Evaluation of the neoplastic infiltration of the skin overlying canine subcutaneous soft tissue sarcomas: an explorative study.

Skin infiltration of subcutaneous sarcomas

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Abstract

Studies regarding the neoplastic infiltration of the skin overlying canine subcutaneous soft tissue sarcoma (sSTS) are lacking. In case of the absence of tumour infiltration, there would be the possibility of leaving this unaffected skin in place, thus simplifying surgery.

The aim of the study was to investigate whether the skin overlying sSTSs is infiltrated by neoplastic cells.

Dogs with sSTSs treated surgically were prospectively enrolled. After excision, the skin was dissected from the tumour along the natural surgical plane of cleavage and histologically evaluated.

Twenty-nine dogs with an sSTS were included (22 grade I, 6 grade II and 1 grade III). The STS-overlying skin was not tumour-infiltrated in 14/29 cases (48.3%). A higher frequency of infiltration was observed in higher grade sSTSs (grades II and III, 100%; p=0.006); nevertheless, 8/22 grade I STSs (36%) also showed cutaneous infiltration. This infiltration involved the dermis of the skin directly in contact with the tumour (multifocal in 11 and diffuse in 4 cases). Although the cutaneous tumour infiltration is less frequent in grade I sSTSs and a wide excision may still be the safest treatment for any sSTS for a greater possibility of local control, this study opens the possibility to a less aggressive cutaneous excision, but still with a local curative intent, as only the skin directly in contact with the sSTS has been proven to be tumour-infiltrated. Additional studies are warranted to confirm that excision of only this skin may guarantee a complete local control, especially in lower-grade sSTSs.

Keywords: histologic margin, skin infiltration, histology, tumour recurrence, soft tissue sarcoma, subcutaneous sarcoma.

Introduction

Soft tissue sarcomas (STSs) represent a heterogeneous group of neoplasms arising from several mesenchymal tissues.[1] In dogs, they account for approximately 15% of all cutaneous and subcutaneous tumours,[2] being characterised by locally aggressive behaviour with a low/moderate tendency to metastasise (approximately 20%), depending on histological grade.[3] The tumours included in the group of STSs are perivascular wall tumours (PWTs), peripheral nerve sheath tumours (PNSTs), undifferentiated sarcomas, myxosarcomas, liposarcomas and fibrosarcomas.[1-4]

The recommended treatment for subcutaneous STS (sSTS) consists of a wide surgical excision in order to reduce the risk of local recurrence.[3-8] The skin overlying the tumour is included in the *en bloc* excision; however, no information regarding its neoplastic infiltration is available in the veterinary literature. Moreover, only a few studies have investigated the histologic pattern of growth of sSTS in the surrounding tissues in dogs.[5,9,10]

The most frequently reported sites of growth of sSTS are the trunk and the limbs. Some locations, especially those distal to the stifle/elbow, are more problematic for a wide lateral resection as well as for the removal of one fascial plane as a deep margin. Wide tumour excisions in these regions often require complicated reconstruction procedures or second intention healing management.[11] In some instances, a local *en bloc* excision preserving the limb function is not feasible, and more aggressive procedures (up to limb amputation) may be necessary.

Incomplete histological margins and histological grade are reported as risk factors for local recurrence and distant metastases.[7,12,13] Differences in the local recurrence rate exist after marginal resection or incomplete histologic excision of sSTSs, depending on

their histological grade.[4,6,8,12,14,15] It has been reported that, even after marginal resection, the recurrence rate for grade I sSTS in the distal limbs is not greater than 7-17%.[6,12,14-16,]

The question regarding the neoplastic infiltration of the skin overlying sSTSs and the need for its removal with wide margins arises. In fact, if this skin was not infiltrated by tumour cells and dissection between the two parts was feasible, this skin could be potentially left in place. Leaving this skin completely or a part of it could simplify the surgery, thus allowing primary closure of the wound and reducing the complications and morbidity caused by reconstructive procedures or second intention healing management.[17]

Therefore, the hypothesis tested herein was that, in sSTSs (as definitively diagnosed at histology postoperatively), the skin overlying the tumour was not infiltrated by neoplastic cells in 100% of cases.

Materials and Methods

Sample collection

Twenty-nine client-owned dogs with a spontaneously arising sSTS which was surgically excised by one European College of Veterinary Surgeons (ECVS) diplomate surgeon or a Fellow of Surgical Oncology between January 2017 and March 2020 were prospectively enrolled in the study. The dogs were operated on at the XXX and XXX. Perioperative standard-of-care management, including analgesia, was assured for all the dogs in both institutions. Written consent was obtained from the owners for the anaesthetic, surgical and histological procedures before proceeding.

A presurgical diagnosis was obtained by fine-needle aspiration biopsy (FNAB) and cytological examination, and/or incisional biopsy and histological evaluation.

The sSTSs were also classified based on their location (head, neck, trunk or limb); in addition, for those of the limbs, it was also required to specify whether they were proximal to the elbow/stifle or at the level or distal to these joints.

Preoperative clinical staging included three radiographic views of the thorax and ultrasound evaluation of the abdomen, or total body computed tomography. Cytological evaluation of the regional lymph nodes was carried out only if they were enlarged; a regional lymphadenectomy concurrent with the excision of the primary tumour was sometimes performed at the surgeon's discretion. All the excised lymph nodes underwent histological evaluation.

Dogs with ulceration of skin overlying the sSTS and those with distant metastases were excluded.

Free mobility of the skin was assigned when the skin was movable and sliding on the mass, in particular at the level of the most prominent part of the tumour. In the case of doubtful mobility, the skin was considered not movable. However, due to the explorative intent of the study, the mobility of the skin overlying the tumour was not taken into account as an inclusion/exclusion criterion.

According to current recommendations,[3] a wide excision (3 cm of lateral margin, 1 fascial plane deep and the overlying skin) of the sSTS was attempted in order to obtain a complete excision. However, when this excision was not feasible (for example, in the distal limbs), the widest excision deemed possible in relation to the function of the region was performed. The wounds were reconstructed using different techniques, including primary closure, tension relieving incisions, local flaps, axial pattern flaps and free grafts, based on both the size and the location of the defect, and the surgeon's preference.

Immediately after excision, the skin overlying the tumour was surgically dissected by means of blunt dissection using Metzenbaum scissors along the natural cleavage plane

between the skin and the subcutis, thus simulating the surgical undermining. When a natural cleavage plane was not found, sharp dissection was performed following the most superficial margin of the tumour, paying attention not to enter the mass. Sutures and ink were applied to allow both sample orientation and deep/lateral margin identification, respectively, on both the tumour and the overlying skin (Figure 1). If a preoperative biopsy had previously been performed, the cutaneous area corresponding to the biopsy site was identified with a suture and included in the histological evaluation.

Trimming protocol

To assess the lateral as well as the deep excision margin infiltration, the excised sample was bisected along its longitudinal and transversal axes (cross-sectioning method); this was the case for tumours of less than 2 centimeters in diameter. Additional parallel slicing was carried out for tumours greater than 2 centimeters; besides, additional sections were also obtained when neoplastic infiltration of the excision margins was macroscopically suspected (Figure 1 A, B).[18] If a preoperative biopsy had previously been performed, the cutaneous area corresponding to the biopsy site (already identified by the surgeon with a suture) was specifically evaluated.
The trimming protocol for the skin overlying the excised tumour consisted of a prior cross sectioning to effectively differentiate and orient the cutaneous lateral margins and the area above the tumour. These quarter sections were then sectioned serially with parallel slicing at intervals of 0.5 centimeters (bread loafing method) (Figure 1 C, D). The skin directly in contact with the sSTS and that lateral to it, i.e. that included as lateral normal tissue, were evaluated (Figure 2).

Histological examination

Formalin-fixed paraffin-embedded samples were stained with hematoxylin-eosin and evaluated by two independent pathologists of the XXX. Well-differentiated tumours were classified according to their predominant histological feature. When no specific feature was detected to differentiate PNSTs from PWTs or other sSTSs, they were considered to be sSTSs without any additional morphological classification. No immunohistochemical evaluation was carried out; histological grade and the mitotic index were assessed according to the Dennis et al. (2011) guidelines.[13]

Based on the distance between the excisional margins and the neoplastic cells, the excision margins were histologically classified as "incomplete" when neoplastic cells reached at least one excisional border, and "complete" when >0 mm separated the margins from the neoplastic cells.[13,19,20]

The totality of the skin dissected from the mass (i.e. that directly in contact with the sSTS and that lateral to it and included during the excision as normal tissue [Figure 2]) was evaluated and categorised as infiltrated and not infiltrated, according to whether or not neoplastic cells invaded at least one of the skin layers.

Statistical analysis

The data were analysed using descriptive statistics and reported as mean, median and range. Distribution was checked graphically using the Shapiro–Wilk test for normality.

Clinical data (tumour location, size and skin mobility) were compared to the histopathological features (histological grade, completeness of histologic excision and tumour infiltration of the overlying skin) using the Fischer's Exact test. The *P* values

obtained were corrected for multiple comparisons using the Benjamini-Hochberg procedure with a false discovery rate of 0.05.[21]

Results

This prospective study included 29 dogs of which there were 13 males (11 intact and 2 neutered) and 16 females (10 intact and 6 spayed). The mean age at presentation was 10 years (range 6-15 years), and the mean body weight was 24 kg (range 15-40 kg) (Table 1).

The sSTS was on the trunk in 7/29 dogs (24.1%) and on a limb in 22/29 dogs (75.9%). In particular, the tumour was proximal to the elbow and stifle in 3/29 dogs (10.3.%), and at the level of or distal to the elbow/stifle joints in the remaining 19/29 (65.5%) dogs. The sSTSs had a median diameter of 5 cm (range 1.5-20 cm) (Table 1). In one dog, the skin over a 15 centimeter sSTS located in the axillary region showed a small area of ulceration which was not considered tumour related and likely caused by pressure necrosis.

Preoperatively, cytology from an FNAB was suggestive of an STS in 18/23 (78.3%) samples. An incisional biopsy was available in 6 cases and, in 3 of these, the morphological classification was consistent with the definitive histological diagnosis; in addition, in 4/6 cases, the histological grading of the sSTS specimen (grade I) agreed with that assigned after histology of the entire mass; for the remaining 2 biopsies (performed elsewhere,) no grading was available.

Histologically, there were 3/29 (10.3%) PNSTs, 20/29 (68.9%) PWTs and 6/29 (20.7%) unclassified sSTSs. Twenty-one of the 29 sSTSs (72.4%) were grade I, 6/29 (20.7%) were grade II and 1/29 (3.4%) was grade III (Table 2). Regional

lymphadenectomy was performed in 12 dogs, and no dog had histologic evidence of nodal metastasis. No dogs showed distant metastasis during staging.

The histological margins were classified as complete in 21/29 dogs (72.4%) and as incomplete in 8/29 (27.6%) dogs (Table 2).

The undermining between the skin and the sSTS after tumour excision was more laborious, actually requiring a sharp dissection in 4/29 (13.8%) cases. In these cases, the histology diagnosed an sSTS which extensively infiltrated the dermis of the skin directly in contact with the sSTS (Table 2). In the 6 dogs in which a biopsy was performed preoperatively, in only one case was the skin extensively infiltrated by neoplastic cells.

The skin overlying the sSTSs (including both that in contact with the tumour and that considered as lateral margin during the tumour excision) was free of neoplastic infiltration in 14/29 dogs (48.3%) while, in the remaining samples (15/29 dogs, 51.7%), the dermis appeared infiltrated in the area of the skin in contact with the tumour only (Table 2, Figure 2). In 4 cases, the infiltration was diffuse at the level of the dermis while in 11 cases, the dermal infiltration was multifocal; no neoplastic infiltration was found at the level of the skin considered as lateral margin during the tumour excision (Figure 2). This neoplastic infiltration was significantly less frequent in the grade I sSTSs as compared to the higher histological grades (grades II and III which were considered as a whole due to the presence of a single grade III sSTS; $p=0.006$, Table 3B). In particular, the skin appeared not to be tumour-infiltrated in 14/22 (63.6%) of the grade I sSTSs.

No significant association was observed between the mobility of the overlying skin and the neoplastic skin infiltration ($p=0.43$). No other significant correlation was found (Table 3 A, B).

Discussion

In this preliminary study, tumour skin infiltration overlying sSTSs was observed in 51.7% of cases, thus confirming the hypothesis that the skin overlying sSTSs was not infiltrated in 100% of cases. However, this tumour infiltration involved only the skin directly in contact with the sSTS. A lower prevalence of tumour infiltration was observed in grade I STSs (36.4%) in comparison with grade II and III STSs (100%).

The higher frequency of neoplastic infiltration of the skin overlying sSTSs graded higher than I may be correlated to their more aggressive behaviour and their possible tendency to more extensively infiltrate the surrounding tissues.[5,9] It has recently been reported that, as compared to mast cell tumours, both grade I and grade II STSs were characterised by more compact growth, with less extensive circumferential and deep invasion, potentially allowing for less aggressive surgery.[10]

The current recommendation for sSTS excision is to provide a margin of normal tissue of 3 cm laterally, including the overlying skin, and one fascial layer deep, with the aim of obtaining complete resection margins and a lower rate of local recurrence.[3,14,20] Clinical retrospective studies evaluating long-term outcome have reported different recurrence rates after the incomplete excision of STSs.[4,8,14,15,20] The recurrence rate for grade I STSs of the distal limbs, excised with incomplete or close margins without any additional adjuvant treatment, has been reported to be approximately 7-17%.[6,12,14-16] Regarding the latter, it should be noted that a universally accepted definition of STS margin infiltration status is still lacking, leading to non-standardised results among studies.[20,22]

Preoperative knowledge of the sSTS grade could guide in planning the best surgical extent with the aim of a more likely complete excision. However, it has been

reported that the STS histological grade coming from an incisional biopsy may be underestimated in 29% and overestimated in 12% of cases.[23] In the present study, the tumour grade was known preoperatively for only 4 biopsies, all of which matched with the final histological grade. Nevertheless, the authors agreed that the definitive histological grading could be reached only after examination of the entire mass after excision. Furthermore, the biopsy itself could lead to a local spread of neoplastic cells, even in the overlying skin; however, in the present study, six preoperative biopsies were performed, but in only 1 case did the bioptic site appear infiltrated. To decrease the risk of dissemination, the biopsy should be performed at the level of the most prominent part of the tumour as this skin is that with the highest chance of being eliminated during the excision of the tumour. Nowadays, the role of a preoperative biopsy is still debatable, for both determining the grade of the tumour and for evaluating the potential infiltration of the overlying tumour skin.

Though a wide margin excision would still represent the safest option, this study opens the possibility to perform a less aggressive cutaneous excision, still having a local curative intent, as only the skin directly in contact with the sSTS has been proven to be infiltrated by neoplastic cells. This preliminary data are encouraging, especially in view of the fact that a wide margin excision cannot always be accomplished owing to tumour size and maintenance of function, for example when the sSTS is at the level of a distal limb. The application of the concept of wide margin excision in the distal limbs often requires complex reconstructions, thus exposing the animal to potential postoperative complications, higher costs and a longer period of clinical management, up to complete healing.[11,17] In the present study, 76.9% of the sSTSs were on a limb, of which 65.4% were distal to the elbow or the stifle, thus reflecting the high prevalence of these tumours

in these areas. Further studies are needed to evaluate what proportion of lateral skin could be safely left in place without compromising local tumour control.

It has been reported that mobile STSs are associated with longer disease-free intervals and survival after surgical excision;[1,6] however, specific evaluation of the mobility of the skin and confirmation of histologically neoplastic infiltration of the skin overlying the sSTS have never been reported. In the present study, it emerged that, when the skin appeared clinically freely movable and/or easy to detach from the tumour along the surgical cleavage plane, neoplastic infiltration was still possible. In fact, in 45% of the cases in which the skin was classified as freely movable, neoplastic infiltration was observed; in addition, in the majority of cases in which the skin was considered as not movable, histologically confirmed tumoral infiltration of the dermis was found (67%). However, as already stated, this infiltration was found at the level of the skin in contact with the tumour. Judging the mobility of the skin may be subjective, especially when the sSTS is very large or is localised in the distal limb as this skin may be under tension or it may appear movable on the lateral portion of the tumour only, but not movable on the top of the sSTS. These difficulties in evaluating the skin mobility, and the relationship between skin mobility and its infiltration status may represent a potential limitation. It is the authors' opinion that this parameter cannot be classified in an absolute manner, especially, as already stated, in the case of very large masses and/or the location on the limb. Similarly, the distinction between an sSTS diffusely invading the dermis secondarily vs. that originating primarily from the dermis may not be so obvious.

The main limitation of the present study is the low number of cases included, especially comprising the group of sSTSs of grades II and III. This reflects the lower incidence of high-grade sSTSs in general.[16]

Another potential limitation regards the fact that the excision of the skin overlying the tumour performed after the surgical removal of the sSTS may have potentially created some artifacts in the histological evaluation. However, this procedure was performed in an attempt to simulate the real surgical scenario as much as possible, i.e. the blunt dissection between the skin and the subcutis performed during surgery in cases in which the skin would have been left in place. This choice was also adopted to match factors, such as cost and efficiency, of the histological evaluation of the overlying skin together with that of the lateral and deep excision margins. The proper orientation of the skin after its dissection allowed the histological evaluation of the different portions which resulted in all of the skin overlying the sSTS being thoroughly analysed.

Another limitation was that these sSTSs were not subclassified by immunohistochemistry to precisely identify the exact histotype.[23,24] In 23 cases, the sSTSs were classified as PNSTs or PWTs according to their morphology while, for the remaining 6, a clear classification was not obtained. However, the exact immunohistochemical characterisation of these STSs was not the goal of this study as the main aim of the study was to prospectively evaluate skin neoplastic infiltration independently of the histotype. Nevertheless, the different histotypes of STSs may potentially account for different behaviours in terms of clinical aggressiveness and surrounding tissue infiltration.[4,5] Regarding this, additional studies are warranted, also considering that, in the veterinary literature, the classification of the different tumours to be included in the STS group may vary among pathologists, thus increasing the difficulty in interpreting the behaviour of the different tumour types.[25]

In conclusion, this study showed that neoplastic infiltration of the skin overlying and in contact with the sSTSs may occur, even if with a lower frequency in grade I sSTSs. Additional studies regarding the possibility of removing only the skin

corresponding to the skin in contact with the sSTS, thus leaving the lateral skin, are warranted.

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The data that support the findings of this study are available from the corresponding author upon reasonable request.

Table 1

Clinical characteristics of the dogs enrolled in the study

Age (years)	**Mean**	**10**	
	Median	**10**	
	Range	**6-15**	
Gender, n (%)	Female	16	(55.2)
	spayed	6	(20.7)
	Male	13	(44.8)
	neutered	2	(6.9)
Breed, n (%)	Crossbreed	13	(44.8)
	Golden Retriever	3	(10.4)
	Rhodesian Ridgeback	2	(6.9)
	Others	11	(37.9)
Weight (kg)	Mean	24	
	Median	25	
	Range	10-40	
Localisation, n (%)	Distal limb (including elbow and stifle)	19	(65.5)
	Proximal	3	(10.4)
	Trunk	7	(24.1)
Tumour diameter, n (%)	< 5cm	13	(44.8)
	> 5cm	16	(55.2)

Skin mobility, n (%)	Movable	20	(69)
	Non-movable	9	(31)

Table 2

Histopathological features of the sSTS samples

Grading, n (%)	**I** **II** **III**	**22** **6** **1**	**(75.9)** **(20.7)** **(3.4)**
Histotype, n (%)	PWT PNST STS	12 4 13	(41.4) (13.8) (44.8)
Mitotic index, n (%)	<9 10-19 > 20	21 5 3 (10.3)	(72.4) (17.3)
Margins, n (%)	Complete Incomplete	21 8	(72.4) (27.6)
Overlying cutis, n (%)	Not infiltrated Infiltrated	14 15 (51.7)	(48.3)

Abbreviations: PWT: perivascular wall tumour; PNST: peripheral nerve sheath tumour; sSTS: subcutaneous soft tissue sarcoma

Table 3 A and B

Association analyses of clinical and histopathological features

A	**Excision margins**				
	Incomplete	**Complete**			
			Total	*p value*	Benjamini-Hochberg significance
Grade					
I	2	16	18	**0.028**	Not significant
II-III	6	5	11		
Total	8	21			
Localisation					
Distal limb	7	12	19	0.2	Not significant
Other site	1	9	10		
Total	8	21			

B	**Overlying skin**				
	Infiltrated	**Non-infiltrated**			
			Total	*p value*	Benjamini-Hochberg significance
Grade					
I	8	14	22	**0.006**	**Significant**
II-III	7	0	7		
Total	15	14			
Margins					

Complete	12	9	21	0.4	Not significant
Incomplete	3	5	8		
Total	15	14			
Mobility					
Movable	9	11	20	0.42	Not significant
Non-movable	6	3	9		
Total	15	14			
Dimension					
< 5 cm	9	4	13	0.134	Not significant
> 5 cm	6	10	16		
Total	15	14			

Figure 1. (A) Macroscopic view of an sSTS after surgical removal. (B) The same specimen after separation of the skin from the sSTS. Note the presence of suture material to identify the lateral margins on both the mass and the skin. (C) Cross-sectioning trimming method of the neoplastic mass. (D) Combined cross sectioning and complete bread loafing of the skin overlying the tumour.

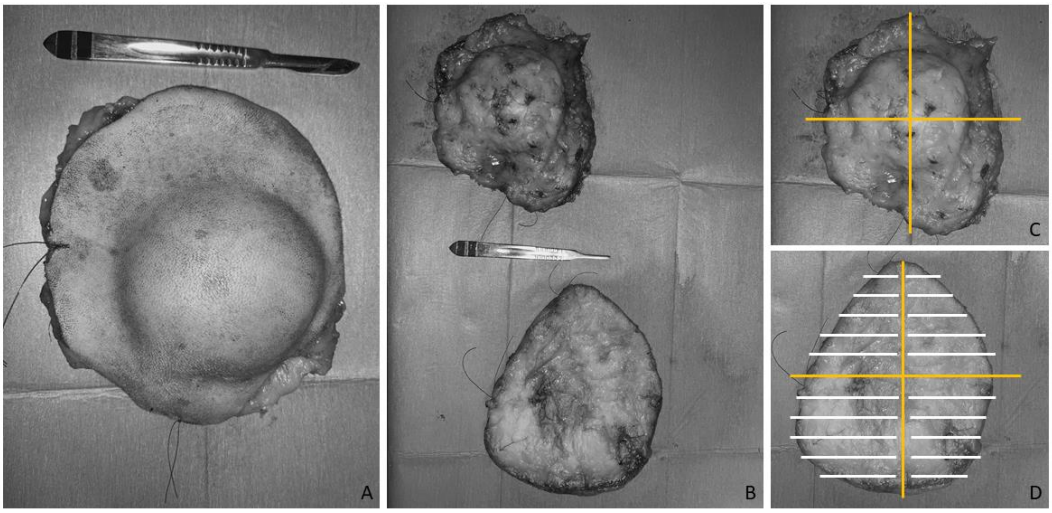

Figure 2. sSTS on the lateral part of the thigh before surgical excision; the overlying skin is divided by the continuous line in the region of the skin in contact with the tumour (A), and by the dotted line in the lateral part of the skin excised during a standard wide margin excision (B).