatic disease was identified in any cat during the follow-up period. In the recurrent group, the median disease-free interval was one hundred and sixty-two days,

with an interquartile range of ninety-three to two hundred and forty-five days. Median survival time for these cats was three hundred and twenty-seven days, with an interquartile range of two hundred and seventy-three to nine hundred and ninety-four days. Survival probabilities within the recurrent group were estimated at ninety percent plus or minus nine percent at six months and thirty-seven percent plus or minus sixteen percent at both one and two years. By the conclusion of the study, seven of ten cats in the recurrent group had died as a direct consequence of the tumour, while three cases were censored due to loss to follow-up or death unrelated to feline injection-site sarcoma. As no tumour-related deaths occurred within the non-recurrent group, survival statistics were not calculated for these animals. Log-rank testing demonstrated a statistically significant difference in survival time between recurrent and non-recurrent groups.

Tumour size, expressed as the major diameter of formalin-fixed specimens, ranged from one point five to eight point zero centimetres. Tumours that recurred were significantly larger than those that did not recur, with recurrent tumours exhibiting a mean diameter of five point three centimetres plus or minus two point one centimetres and a median diameter of five point zero centimetres. In contrast, non-recurrent tumours had a mean diameter of two point seven centimetres plus or minus one point one centimetres and a median diameter of two point five centimetres. This difference was statistically significant, with the largest tumour diameters observed predominantly within the recurrent group. Each unit increase in tumour diameter was associated with a one point seven two-fold increase in the likelihood of recurrence and a one point eight eight-fold increase in the risk of tumour-related death, as determined by Cox proportional hazards modelling.

Histologic Examination

Tumours were graded using the soft tissue sarcoma grading system. Two cases were classified as grade one sarcomas, with one occurring in each clinical group. Ten tumours were categorised as grade two sarcomas, evenly distributed between recurrent and non-recurrent cases. The remaining twelve tumours were assigned grade three status, of which four were recurrent and eight were non-recurrent. Statistical analysis revealed no significant differences in overall tumour grade between groups, nor did grade predict the risk of recurrence or survival outcome.

In contrast, mitotic score differed significantly between recurrent and non-recurrent tumours. Seven of ten recurrent tumours exhibited a mitotic score of three, whereas only two of fourteen non-recurrent tumours fell within this category. Consequently, mitotic count was further evaluated as a continuous variable. Recurrent tumours demonstrated a markedly higher mitotic count, with a mean of twenty-seven mitoses per ten high-power fields and a median of twenty-nine mitoses. Non-recurrent tumours showed substantially lower values, with a mean of thirteen and a median of twelve mitoses per ten high-power fields. These differences were statistically significant. Cox regression analysis confirmed



mitotic count as a significant predictor of both tumour recurrence and tumour-related mortality.

Depth of infiltration was assessed in twenty-three of the twenty-four tumours. None of the tumours was restricted to the dermis. Fifteen tumours infiltrated the subcutaneous tissue, while eight extended into the underlying musculature. No statistically significant associations were identified between depth of infiltration and either recurrence risk or survival time.

Evaluation of surgical margins was possible in nineteen tumours. Margins were classified as infiltrated in six cases and non-infiltrated in thirteen cases. The distribution of margin status did not differ significantly between recurrent and non-recurrent tumours, and no association was detected between margin infiltration and tumour recurrence.

Immunohistochemistry

Matrix Metalloproteinase-2

Immunohistochemical staining for matrix metalloproteinase-2 demonstrated marked variability in the proportion of positive tumour cells across both recurrent and non-recurrent groups. Expression was detected in multifocal to confluent patterns in twenty-two of the twenty-four tumours examined. The two tumours that lacked detectable staining belonged to the recurrent group. Within recurrent tumours, the proportion of MMP-2–positive neoplastic cells ranged from zero to eighty-three point three percent, with a mean value of forty-four point eight percent plus or minus thirty-three point nine percent and a median of forty-six point seven percent. In non-recurrent tumours, positive cell percentages ranged from one point seven to ninety-six point seven percent, with a mean of thirty-seven point six percent plus or minus thirty-two point six percent and a median of twenty-five point eight percent.

Immunoreactivity for MMP-2 was primarily localised within the cytoplasm of mononucleated spindle-shaped tumour cells, while multinucleated giant cells exhibited only occasional positivity. Statistical analysis revealed no significant difference in MMP-2 expression between recurrent and non-recurrent groups. Consistent with these findings, Cox proportional hazards modelling showed that the proportion of MMP-2–positive cells was not associated with an increased risk of tumour recurrence or tumour-related death.

In addition to tumour cell staining, MMP-2 immunoreactivity was consistently observed within endothelial cells of small intratumoural and peritumoural blood vessels. This endothelial expression was particularly prominent within capillaries associated with lymphocytic aggregates.

Matrix Metalloproteinase-9

Expression of matrix metalloproteinase-9 was highly heterogeneous among tumours and appeared in multifocal to confluent distributions. Five tumours, including two recurrent and three non-recurrent cases, were entirely negative for MMP-9 immunostaining. In recurrent tumours, the percentage of positive neoplastic cells ranged from zero to fifty-five percent, with a mean of fourteen point nine percent plus or minus twenty-two point nine percent and a median of one point seven percent. Non-recurrent tumours exhibited values ranging from zero to fifty percent, with a mean of twenty-one point two percent plus or minus nineteen point one percent and a median of eighteen point three percent.

MMP-9 immunoreactivity was predominantly localised within the cytoplasm of multinucleated giant cells, whereas mononucleated spindle cells were infrequently positive and generally exhibited weak staining intensity. Comparative analysis demonstrated no statistically significant differences in MMP-9 expression between recurrent and non-recurrent groups. Cox regression analysis further confirmed that MMP-9 expression did not predict tumour recurrence or survival outcome.

Tissue Inhibitor of Metalloproteinases-2

Tissue inhibitor of metalloproteinases-2 was expressed in all tumours examined. In recurrent cases, the proportion of positive tumour cells ranged from five point zero to ninety-three point three percent, with a mean of fifty-nine point one percent plus or minus thirty-one point nine percent and a median of sixty-five point zero percent. In non-recurrent tumours, positive cell percentages ranged from twenty-two point zero to ninety-three point zero percent, yielding a mean of sixty-three point one percent plus or minus twenty-six point one percent and a median of sixty-three point five percent.

Cytoplasmic staining for TIMP-2 was evident in both mononucleated spindle cells and multinucleated giant cells. No statistically significant differences were identified between recurrent and non-recurrent groups. Cox proportional hazards modelling indicated that TIMP-2 expression was not associated with the risk of recurrence or tumour-related mortality.

Ki-67

Ki-67 immunoreactivity was observed mainly within mononucleated tumour cells, with only rare nuclear staining detected in multinucleated giant cells. The number of Ki-67–positive nuclei per high-power field ranged from four to thirty-eight. In recurrent tumours, the mean number of positive nuclei was twenty-one point four plus or minus fourteen point four, with a median of eighteen point seven. In non-recurrent tumours, the mean was twenty-three point seven plus or minus seventeen point three, with a median of twenty-six point five.

No correlation was identified between Ki-67 expression and mitotic count. Furthermore, the number of Ki-67–positive nuclei did not differ significantly between recurrent and non-recurrent groups. Cox regression analysis demonstrated that Ki-67 was not predictive of tumour recurrence or survival.

Receiver Operating Characteristic Analysis

Variables that demonstrated statistical significance in bivariate analyses were selected for receiver operating characteristic analysis to evaluate their ability to predict tumour recurrence. Tumour size and mitotic activity were assessed, with mitotic activity analysed both as a continuous mitotic count value and as a categorical score.

The area under the receiver operating characteristic curve for tumour major diameter was zero point eight six one, indicating high diagnostic accuracy. Analysis using the Youden index identified an optimal cut-off value of three point seven five centimetres for predicting recurrence, corresponding to a sensitivity of eighty-eight percent, a specificity of eighty-five percent, and an overall accuracy of eighty-six percent. Cats with tumours equal to or exceeding this threshold exhibited significantly shorter survival times compared with those bearing smaller tumours.

Mitotic count yielded an area under the curve of zero point eight eight two, also indicative of high diagnostic accuracy. The optimal cut-off value for mitotic count was twenty mitoses per ten high-power fields, which provided a sensitivity of seventy percent, a specificity of eighty-six percent, and an accuracy of eighty percent. Tumours with mitotic counts at or above this threshold were associated with reduced median survival times. No statistically significant differences were observed when comparing the diagnostic performance of tumour diameter and mitotic count, nor between mitotic count and mitotic score.

Discussion

The objective of this retrospective investigation was to evaluate the expression of matrix metalloproteinase-2, matrix metalloproteinase-9, and one of their endogenous inhibitors, tissue inhibitor of metalloproteinases-2, within feline injection-site sarcomas, with the aim of determining whether these molecules could function as predictors of tumour biological behaviour. In parallel, the prognostic relevance of tumour size, soft tissue sarcoma grade, depth of infiltration, surgical margin status, and Ki-67 proliferative index was also examined.

Matrix metalloproteinases and their inhibitors have been widely recognised as key contributors to tumour aggressiveness across a range of neoplastic diseases. Matrix metalloproteinase-2, a gelatinase capable of degrading multiple extracellular substrates including denatured collagen, was detected in nearly all feline injection-site sarcomas analysed, with the exception of two recurrent tumours. Despite its widespread expression,

no association was identified between MMP-2 immunoreactivity and either tumour recurrence or survival outcome. The involvement of MMP-2 in angiogenesis remains controversial, as it has been shown to exert both pro-survival and pro-angiogenic effects as well as pro-apoptotic and anti-angiogenic actions, depending on the biological context. In the present study, consistent endothelial expression of MMP-2 was observed, particularly at the tumour periphery and within lymphocytic aggregates, supporting a role in angiogenesis and leukocyte migration within feline injection-site sarcomas.

Matrix metalloproteinase-9 shares overlapping substrate specificity with MMP-2, with the exception of collagen type I, and its expression may be induced in various cell types during inflammatory processes. Increased MMP-9 activity has been documented in malignant cell lines, and experimental models have demonstrated reduced tumour proliferation in the absence of MMP-9, suggesting a growth-promoting role. In the current study, MMP-9 expression was highly variable and predominantly localised within multinucleated giant cells. As with MMP-2, no statistically significant differences were observed between recurrent and non-recurrent tumours. The absence of prognostic significance for both gelatinases may reflect stage-dependent expression patterns during tumour progression. Evidence from failed clinical trials involving metalloproteinase inhibitors has suggested that these enzymes may play a more critical role during early tumour development rather than in advanced disease stages. Furthermore, the functional complexity of matrix metalloproteinases extends beyond extracellular matrix degradation, with both MMP-2 and MMP-9 reported to exert tumour-suppressive effects under certain conditions. Given the large number of metalloproteinases involved in tissue remodelling, it is plausible that MMP-2 and MMP-9 contribute to tumour invasiveness without serving as decisive prognostic determinants when assessed in isolation.

Tissue inhibitor of metalloproteinases-2 was consistently expressed in all tumours examined, with no differences observed between clinical outcome groups. This finding was unexpected, as TIMPs are classically viewed as regulators of metalloproteinase activity and extracellular matrix turnover. However, increasing evidence indicates that TIMPs possess biological functions independent of metalloproteinase inhibition. Specifically, TIMP-2 has been shown to influence endothelial and fibroblast differentiation, which may paradoxically support tumour progression. The absence of metastatic disease in the present cohort may be related to the lack of systematic radiographic or advanced imaging evaluation in all cats prior to death.

Tumour size emerged as a significant prognostic factor, with recurrent tumours exhibiting significantly larger diameters than non-recurrent lesions. These findings reinforce the importance of early detection and prompt surgical intervention in feline injection-site sarcoma management. The optimal cut-off value identified in this study was three point seven five centimetres; however, this threshold pertains specifically to formalin-fixed specimens and cannot be directly extrapolated to in vivo clinical measurements.

Although feline injection-site sarcomas are commonly classified as soft tissue sarcomas, the applicability of the soft tissue sarcoma grading system as a prognostic tool has remained controversial. In this study, overall tumour grade did not predict recurrence or survival. Among the individual grading parameters, only mitotic activity demonstrated a statistically significant association with clinical outcome. Mitotic count, when analysed as a continuous variable, was a strong predictor of both tumour recurrence and tumour-related mortality. Receiver operating characteristic analysis confirmed the prognostic utility of mitotic count, with an optimal threshold of twenty mitoses per ten high-power fields. Tumours exceeding this value were associated with an increased risk of recurrence and reduced survival time. In contrast, tumour necrosis and degree of differentiation did not differ between outcome groups, suggesting limited prognostic relevance in feline injection-site sarcomas.

Surgical margin status did not significantly influence recurrence risk in the present cohort. Tumours were classified as having infiltrated or non-infiltrated margins, with close and clean margins grouped together due to sample size limitations. These findings suggest that this binary classification may not be sufficient as a prognostic indicator and further support recommendations for wide surgical excision when managing these tumours.

The Ki-67 proliferative index did not differ between recurrent and non-recurrent tumours and did not correlate with mitotic count. The lack of association between Ki-67 expression and mitotic activity has been reported previously in both human and veterinary oncology and underscores the independent biological significance of these proliferative markers.

Conclusion

In summary, matrix metalloproteinase-2, matrix metalloproteinase-9, and tissue inhibitor of metalloproteinases-2 appear to play roles in the biological behaviour of feline injection-site sarcomas, as indicated by their widespread expression; however, none of these markers demonstrated sufficient prognostic value when evaluated individually. In contrast, tumour size and mitotic count emerged as the most reliable prognostic indicators within this cohort. Specifically, a major tumour diameter of three point seven five centimetres in formalin-fixed specimens and a mitotic count of twenty mitoses per ten high-power fields represent useful threshold values for predicting recurrence and informing prognosis. Given the retrospective nature of the study and the relatively small sample size, further investigations involving larger case series are warranted to refine prognostic assessment in feline injection-site sarcomas.

References

1. Akkoc A, Inan S, Sonmez G. Matrix metalloproteinase (MMP-2 and MMP-9) and steroid receptor expressions in feline mammary tumors. Biotech Histochem. 2012;87(4):312–319.

2. Aupperle H, Baldauf K, März I. An immunohistochemical study of feline myocardial fibrosis. J Comp Pathol. 2011;145(2–3):158–173.
3. Benassi MS, Gamberi G, Magagnoli G, et al. Metalloproteinase expression and prognosis in soft tissue sarcomas. Ann Oncol. 2001;12(1):75–80.
4. Björklund M, Koivunen E. Gelatinase-mediated migration and invasion of cancer cells. Biochim Biophys Acta. 2005;1755(1):37–69.
5. Bray J, Polton G. Neoadjuvant and adjuvant chemotherapy combined with anatomical resection of feline injection-site sarcoma: results in 21 cats. Vet Comp Oncol. 2016;14(2):147–160.
6. Carminato A, Vascellari M, Marchioro W, Melchiotti E, Mutinelli F. Microchip-associated fibrosarcoma in a cat. Vet Dermatol. 2011;22(6):565–569.
7. Chakrabarti S, Patel KD. Matrix metalloproteinase-2 (MMP-2) and MMP-9 in pulmonary pathology. Exp Lung Res. 2005;31(6):599–621.
8. Coindre JM. Grading of soft tissue sarcomas: review and update. Arch Pathol Lab Med. 2006;130(10):1448–1453.
9. Couto SS, Griffey SM, Duarte PC, Madewell BR. Feline vaccine-associated fibrosarcoma: morphologic distinctions. Vet Pathol. 2002;39(1):33–41.
10. Dean RS, Pfeiffer DU, Adams VJ. The incidence of feline injection site sarcomas in the United Kingdom. BMC Vet Res. 2013;9:17.
11. Dennis MM, McSporran KD, Bacon NJ, Schulman FY, Foster RA, Powers BE. Prognostic factors for cutaneous and subcutaneous soft tissue sarcomas in dogs. Vet Pathol. 2011;48(1):73–84.
12. Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer. 2002;2(3):161–174.
13. Field A. Discovering Statistics Using SPSS. London, UK: SAGE; 2009.
14. Giantin M, Aresu L, Benali S, et al. Expression of matrix metalloproteinases, tissue inhibitors of metalloproteinases and vascular endothelial growth factor in canine mast cell tumours. J Comp Pathol. 2012;147(4):419–429.
15. Giudice C, Stefanello D, Sala M, et al. Feline injection-site sarcoma: recurrence, tumour grading and surgical margin status evaluated using the three-dimensional histological technique. Vet J. 2010;186(1):84–88.
16. Greiner M, Pfeiffer D, Smith RD. Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. Prev Vet Med. 2000;45(1–2):23–41.
17. Gross TL, Ihrke PJ, Walder EJ, et al. Skin Diseases of the Dog and Cat. 2nd ed. Oxford, UK: Blackwell; 2005.
18. Hendrick MJ, Brooks JJ. Postvaccinal sarcomas in the cat: histology and immunohistochemistry. Vet Pathol. 1994;31(1):126–129.
19. Hershey AE, Sorenmo KU, Hendrick MJ, Shofer FS, Vail DM. Prognosis for presumed feline vaccine-associated sarcoma after excision: 61 cases (1986–1996). J Am Vet Med Assoc. 2000;216(1):58–61.

20. Jalava P, Kuopio T, Juntti-Patinen L, Kotkansalo T, Kronqvist P, Collan Y. Ki67 immunohistochemistry: a valuable marker in prognostication but with a risk of misclassification. Histopathology. 2006;48(6):674–682.
21. Kamstock DA, Ehrhart EJ, Getzy DM, et al. Recommended guidelines for submission, trimming, margin evaluation, and reporting of tumor biopsy specimens in veterinary surgical pathology. Vet Pathol. 2011;48(1):19–31.
22. Kass PH, Barnes WG Jr, Spangler WL, Chomel BB, Culbertson MR. Epidemiologic evidence for a causal relation between vaccination and fibrosarcoma tumorigenesis in cats. J Am Vet Med Assoc. 1993;203(3):396–405.
23. Kessenbrock K, Plaks V, Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. Cell. 2010;141(1):52–67.
24. Klein T, Bischoff R. Physiology and pathophysiology of matrix metalloproteases. Amino Acids. 2011;41(2):271–290.
25. Martano M, Morello E, Buracco P. Feline injection-site sarcoma: past, present and future perspectives. Vet J. 2011;188(2):136–141.
26. Martano M, Morello E, Iussich S, Buracco P. A case of feline injection-site sarcoma at the site of cisplatin injections. J Feline Med Surg. 2012;14(10):751–754.
27. McEntee MC, Page RL. Feline vaccine-associated sarcomas. J Vet Intern Med. 2001;15(3):176–182.
28. Meuten DJ, Moore FM, George JW. Mitotic count and the field of view area: time to standardize. Vet Pathol. 2016;53(1):7–9.
29. Moore CS, Crocker SJ. An alternate perspective on the roles of TIMPs and MMPs in pathology. Am J Pathol. 2012;180(1):12–16.
30. Nagase H, Visse R, Murphy G. Structure and function of matrix metalloproteinases and TIMPs. Cardiovasc Res. 2006;69(3):562–573.
31. Petznick A, Madigan MC, Garrett Q, Sweeney DF, Evans MD. Contributions of ocular surface components to matrix-metalloproteinases (MMP)-2 and MMP-9 in feline tears following corneal epithelial wounding. PLoS One. 2013;8(8):e71948.
32. Porcellato I, Giontella A, Mechelli L, Del Rossi E, Brachelente C. Feline eosinophilic dermatoses: a retrospective immunohistochemical and ultrastructural study of extracellular matrix remodelling. Vet Dermatol. 2014;25(2):86–94.
33. Romanelli G, Marconato L, Olivero D, Massari F, Zini E. Analysis of prognostic factors associated with injection-site sarcomas in cats: 57 cases (2001–2007). J Am Vet Med Assoc. 2008;232(8):1193–1199.
34. Roomi MW, Kalinovsky T, Monterrey J, Rath M, Niedzwiecki A. In vitro modulation of MMP-2 and MMP-9 in adult human sarcoma cell lines. Int J Oncol. 2013;43(6):1787–1798.
35. Shapiro S, Khodalev O, Bitterman H, Auslender R, Lahat N. Different activation forms of MMP-2 oppositely affect the fate of endothelial cells. Am J Physiol Cell Physiol. 2010;298(4):C942–C951.

36. Sorensen KC, Kitchell BE, Schaeffer DJ, Mardis PE. Expression of matrix metalloproteinases in feline vaccine site-associated sarcomas. Am J Vet Res. 2004;65(3):373–379.
37. Stefanidakis M, Koivunen E. Cell-surface association between matrix metalloproteinases and integrins. Blood. 2006;108(5):1441–1450.
38. Stetler-Stevenson WG. The tumor microenvironment: regulation by MMP-independent effects of tissue inhibitor of metalloproteinases-2. Cancer Metastasis Rev. 2008;27(1):57–66.
39. Stetler-Stevenson WG, Gavil NV. Normalization of the tumor microenvironment. Connect Tissue Res. 2014;55(1):13–19.
40. Swets JA. Measuring the accuracy of diagnostic systems. Science. 1988;240(4857):1285–1293.
41. Tamamoto T, Ohno K, Takahashi M, et al. Plasma matrix metalloproteinase-9 activity in cats with lymphoma. Vet Comp Oncol.
42. Trojani M, Contesso G, Coindre JM, et al. Soft-tissue sarcomas of adults. Int J Cancer. 1984;33(1):37–42.
43. van Lelyveld S, Warland J, Miller R, et al. Comparison between Ki-67 index and mitotic index for predicting outcome in canine mast cell tumours. J Small Anim Pract. 2015;56(5):312–319.