UC Davis
UC Davis Previously Published Works

Title

Retrospective immunohistochemical investigation of suspected non-visceral leiomyosarcoma in dogs

Permalink

Journal

ISSN

Authors

Brady, Rachel V
Rebhun, Robert B
Skorupski, Katherine A
et al.

Publication Date

DOI

Peer reviewed

Retrospective immunohistochemical investigation of suspected non-visceral leiomyosarcoma in dogs

Rachel V. Brady, [iD] **Robert B. Rebhun,** [iD] **Katherine A. Skorupski, Jenna H. Burton,** [iD]
Sami Al-Nadaf, Eunju Choi,* Jennifer L. Willcox[1]*

Abstract. Visceral leiomyosarcoma is well described in dogs, but information about non-visceral locations and prevalence is lacking. The diagnosis of leiomyosarcoma is challenging without a gold standard, and often includes the use of immunohistochemical (IHC) stains. We used defined histopathologic patterns, histochemical staining, and IHC staining for smooth muscle actin (SMA), desmin, and laminin to characterize suspected non-visceral leiomyosarcoma in dogs at a single academic institution. In a retrospective search, we identified 24 dogs with a definitive or suspected histologic diagnosis of leiomyosarcoma in a non-visceral location. Histopathology results and clinical details were obtained. Biopsy sections were reviewed by a single pathologist using standardized histologic criteria, including light microscopic appearance, immunohistochemistry (more than two-thirds of neoplastic cells labeled with SMA and desmin or laminin), and histochemical staining (minimal-to-mild matrix deposition by Masson trichrome). Of the 24 cases of possible non-visceral leiomyosarcomas, 4 were consistent with a definitive diagnosis of non-visceral leiomyosarcoma (3) or leiomyoma (1) based on the established criteria. Only the leiomyoma had more than two-thirds of neoplastic cells label with all 3 markers; all 3 leiomyosarcomas had more than two-thirds of neoplastic cells label with SMA and laminin. Our data highlight the uncommon nature of non-visceral leiomyosarcoma and the importance of IHC for their diagnosis. A definitive diagnosis could not be made based on SMA alone, and desmin was not useful in this cohort. Further studies are needed to clarify the histopathologic, IHC, and clinical features of canine non-visceral SMA-positive mesenchymal tumors.

Keywords: canine; desmin; laminin; mesenchymal; smooth muscle actin; soft tissue sarcoma.

Leiomyosarcoma is a malignant mesenchymal tumor of smooth muscle origin that has been classified inconsistently within the soft tissue sarcoma (STS) group, with examples of inclusion[16,34] and specific exclusion[15,17] in the veterinary literature. In dogs, leiomyosarcoma occurs most often in visceral organs including the gastrointestinal (GI) tract, genital tract, urinary tract, liver, and spleen.[13,25–27,37,46,48,52] Visceral leiomyosarcoma frequently requires immunohistochemical (IHC) staining for definitive diagnosis, and subsequently it is likely that an unknown subset of GI tumors in older studies represent alternative diagnoses such as gastrointestinal stromal tumor (GIST).[4,11,19,23,32,40,45] The prevalence of visceral leiomyosarcomas has allowed prognostic studies in these organ systems.[27,32,45,46] More atypical locations such as the oral cavity, skin, and large peripheral blood vessels[5,7,31,42,44] have been reported sporadically. Little is known about whether there is a similar need for IHC stains for diagnosis of non-visceral leiomyosarcomas or their clinical behavior. In people, the biologic behavior and outcome of sarcomas in general[6] and leiomyosarcoma in particular[8,20,33] is predicted in part by anatomic location, suggesting that anatomic location should be investigated for prognostic significance in the dog.

Leiomyosarcomas can present a diagnostic challenge given that their histopathologic characteristics may mirror other mesenchymal neoplasms, such as fibrosarcoma, myofibroblastic neoplasm, and GIST,[24] and no gold standard exists for diagnosis. Although descriptive features of leiomyosarcoma are reported (infiltrative, broad, and long fascicles of densely packed plump spindle cells with abundant eosinophilic cytoplasm, granular chromatin, and elongate or "cigar-shaped" nuclei[24]), the level of differentiation and atypia may limit the utility of microscopic characteristics alone, which

Veterinary Medical Teaching Hospital (Brady), and Departments of Surgical and Radiological Sciences (Rebhun, Skorupski, Burton, Al-Nadaf, Willcox) and Pathology, Microbiology and Immunology (Choi), School of Veterinary Medicine, University of California–Davis, Davis, CA, USA. Current addresses: Department of Clinical Sciences (Brady), Flint Animal Cancer Center (Burton), College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, USA.

*These authors contributed equally to this work.

[1]Corresponding author: Jennifer L. Willcox, Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California–Davis, Davis, CA 95616-5270, USA.
jlwillcox@ucdavis.edu

highlights the diagnostic challenge that leiomyosarcoma presents. Diagnosis is especially challenging in non-visceral locations, with many other differentials considered for sites such as the dermis and subcutis. In these instances, the need for IHC staining for confirmatory diagnosis is emphasized to rule-in or rule-out other more common tumor types.

Although no gold standard criteria exist for the diagnosis of leiomyosarcoma in dogs, various immunolabels have been utilized to aid in their identification. As a mesenchymal neoplasm, leiomyosarcoma largely immunolabels with vimentin,[30,41,44,45] which lacks specificity for diagnosis given that most sarcomas are vimentin positive. Immunostaining for smooth muscle actin (SMA), a cytosolic intermediate filament, is often used clinically as a standalone IHC stain for leiomyosarcoma. Importantly, other tumors such as GIST (including extraintestinal GIST), myofibroblastic neoplasms, and perivascular wall tumors, as well as benign lesions such as nodular fasciitis and granulation tissue, can also immunolabel with SMA.[3,4,9,10,29,41,44] Desmin, a cytoskeletal intermediate filament, has also been considered a specific marker that demonstrates muscle differentiation and hence is used to diagnose rhabdomyosarcomas and leiomyosarcomas.[2,10,41] Laminin, a large extracellular matrix protein found in the basal lamina of many cell types including blood vessels, has also been identified as a useful marker for specific variants of smooth muscle tumors.[3] The use of laminin to aid in the diagnosis of leiomyosarcoma has only been described for variants of perivascular wall tumors.[3,31] Stains for other IHC targets, such as calponin and heavy-caldesmon, both of which are constituents of smooth muscle thin filament and markers used in humans, are not routinely available in clinical veterinary practice.[24] Furthermore, although SMA and desmin are considered markers for leiomyosarcoma, the cutoffs or thresholds for percent positive cells for definitive diagnosis of a smooth muscle tumor are not established.

Evidence in both the human[8,20] and veterinary[18,27,31,46] literature demonstrates that leiomyosarcomas may exhibit different biologic behavior based on their anatomic location of origin. In dogs, for example, esophageal leiomyosarcomas are considered to be mostly low-grade and benign,[18] whereas splenic and hepatic leiomyosarcomas are considered largely malignant.[27,46] Smooth muscle tumors of the skin, reported as angioleiomyosarcoma and piloleiomyosarcoma, appear to be as benign as the esophageal lesions, and are suspected to have a better prognosis given that resection is less complex than from the esophagus.[31] Hence, there is justification to characterize the behavior of leiomyosarcomas depending on location. Although visceral leiomyosarcomas have been relatively well-characterized in the veterinary literature, there is a paucity of descriptions of those occurring in non-visceral locations. Additionally, given the proclivity for other, more common tumor types to arise in non-visceral sites, as well as the lack of a gold standard for diagnosis, clearly defined histologic parameters and IHC staining are crucial in defining this population. Our aim, therefore, was to characterize suspected non-visceral leiomyosarcomas in dogs at a single academic institution utilizing a defined histopathologic pattern, histochemical staining, and IHC staining for SMA, desmin, and laminin.

Materials and methods

Case selection

The veterinary electronic medical records database at the University of California, Davis Veterinary Medical Teaching Hospital (UCD-VMTH) was searched (1987–2019) for canine cases with a diagnosis of suspected non-visceral leiomyosarcoma made by histopathology. Any suspected leiomyosarcoma was excluded if it arose from the abdominal or retroperitoneal cavity, esophagus, rectum, or internal genitalia. Cases were included when a definitive or suspected histologic diagnosis of leiomyosarcoma was made, which included those in which a smooth muscle origin was considered and IHC staining had not definitively confirmed another sarcoma subtype. Those cases without tissue available for evaluation and IHC assessment were excluded. Information captured from the medical record included signalment, description of the tumor, histopathologic characteristics, and any previous IHC stains used. Additional clinical details were captured, including (when available) clinical signs, results of initial laboratory tests, presence or absence of metastasis, treatment provided, surgical findings, and outcome information.

Histologic evaluation

Sections of formalin-fixed, paraffin-embedded (FFPE) biopsy specimens were evaluated by a single pathologist (E Choi) using standardized criteria as described. All slides from the identified cases were evaluated. Prior to the use of IHC, tumors were evaluated microscopically for characteristics deemed to be consistent with a possible smooth muscle tumor, including spindle cells forming variably dense streams, bundles, and/or fascicles. Tumors forming whorls were not considered compatible with smooth muscle tumors and were excluded from further characterization. For the tumors with consistent cellular morphology, a representative slide was chosen to perform the IHC staining.

A FFPE tumor specimen was cut into 4-μm-thick sections for IHC staining (Table 1). Antigen retrieval was performed, and sections were blocked in 10% normal horse serum in PBS-Tween 20 (0.02%) for 20 min. The primary antibody was applied without rinsing and incubated for 1 h. All post–antigen-retrieval incubations were in a humidity chamber at room temperature. The secondary polymer was applied for 30 min to label. Labels were visualized with NovaRED for peroxidase (SK-4800; Vector), per the manufacturer's instructions. Sections were counterstained with hematoxylin 1 (Fisher) for 10 s and bluing reagent (Fisher) for 2 min, air dried, and coverslipped. Sections of canine small intestine,

Table 1. Details of immunohistochemical stains performed on sections of suspected non-visceral leiomyosarcoma in dogs.

Antibody	Source	Antigen retrieval	Dilution	Secondary polymer
SMA	BioGenex (MU128-UC)	Heat-induced epitope retrieval in a Black & Decker steamer for 30 min at 95°C in EDTA buffer (pH 9.0)	1:300	BioCare polymer-HRP (mouse MC541H)
Desmin	Cell Marque (243M-16)	Heat-induced epitope retrieval in a Black & Decker steamer for 30 min at 95°C in target retrieval solution, pH 6 (Dako S1699)	1:100	BioCare polymer-HRP (mouse MC541H)
Laminin	BioGenex (PU078-UP)	10-min incubation in 0.2% protease enzyme diluted in PBS at 37°C	1:50	Rabbit-on-canine HRP polymer (Biocare RC542H)

SMA = smooth muscle actin.

Table 2. Scoring system used to evaluate suspect leiomyosarcomas in dogs.

	Score			
Criterion	0	1	2	3
Immunolabeling by smooth muscle actin and/ or desmin and/or laminin	None	Up to 1/3 of neoplastic cells	Up to 2/3 of neoplastic cells	More than 2/3 of neoplastic cells
Amount of collagen fibrils between neoplastic cells, based on Masson trichrome staining	Minimal	Small	Moderate	Abundant

skeletal muscle, and skin stained for SMA, desmin, and laminin, respectively, were used as positive controls. Blood vessels served as the internal positive control. For negative controls, primary antibodies were replaced with homologous nonimmune sera. The samples were processed in batches for each antibody, and each batch had a positive and negative control.

Representative slides of each IHC stain were scored using a semiquantitative method by a single pathologist (E Choi) based on percent immunolabeled neoplastic cells (Table 2). For tumors with a SMA immunolabeling score of 3, Masson trichrome (MT) staining was performed to assess the degree of extracellular matrix (collagen) deposition between neoplastic cells. The amount of collagen was given a score of 0–3: 0 = minimal, 1 = mild, 2 = moderate, and 3 = abundant material between neoplastic cells.

Samples that were morphologically compatible were determined to be consistent with leiomyosarcoma or leiomyoma if both SMA and desmin or both SMA and laminin scores were 3, with a minimal-to-mild amount of collagen between neoplastic cells (MT score of 0 or 1), unless otherwise defined. Good demarcation, lack of invasion, and lack of mitotic figures supported the diagnosis of a leiomyoma over a leiomyosarcoma.

Results

Suspected non-visceral leiomyosarcomas were identified from 42 dogs according to the search criteria (Fig. 1); 7 lacked available tissue samples for further evaluation, and 11 were excluded because of histologic whorls. The 24 remaining cases fulfilled the defined morphology and were included in our study. The masses were from a variety of non-visceral locations including the subcutis ($n=15$), dermis ($n=3$), footpad ($n=1$), orbit (retrobulbar $n=1$, conjunctiva $n=1$, eyelid $n=1$), pharynx ($n=1$), and intradural cervical region ($n=1$; Table 3, Suppl. Table 1).

SMA, desmin, and laminin immunolabeling were identified in 16, 7, and 15 cases, respectively (Fig. 2). Cases 1–18 had immunolabeling with >2 markers; 6 cases (19–24; Table 3) were negative for all 3 markers.

Ultimately, 4 of 24 tumors (cases 1–4) were consistent with leiomyoma or leiomyosarcoma based on the diagnostic criterion of more than two-thirds of neoplastic cells labeled with SMA and laminin (score of 3) and low MT staining (score of 0 or 1). Two were from the dermis of the distal forelimb (carpus and metacarpal footpad), one was from the pharynx, and one from the third eyelid. The 2 dermal masses had features suggestive of anatomic origin. Case 1, the carpal mass, strongly resembled erector pili muscle and had histologic features supportive of a benign neoplasm suggestive of a piloleiomyoma. Case 3, the metacarpal footpad mass, was closely associated with numerous vascular walls and had features of malignancy supporting the diagnosis of an angioleiomyosarcoma (Fig. 2). Interestingly, this mass had variable MT staining ranging from minimal between tightly packed neoplastic cells to abundant perivascular collagen deposition. The pharyngeal (case 2) and third eyelid masses (case 4) were considered to have originated from resident smooth muscle bundles in those locations.

Seven cases (cases 5–11) received an SMA score of 3 but did not fall within our diagnostic criteria. Case 5, a
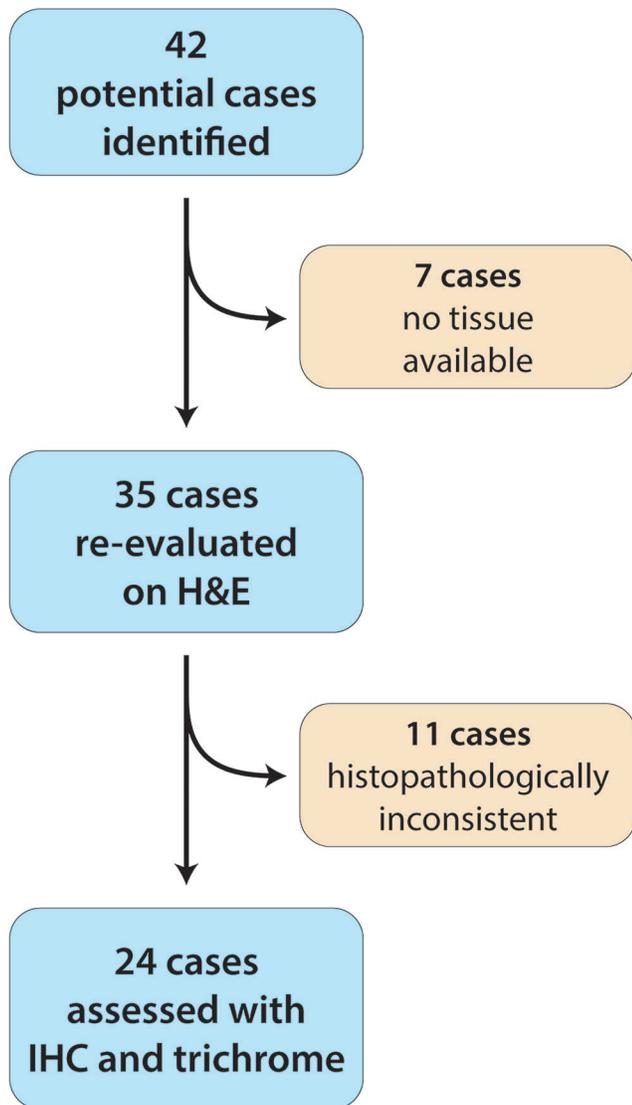
Figure 1. Flowchart of biopsy samples of suspected non-visceral leiomyosarcomas in dogs. IHC = immunohistochemistry.

subcutaneous and intramuscular mass of the thigh, scored 3 for SMA and laminin but received an MT score of 2 (Fig. 2). Additionally, 4 cases scored 3 for SMA but received a score of 2 (cases 6, 7) or a score of 1 (cases 8, 9) for laminin. Two cases (cases 10, 11) scored 3 for SMA but did not stain with laminin (Fig. 2). Of these, only 2 cases received a score of 1 for desmin (cases 7, 9). The MT score was variable in these cases and, as expected, was not correlated with the laminin score.

In 4 cases (cases 12–15), SMA labeled less than two-thirds of the neoplastic population (score of 2). In these 4 cases, 1 case scored 3 for laminin (case 12) and 3 cases scored 2 (cases 13–15). None of these cases stained with desmin. In the eyelid mass from case 12, SMA only stained neoplastic cells locked in dense fascicles, which accounted for

40% of the entire population (score of 2) and spared broad streams and bundles, supporting heterogeneity of the neoplastic cells. Interestingly, this dog developed a newly identified rib osteosarcoma within a year of the eyelid mass diagnosis, which showed similar immunolabeling of neoplastic cells but a different histologic pattern.

Two cases (cases 16, 17) were considered to have a SMA score of 1, a laminin score of 1, and a desmin score of 1 or 2. Both were subcutaneous masses, one from the pelvic limb, and the other from the left hip. One case (case 18) was negative for SMA but scored 2 for desmin and laminin. Six cases (cases 19–24) were diffusely negative (score of 0) for SMA, desmin, and laminin.

Discussion

Leiomyosarcoma, although uncommon in non-visceral organ systems, is a diagnosis that is routinely considered by the pathologist when evaluating a sarcoma because of its histologic similarities to other sarcomas, such as fibrosarcoma, peripheral nerve sheath tumors, perivascular wall tumors, and myofibroblastic tumors. However, diagnostic criteria for subtypes of sarcomas, including leiomyosarcoma, are not defined clearly. We attempted here to utilize a combination of stringently defined histopathologic and IHC characteristics to identify non-visceral leiomyosarcomas. Our data highlight the diagnostic challenge that non-visceral smooth muscle tumors present, the importance of IHC staining, and the questions that remain about the most accurate approach given the lack of a gold standard for definitive diagnosis. In our sample set of 31 suspected leiomyosarcomas with available tissue samples, 11 were excluded based on their cellular morphologic characteristics, and 11 of the 24 remaining cases were diffusely strongly positive for SMA, the IHC stain utilized most commonly for the diagnosis of smooth muscle tumors. With the diagnosis of smooth muscle origin defined in our study as positive staining with both SMA and desmin or SMA and laminin, and minimal-to-low trichrome-positive material deposition between neoplastic cells, only 4 cases fulfilled our criteria. The only case that scored 3 for all 3 IHC stains was diagnosed as a leiomyoma, a benign smooth muscle tumor likely originating from the erector pili muscle. Only 5 other tumors had joint SMA, desmin, and laminin labeling and, of these, one was considered a leiomyosarcoma. The others, given their lesser percentage of immunolabeled cells or abundant collagen deposition, did not meet our diagnostic criteria for leiomyoma or leiomyosarcoma. This finding may imply that only 2 of 24 potential smooth muscle tumors are indeed smooth muscle origin, but alternatively, it is possible that 1) laminin and especially desmin are not reliable markers to aid in the diagnosis of leiomyosarcoma in dogs, or 2) our diagnostic criteria were too stringent. Interestingly, human leiomyosarcomas may have loss of desmin immunolabeling, which

Table 3. Clinical information and staining results for suspected non-visceral leiomyosarcomas in dogs.

Case	Location	Treatment	Metastasis	Recurrence	DFS	Status	IHC score SMA	Desmin	Laminin	MT score	Consistent with smooth muscle origin
1	Dermis, carpus	Excisional biopsy	No staging	Unknown	0	Unknown	3	3	3	0	Leiomyoma (piloleiomyoma)
2	Pharynx	Incomplete excision	Concern for liver/spleen masses on AUS at time of recurrence, not sampled	Yes	292	Euthanized	3	1	3	1	Leiomyosarcoma
3	Dermis, footpad	Marginal excision	No staging	Historic recurrence	17	Unknown	3	0	3	Variable	Leiomyosarcoma (angioleiomyosarcoma)
4	Bulbar third eyelid	Incomplete excision (3rd eyelid removal)	Unremarkable TXR/AUS at diagnosis	No	21	Unknown	3	0	3	0	Leiomyosarcoma
5	Subcutis, pelvic limb	Complete excision	Unremarkable TXR at diagnosis, cardiac/pulmonary masses at autopsy	No	52	Died from cardiopulmonary SMA-sarcoma	3	0	3	2	No
6	Subcutis, carpus	Complete excision (amputation)	Unremarkable TXR at diagnosis	Unknown	0	Unknown	3	0	2	0	No
7	Subcutis, calvaria	None	None on autopsy	Unknown	0	Died as a result of cerebellar compression and hemorrhage from mass	3	1	2	1	No
8	Subcutis, popliteal	None	None on autopsy	Unknown	0	Euthanized	3	0	1	2	No
9	Intradural C3	None	None on autopsy	Unknown	0	Euthanized as a result of mass	3	1	1	3	No
10	Subcutis, base of penis	Two doses doxorubicin	Unremarkable TXR/AUS at diagnosis	Historic recurrence	55	Unknown	3	0	0	1	No
11	Subcutis, neck	Complete excision, 4 doses doxorubicin	Unremarkable TXR/AUS (multiple times)	No	1,457	Unknown	3	0	0	3	No
12	Eyelid	Exenteration, 6 doses carboplatin for rib osteosarcoma	Unremarkable TXR/AUS (multiple times)	No	316	Unknown	2	0	3	ND	No
13	Subcutis, periorbital	None	No staging	No	9	Unknown	2	0	2	ND	No
14	Subcutis, body wall	1 dose doxorubicin	Pulmonary metastasis on CT at diagnosis	No	78	Euthanized as a result of cancer progression	2	0	2	ND	No
15	Dermis, neck	Marginal excision	Unremarkable TXR at diagnosis	No	49	Unknown	2	0	2	ND	No
16	Subcutis, pelvic limb	None	Pulmonary metastasis on autopsy	Unknown	0	Euthanized as a result of epistaxis	1	2	1	ND	No
17	Subcutis, left hip	Complete excision	Unremarkable TXR/AUS at diagnosis	No	2	Unknown	1	1	1	ND	No
18	Subcutis, distal femur	None	Pulmonary metastasis on autopsy	Unknown	0	Euthanized as a result of mass	0	2	2	ND	No
19	Subcutis, metatarsus	Complete excision+RT after multiple regrowths over 2 mo	Unremarkable TXR (multiple times)	No	1,548	Unknown	0	0	0	ND	No
20	Subcutis, body wall	None	Unremarkable TXR/AUS at diagnosis	No	210	Died	0	0	0	ND	No
21	Dermis, base of tail	1 dose carboplatin	Unremarkable thoracic CT/AUS at diagnosis; metastases in sublumbar LNs on autopsy	Unknown	0	Euthanized	0	0	0	ND	No
22	Retrobulbar	Incomplete excision	Unremarkable TXR at diagnosis	No	33	Died prior to induction for first RT	0	0	0	ND	No
23	Subcutis, ventral to orbit	Incomplete excision	No staging	No	1	Unknown	0	0	0	ND	No
24	Subcutis, carpus	Two incomplete excisions, RT	Unremarkable TXR at diagnosis, pulmonary and costal pleural metastases on autopsy	No	270	Euthanized as a result of metastatic disease	0	0	0	ND	No

Historical recurrence: indicates the biopsy sample available for review was a regrowth of a previous mass. Status: if the cause of death could reasonably be attributed to the tumor, this is noted. Immunolabeling by immunohistochemical (IHC) stain (smooth muscle actin [SMA], desmin, laminin): 0=none, 1=up to 1/3 of neoplastic cells, 2=up to 2/3 of neoplastic cells, 3=more than 2/3 of neoplastic cells. MT score: 0=minimal, 1=mild, 2=moderate, 3=abundant material between neoplastic cells.

AUS=abdominal ultrasound; CT=computed tomography; DFS=disease-free survival, as defined from the date of diagnosis to either recurrence, death, or last known follow-up; LNs=lymph nodes; MT=Masson trichrome; ND=not done; RT=radiation therapy; TXR=thoracic radiographs.
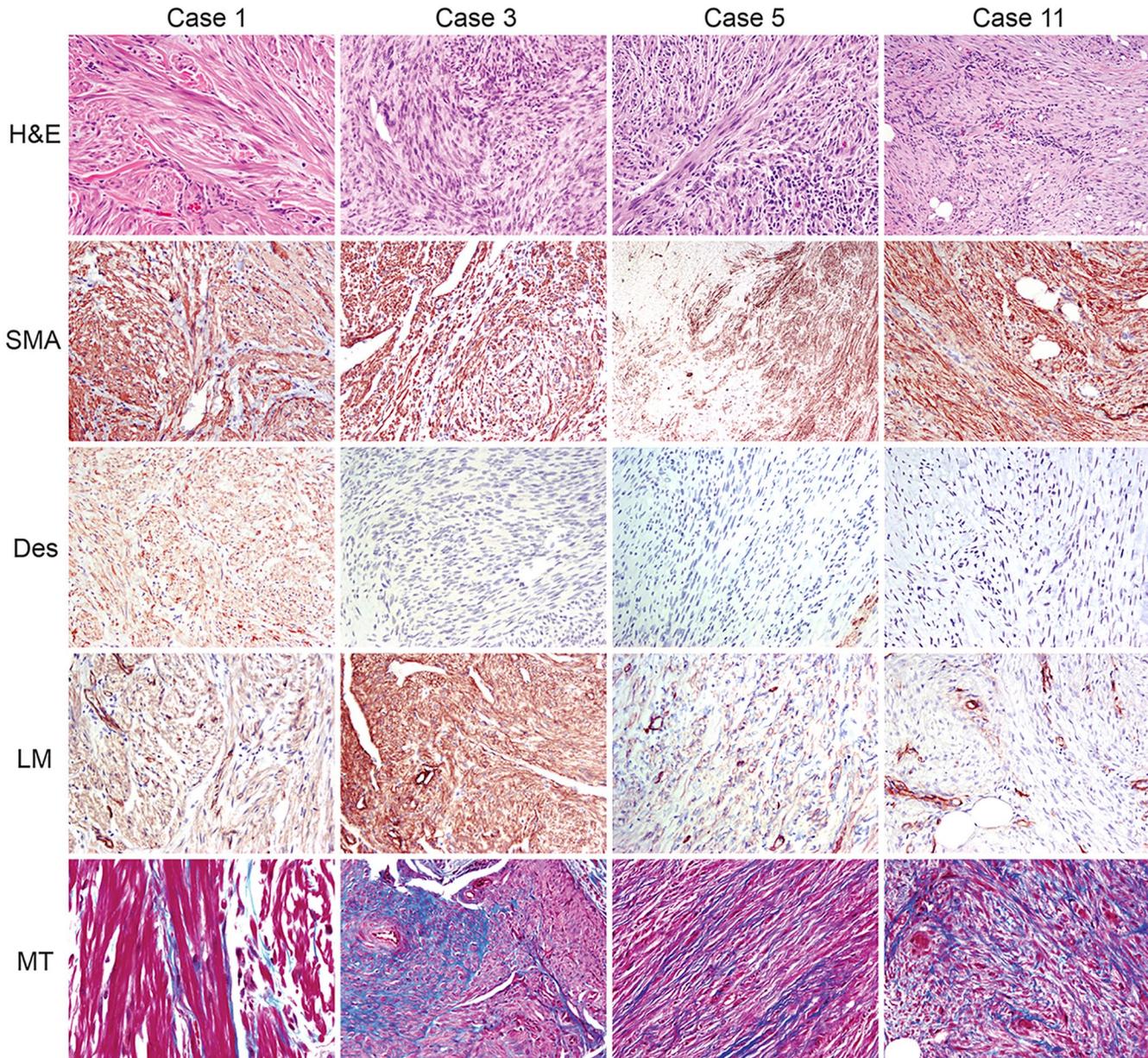
Figure 2. Representative images of masses considered smooth muscle tumors and other tumors. **Case 1** was compatible with a leiomyoma and received smooth muscle actin (SMA), desmin (Des), and laminin (LM) scores of 3 and a Masson trichrome (MT) score of 0. **Case 3** was compatible with an angioleiomyosarcoma, with SMA and laminin scores of 3, a desmin score of 0, and minimal-to-abundant collagen deposition. **Case 5** was considered an inflammatory myofibroblastic tumor given the presence of inflammation, SMA and laminin scores of 3, a desmin score of 0, and a MT score of 2; although the SMA score was 3, a small area lacked SMA labeling. **Case 11** was considered nodular fasciitis given the prominent vasculature, a SMA score of 3, desmin and laminin scores of 0, and a MT score of 3.

is thought to be associated with loss of myogenic differentiation.[14] It is possible that neoplastic cells in these tumors may lose desmin expression, and one could theorize that this reduced expression may occur in dogs.

Laminin was chosen as the third IHC stain to compare the staining pattern in conjunction with SMA and desmin. Laminin is a major basement membrane protein and is therefore present in many different tissue and cell types, including epithelial and mesenchymal cells. Although laminin is not a specific marker for smooth muscle differentiation, the amount of laminin is suggested to decrease with myofibroblastic differentiation, and laminin is not expected to be expressed in human low-grade myofibroblastic sarcomas.[35] Indeed, in our sample set, a subset of tumors strongly expressed SMA whereas none to less than two-thirds of neoplastic cells expressed laminin. This may suggest that a

subset of these tumors should be better classified as myofibroblastic tumors, and their behavior should be studied separately. In humans, myofibroblastic cells can form a variety of benign and malignant tumors, including inflammatory myofibroblastic tumor, nodular fasciitis, and myofibroblastic sarcoma.

Inflammatory myofibroblastic tumors are characterized by spindle cell proliferation with a variable inflammatory component and occur mostly in the abdominal cavity of children.[12] A subset of inflammatory myofibroblastic tumors has *ALK* gene arrangement.[49] Given histologic similarities, inflammatory myofibroblastic tumors have been reported in dogs,[29,47] although *ALK* gene arrangement has not been investigated, to our knowledge. Our subset had 2 cases (case 8, 10) that could fit this diagnosis, although the diagnostic criteria are not well established in veterinary medicine.

Nodular fasciitis is a benign mass with fusion genes involving *USP6* in people.[39] Nodular fasciitis has also been recognized in the dog,[9,22] but gene fusion has not been investigated, to our knowledge. Histologically, nodular fasciitis is considered when the proliferating cells are loosely formed with prominent reactive blood vessels and small numbers of scattered inflammatory cells. These can grow rapidly and have mitotic figures, but the overall size of the mass tends to be small. Variable SMA, desmin, and laminin staining is expected given that these tumors are composed of mixed fibroblasts and myofibroblasts. Two cases from our set had histopathologic features suggestive of nodular fasciitis (cases 11, 13).

Low-grade myofibroblastic sarcoma (an entity distinct from *ALK*-positive myofibroblastic tumors[43]) is a low-grade malignancy in people that commonly recurs.[51] High-grade myofibroblastic sarcomas are considered undifferentiated pleomorphic sarcomas, an entity that falls under the STS classification in dogs. Generally, electron microscopy is used for diagnosis because IHC will do little to distinguish among the other differentials. In our subset, low-grade myofibroblastic sarcomas were considered for most of the cases not considered leiomyoma or leiomyosarcoma.

Stringent but clearly defined diagnostic criteria were used to ultimately determine whether a tumor should be deemed smooth muscle in origin. We considered this stringency critical given that other sarcomas may express these IHC markers. For example, SMA can be expressed in other tumors, such as myofibroblastic tumors,[29] osteosarcoma,[36] rhabdomyosarcoma,[28] perivascular wall tumors,[3] and GISTs.[45] To complement IHC stains, MT was used to confirm minimal collagen production by the neoplastic cells, a feature not expected in smooth muscle tumors. Our stringent criteria may have led to fewer diagnoses, albeit with higher confidence.

Of note, case 3, angioleiomyosarcoma, was considered an exception in this case given that its close association with vasculature suggested that the anatomic origin was the vascular wall. This case had variable collagen deposition and we speculated that the areas with abundant collagen may be similar to vascular fibrosis seen in vasculopathies. Whether this tumor should be considered a smooth muscle tumor or perivascular wall tumor is debatable and further research is required.

There are several important limitations to our study, the most important of which is small sample size, which precluded statistical comparisons and limited our ability to draw conclusions regarding the clinical behavior of non-visceral leiomyosarcomas. Commercial and primary-care diagnostic laboratories may have a wider database of dermal and subcutaneous masses than tertiary institutions. Another limitation is the lack of a gold standard for comparison for leiomyosarcoma and, therefore, reliance on unvalidated and arbitrary IHC scoring criteria and morphologic characteristics for diagnostic criteria. The importance of a threshold at which staining is called positive or negative is established in the human literature[38]; however, even in human pathology, most work has been with carcinomas of unknown origin rather than sarcomas. The movement toward using a panel of IHC stains rather than a single stain and identification of specific mutations associated with neoplastic thresholds may explain the lack of defined thresholds to identify mesenchymal tumors that may be of smooth muscle origin. Future studies should aim to better characterize the staining pattern of smooth muscle tumors in known common sites of origin, such as the GI tract and reproductive tract, to gain more reliable information on the diagnostic criteria for these tumors.

Other common limitations for IHC studies include false-negative results, which can arise from inadequate antigen preservation or expression. Especially with older, formalin-fixed samples, and samples that were maintained in formalin for longer than optimal before processing, negative immunostaining may reflect the age, storage condition, or suboptimal processing of the sample rather than being a true negative. Although well-differentiated tumors often maintain expression of several markers, these same markers may be lost in most cells in poorly differentiated tumors, or gain other traits, that together, can preclude a definitive interpretation.[10] Therefore, the absence of labeling does not always exclude a diagnosis, although true loss of expression may suggest that the behavior of the neoplastic cells would change accordingly. It is also possible that tumors that should be classified as leiomyosarcomas were not identified in our initial search given the previously mentioned limitations in diagnosis (e.g., H&E appearance overlapping with other sarcomas, lack of routine IHC use). Therefore, a set of leiomyosarcomas was likely not captured by our search terms. Other limitations are inherent to the retrospective nature of our study, including the variable availability of clinical follow-up for these cases.

Our study highlights the uncommon nature of canine non-visceral leiomyosarcoma and potential differentials that should be considered. IHC staining is useful and important; however, pathologists should recognize that there can be different tumors with overlapping staining patterns. SMA alone did not lead to a definitive diagnosis, and desmin may not be

useful for the diagnosis of leiomyosarcoma because of potential loss of staining in this cohort. The loss of desmin expression should be investigated in leiomyosarcoma as a potential marker of more aggressive behavior. Although the importance of histologic and molecular subtype of sarcomas has been demonstrated in people,[1,21,50] our study emphasizes the need for continued efforts in veterinary species to ensure that these subtypes are accurately classified to potentially predict behavior and guide treatment more definitively. Future studies with alternative tools such as electron microscopy and molecular characterization in addition to IHC staining are required in veterinary medicine to better document the spectrum of IHC staining variation and clinical behavior of sarcoma subtypes, including non-visceral SMA-positive mesenchymal tumors such as leiomyosarcomas and myofibroblastic tumors.

ORCID iDs

Rachel V. Brady https://orcid.org/0000-0002-5688-3872

Robert B. Rebhun https://orcid.org/0000-0002-8047-3494

Jenna H. Burton https://orcid.org/0000-0002-1895-8298

Supplemental material

Supplemental material for this article is available online.

References

1. Anderson ND, et al. Lineage-defined leiomyosarcoma subtypes emerge years before diagnosis and determine patient survival. Nat Commun 2021;12:4496.
2. Andreasen CB, Mahaffey EA. Immunohistochemical demonstration of desmin in canine smooth muscle tumors. Vet Pathol 1987;24:211–215.
3. Avallone G, et al. The spectrum of canine cutaneous perivascular wall tumors: morphologic, phenotypic and clinical characterization. Vet Pathol 2007;44:607–620.
4. Bettini G, et al. Gastrointestinal spindle cell tumours of the dog: histological and immunohistochemical study. J Comp Pathol 2003;129:283–293.
5. Boy SC, et al. Diagnosis and treatment of primary intraoral leiomyosarcomas in four dogs. Vet Rec 2005;156:510–513.
6. Brennan MF, et al. Lessons learned from the study of 10,000 patients with soft tissue sarcoma. Ann Surg 2014;260:416–422.
7. Callanan JJ, et al. Primary pulmonary artery leiomyosarcoma in an adult dog. Vet Pathol 2000;37:663–666.
8. Carvalho JC, et al. Cluster analysis of immunohistochemical markers in leiomyosarcoma delineates specific anatomic and gender subgroups. Cancer 2009;115:4186–4195.
9. Conceição LG, et al. Cutaneous nodular fasciitis in the dog. Morphological and immunohistochemical study. Braz J Vet Pathol 2009;2:41–44.
10. Cooper BJ, Valentine BA. Tumors of muscle. In: Meuten DJ, ed. Tumors in Domestic Animals. 5th ed. Wiley, 2017:425–466.
11. Dailey DD, et al. DOG1 is a sensitive and specific immunohistochemical marker for diagnosis of canine gastrointestinal stromal tumors. J Vet Diagn Invest 2015;27:268–277.
12. Dalton BGA, et al. Inflammatory myofibroblastic tumors in children. J Pediatr Surg 2016;51:541–544.
13. Day MJ, et al. A review of pathological diagnoses made from 87 canine splenic biopsies. J Small Anim Pract 1995;36:426–433.
14. Demicco EG, et al. Progressive loss of myogenic differentiation in leiomyosarcoma has prognostic value. Histopathology 2015;66:627–638.
15. Dennis MM, et al. Prognostic factors for cutaneous and subcutaneous soft tissue sarcomas in dogs. Vet Pathol 2011;48:73–84.
16. Ehrhart N. Soft-tissue sarcomas in dogs: a review. J Am Anim Hosp Assoc 2005;41:241–246.
17. Ettinger SN. Principles of treatment for soft-tissue sarcomas in the dog. Clin Tech Small Anim Pract 2003;18:118–122.
18. Farese JP, et al. Oesophageal leiomyosarcoma in dogs: surgical management and clinical outcome of four cases. Vet Comp Oncol 2008;6:31–38.
19. Frost D, et al. Gastrointestinal stromal tumors and leiomyomas in the dog: a histopathologic, immunohistochemical, and molecular genetic study of 50 cases. Vet Pathol 2003;40:42–54.
20. Grossmann AH, et al. Classification, molecular characterization, and the significance of *Pten* alteration in leiomyosarcoma. Sarcoma 2012;2012:380896.
21. Grünewald TG, et al. Sarcoma treatment in the era of molecular medicine. EMBO Mol Med 2020;12:e11131.
22. Gwin RM, et al. Ophthalmic nodular fasciitis in the dog. J Am Vet Med Assoc 1977;170:611–614.
23. Hayes S, et al. Classification of canine nonangiogenic, nonlymphogenic, gastrointestinal sarcomas based on microscopic, immunohistochemical, and molecular characteristics. Vet Pathol 2013;50:779–788.
24. Hendrick MJ, et al. Mesenchymal tumors of the skin and soft tissues. In: Meuten DJ, ed. Tumors in Domestic Animals. 5th ed. Wiley, 2017:142–175.
25. Heng HG, et al. Smooth muscle neoplasia of the urinary bladder wall in three dogs. Vet Radiol Ultrasound 2006;47:83–86.
26. Johnson GC, et al. Comparison of argyrophilic nucleolar organizer regions (AgNORs) and mitotic index in distinguishing benign from malignant canine smooth muscle tumors and in separating inflammatory hyperplasia from neoplastic lesions of the urinary bladder mucosa. J Vet Diagn Invest 1995;7:127–136.

27. Kapatkin AS, et al. Leiomyosarcoma in dogs: 44 cases (1983–1988). J Am Vet Med Assoc 1992;201:1077–1079.

28. Kimura M, et al. Gingival rhabdomyosarcoma accompanied by an immature myogenic population immunoreactive for α-smooth muscle actin in a dog. J Comp Pathol 2013;149:48–52.

29. Knight C, et al. Inflammatory myofibroblastic tumors in two dogs. Vet Pathol 2009;46:273–276.

30. LaRock RG, Ginn PE. Immunohistochemical staining characteristics of canine gastrointestinal stromal tumors. Vet Pathol 1997;34:303–311.

31. Liu SM, Mikaelian I. Cutaneous smooth muscle tumors in the dog and cat. Vet Pathol 2003;40:685–692.

32. Maas CPHJ, et al. Reclassification of small intestinal and cecal smooth muscle tumors in 72 dogs: clinical, histologic, and immunohistochemical evaluation. Vet Surg 2007;36:302–313.

33. Martin-Liberal J. Leiomyosarcoma: principles of management. Intractable Rare Dis Res 2013;2:127–129.

34. Mauldin GN. Soft tissue sarcomas. Vet Clin North Am Small Anim Pract 1997;27:139–148.

35. Myong N-H, Min J-W. Low-grade myofibroblastic sarcoma arising in fibroadenoma of the breast—a case report. Diagn Pathol 2016;11:33.

36. Nagamine E, et al. Diversity of histologic patterns and expression of cytoskeletal proteins in canine skeletal osteosarcoma. Vet Pathol 2015;52:977–984.

37. Ogden JA, et al. Outcomes associated with vaginectomy and vulvovaginectomy in 21 dogs. Vet Surg 2020;49:1132–1143.

38. Oien KA. Pathologic evaluation of unknown primary cancer. Semin Oncol 2009;36:8–37.

39. Patel NR, et al. USP6 activation in nodular fasciitis by promoter-swapping gene fusions. Mod Pathol 2017;30:1577–1588.

40. Pellegrino V, et al. Canine gastrointestinal spindle cell tumors efficiently diagnosed by tissue microarray-based immunohistochemistry. Vet Pathol 2018;55:678–681.

41. Pérez J, et al. Immunohistochemical characterization of hemangiopericytomas and other spindle cell tumors in the dog. Vet Pathol 1996;33:391–397.

42. Pierini A, et al. Primary leiomyosarcoma of the jugular vein in a dog. Open Vet J 2017;7:61–64.

43. Qiu X, et al. Inflammatory myofibroblastic tumor and low-grade myofibroblastic sarcoma: a comparative study of clinicopathologic features and further observations on the immunohistochemical profile of myofibroblasts. Hum Pathol 2008;39:846–856.

44. Rabanal RH, et al. Immunocytochemical diagnosis of skin tumours of the dog with special reference to undifferentiated types. Res Vet Sci 1989;47:129–133.

45. Russell KN, et al. Clinical and immunohistochemical differentiation of gastrointestinal stromal tumors from leiomyosarcomas in dogs: 42 cases (1990–2003). J Am Vet Med Assoc 2007;230:1329–1333.

46. Spangler WL, et al. Primary mesenchymal (nonangiomatous/nonlymphomatous) neoplasms occurring in the canine spleen: anatomic classification, immunohistochemistry, and mitotic activity correlated with patient survival. Vet Pathol 1994;31:37–47.

47. Swinbourne F, et al. Inflammatory myofibroblastic tumour in the nasal cavity of a dog. J Small Anim Pract 2014;55:121–124.

48. Thacher C, Bradley RL. Vulvar and vaginal tumors in the dog: a retrospective study. J Am Vet Med Assoc 1983;183:690–692.

49. Trahair T, et al. Crizotinib and surgery for long-term disease control in children and adolescents with ALK-positive inflammatory myofibroblastic tumors. JCO Precis Oncol 2019;3:PO.18.00297.

50. van Vliet M, et al. Soft tissue sarcomas at a glance: clinical, histological, and MR imaging features of malignant extremity soft tissue tumors. Eur Radiol 2009;19:1499–1511.

51. Wang L, et al. Low-grade myofibroblastic sarcoma: clinical and imaging findings. BMC Med Imaging 2019;19:36.

52. Weinstein MJ, et al. Nonangiogenic and nonlymphomatous sarcomas of the canine spleen: 57 cases (1975–1987). J Am Vet Med Assoc 1989;195:784–788.