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Histomorphological and immunophenotypic diagnoses of gastrointestinal stromal tumors and other sarcomas that affect the intestine of dogs

Diagnósticos histomorfológico e imunofenotípico de tumores estromais gastrointestinais e outros sarcomas que acometem o intestino de cães

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Abstract
In view of the morphological similarity between gastrointestinal stromal tumors (GIST) and other sarcomas of the intestine of dogs, the aim was to carry out the histomorphological and immunohistochemical diagnosis of these tumors, associating breed, sex and age, location and tumor invasion. 217 cases were evaluated by histopathology and 36 diagnosed by immunohistochemistry were included (24 GIST and 12 other intestinal sarcomas). Mixed breed dogs were the most diagnosed with GIST, mainly elderly females (9.5±2.2 years); in the other intestinal sarcomas, crossbreeds and Dachshunds, males and females, were equally affected. The cecum was the most affected by GISTs, with tumor invasion of the intestinal layers in all cases. The small intestine was the most affected by the other intestinal sarcomas, with invasion of the layers in most of these tumors. GISTs expressed markers such as CD117 and DOG-1, unlike other intestinal sarcomas. GIST and other intestinal sarcomas denoted histomorphological and immunophenotypic characteristics similar to histopathology, justifying the association of immunohistochemistry for the definitive diagnosis.
Keywords: CD117; DOG-1; GIST; veterinary oncology

Resumo
Tendo em vista a semelhança morfológica entre tumores estromais gastrointestinais (GIST) e outros sarcomas do intestino de cães, objetivou-se realizar o diagnóstico histomorfológico e imunoistoquímico desses tumores, associando raça, sexo e idade, localização e invasão tumoral. Foram avaliados 217 casos à histopatologia e incluídos 36 diagnosticados por imuno-histoquímica (24 GIST e 12 outros sarcomas intestinais). Cães sem raça definida foram os mais diagnosticados com GIST, principalmente fêmeas idosas (9,5±2,2 anos); nos demais sarcomas intestinais, mestiços e Dachshunds, machos e fêmeas, foram igualmente acometidos. O ceco foi o mais acometido pelos GISTs, com invasão tumoral das camadas intestinais em todos os casos. O intestino delgado foi o mais acometido pelos demais sarcomas intestinais, com invasão das camadas na maioria desses ttumores. GISTs expressaram marcadores como CD117 e DOG-1, ao contrário de outros sarcomas intestinais. O GIST e outros sarcomas intestinais denotaram características histomorfológicas e imunofenotípicas semelhantes à histopatologia, justificando a associação da imuno-histoquímica para o diagnóstico definitivo.
Palavras-chave: CD117; DOG-1; GIST; oncologia veterinária

1. Introduction

Gastrointestinal neoplasms, uncommon in dogs, represent 2% of all tumors in this species[1,2,3,4]. Among the intestinal neoplasms, the gastrointestinal stromal tumor (GIST - epithelioid, fusiform, mixed or anaplastic)[5,6] stands out, considered mesenchymal, with development from the differentiation of interstitial Cajal cells or their precursors[7,8]. GIST expresses receptor tyrosine kinase (KIT) encoded by the c-KIT proto-oncogene on its surface[8,9]. Mutations can occur in the c-KIT gene, affecting the juxtamembrane domain encoded by exon 11, and in the platelet-derived growth factor receptor (PDGRFA),

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compromising the extracellular domain of KIT encoded by exon 9, being activated by factor of stem cells [10,11,12].

Histologically, epithelial cell bundles, fusiform or mixed, are identified, mostly involving the tunica and smooth intestinal muscles, with variable presence of mitosis and different classifications[13]. On the other hand, the immunohistochemical evaluation is more reliable when evaluating the expression of CD117 (tyrosine kinase marker of c-KIT), CD34 (specific marker of GIST and interstitial Cajal cells) and SMA (smooth muscle actin)[14], associated with markers that differentiate the neoplasm from other gastrointestinal tumors or tumors of nervous origin[15]. Still, immunohistochemistry helps to identify a small percentage of GIST that may express insignificant or absent amounts of CD117 and PDGRFA[16], which has contributed to research in veterinary and human medicine, using markers such as DOG-1 to aid in the definitive diagnosis of these tumors[9].

Based on the difficulty in establishing the definitive diagnosis of intestinal neoplasms by histopathology and in the face of negative GIST CD117, researchers compared the expression of the anti-DOG-1 antibody between GIST and other intestinal sarcomas and identified a gene capable of being expressed in greater amounts in the GIST when compared to CD117[17]. Subsequently, researches investigated the functions of DOG-1, being considered a transmembrane protein associated with the channeling of chloride ions, with excitatory and inhibitory functions, contributing to the electrical activity of the interstitial Cajal cells with an effect on the motility of the gastrointestinal tract[18,19,20].

Due to the scarcity of studies that prove the real incidence of intestinal neoplasms in dogs, the present research aimed, at the histomorphological and immunohistochemical diagnosis of GIST and other intestinal sarcomas, associating breed, sex and age, location and tumor invasion.

2. Material and methods

From June 2014 to December 2020, an analysis of GIST and other intestinal sarcomas by histopathology and immunohistochemistry was performed in dogs, from cases diagnosed in a Veterinary Pathology Laboratory. According to the laboratory, the samples were stained with hematoxylin and eosin (HE) and analyzed by optical microscopy and later evaluated by different immunohistochemical markers, and the definitive diagnosis included GIST and other intestinal sarcomas.

Data from the review of those affected by GIST or other intestinal sarcomas were analyzed, including breed, sex and age, and the results were expressed descriptively. Of the dogs diagnosed with GIST or other intestinal sarcomas by histopathological examination, the macroscopic location and mucosal invasion were analyzed, with descriptive data.

Tumors were investigated with immunohistochemical markers containing Ki-67, CD117, DOG-1, S-100, 1A4, HHF35, desmin, vimentin, CD31, MyoD1, AE1AE3, CD99, GFAP and p53, allowing the differentiation of GIST from other intestinal sarcomas. For this, the tissues processed for histopathological analysis and embedded in paraffin were placed on previously marked slides. Antigenic retrieval by the moist heat method was performed in a steam pan (20-30 minutes), followed by incubation with primary antibodies at 4°C. In all immunostaining protocols, diaminobenzidine tatrahydrochloride (DAB) was used to establish brown staining of positive tissue, while counterstaining was performed using Harris' hematoxylin.

The samples submitted to Ki-67 analysis to evaluate the proliferative index were evaluated by light microscopy, using an ocular grating with a diameter of 1 mm. The immunoexpressions were established by percentage of labeled cells, considering the number of positive cells and the total number of cells within the grating, evaluated in five random fields of higher magnification (40x objective). Subsequently, samples were classified into scores: 0 (no immunolabeling), 1 (< 5% immunolabeled cells), 2 (5-8% immunolabeled cells), 3 (> 8% immunolabeled cells)[22]. Data were statistically analyzed by Sperman correlation using GraphPad Prism® software (version 9.1 - GraphPad Software, Inc 2021), and variables were considered significant when p value ≤ 0.05.

Data on the antibodies used to define gastrointestinal stromal tumors and other intestinal sarcomas in dogs are shown in Table 1.

Table 1. Antibodies used to define the diagnosis of gastrointestinal stromal tumors (GIST) and other intestinal sarcomas that affect the intestine of dogs, from June 2014 to December 2020

Antibody	Clone	Dilution	Laboratory
Ki-67	MIB-1	1:400	Dako
C-kit	CD117 Dako	1:300	Dako
DOG-1	EP332	1:100	BioSb
S-100	Mmab	1:800	Dako
1A4	Policlonal Dako	1:200	Dako
HHF-35	HHF-35	1:400	CellMarque
Desmin	D33	1:200	Dako
Vimentin	V9	1:1000	Dako
MyoD1	5.8[a]	1:250	BioSb
Cox-2	EP293	1:200	BioSb
AE1/AE3	AE1/AE3	1:300	Dako
CD99	12E7	1:100	Dako
GFAP	6F2	1:300	Dako
P53	DO7	1:150	Dako

3. Results

During the studied period, 217 GIST histopathological examination result were evaluated, of which 36 (16.5%) were definitively diagnosed through immunohistochemistry; 24 of these had a consistent diagnosis of GIST and 12 were compatible with other intestinal sarcomas. Thus, when analyzing the effectiveness of diagnostic methods, histopathology was conclusive in 66.6% of GISTs when evaluated together with immunohistochemistry, while 33.4% of tumors showed diagnostic alteration after analysis of immunohistochemical markers.

Of the 24 dogs diagnosed with GIST by immunohistochemistry, the main breeds affected were mixed breed dogs (8), Poodle (3), Dachshund (3), Golden Retriever (2), French Bulldog (1), English Bulldog (1), Jack Russel Terrier (1), Beagle (1), Lhasa Apso (1), Pitbull (1), West Highland Terrier (1), and unreported breed (1). Of these, 14 were females and ten were males, with a mean age of 10.5±3.0 years. Of the 12 dogs with other intestinal sarcomas by immunohistochemistry, most were undefined breed (4), followed by Dachshund (2), Chow Chow (1), Dogue de Bordeaux (1), Lhasa Apso (1), Shih Tzu (1), Yorkshire (1), and unreported breed (1). Of these, six were females and six were males, with a mean age of 9.5±2.2 years.

As for the tumor localization of the GIST, five dogs had the intestinal segment discriminated in the immunohistochemical reports, the cecum (5) being the most described region. Of the 24 GIST, nine animals had tumor invasion of the muscular intestinal layer, eight of the submucosal/muscular layer, two of the muscular/serous layer, one had diffuse invasion, and four did not have the region discriminated. In addition, three presented ulceration caused by the neoformation. In relation to the 12 other intestinal sarcomas, the tumor location was reported in three dogs, and the small intestine was the most commonly described segment. Neoplastic invasion occurred in 11 dogs, with the submucosa/muscular layer being one of the most affected (5), followed by the muscular layer (3), submucosa (1), serosa (1) and the diffuse layer (1). In addition, one dog did not have the site of invasion determined. Ulceration was observed in three os these neoplasms.

Immunohistochemical tests to confirm the diagnosis of GIST demonstrated 70% positive and 29.1% negative immunoexpressions for CD117, while 100% of tumors were positive by DOG-1, including those negative for CD117. In addition, 54.1% and 66.6% of GISTs were positive for smooth muscle actin (SMA) by immunolabeling for HHF35 and 1A4, respectively; 95% were evaluated for desmin expression and only 12% were positive for this marker. As for S100, 95% of the tumors were evaluated, and 70.8% showed positive markers. In addition, 33% were evaluated and showed immunolabeling for vimentin and 8.3% for MyoD1. Some tumors were evaluated for AE1AE3, GFAP and CD99 expression, which were negative in 25%, 12.5% and 4.1% of the cases, respectively. As for the other intestinal sarcomas, all were negative for CD117 and DOG-1. While 75% of the tumors were positive for S-100; 25% expressed 1A4 and HHF35; 4.1% marked positive for desmin and only 58% were positive for vimentin. In addition, 4.1% expressed CD31, being classified as intestinal hemangiosarcoma. Some tumors were evaluated for AE1AE3, GFAP and CD99 expression, and these were negative in 16.1%, 4.1% and 4.1% of cases, respectively.

Of the 24 dogs diagnosed with GIST, eight showed Ki-67 staining with score 2 (5-8% of immunolabeled cells), nine with score 3 (> 8% of immunolabeled cells), and six animals did not have the index determined. Regarding the other 12 intestinal sarcomas, five had the proliferative index determined, all with a score of 3 (> 8% of immunolabeled cells).

The results of the immunohistochemical analyses are described in Tables 2 and 3.

4. Discussion

The diagnostic methods evaluated in the present study demonstrated differences regarding the definition of GIST, since some neoplasms identified only by histopathology showed a change in diagnosis when analyzed jointly by immunohistochemical markers. These results corroborated the descriptions of Shales et al.[23], that other intestinal sarcomas may present histomorphological characteristics analogous to GIST, requiring differentiation by specific and reliable techniques such as immunohistochemistry. What is more, according to Hayes et al.[24], Yamamoto et al.[25] and Dailey et al.[26], the definition of the diagnosis is essential due to the distinct behaviors of these neoplasms, which can directly influence the therapeutic choice and consequently the prognosis of affected patients.

According to Robertson and Patil[27], immunohistochemistry allows the identification of malignant and undifferentiated diseases through markers that help identify the affected cell lineage, however, it must be associated with histopathological analysis and clinical history of the patient, as performed in this study. If immunohistochemistry does not identify the cellular origin, mutational analysis can be performed to elucidate the diagnosis[28].

The results of the present study also highlighted the importance of fully describing information regarding patient reviews and neoplastic characteristics on histopathology and immunohistochemistry request forms made by veterinary medical professionals to specialized diagnostic centers. Combined with the infrequent occurrence of intestinal neoplasms in dogs and the difficulty of differentiating GIST with other mesenchymal neoplasms in the past[7], the scarcity of consistent data limits scientific publications and restricts discussions relevant to directing diagnosis, therapeutic options, and prognosis.

Table 2. Immunohistochemical panels used to define gastrointestinal stromal tumors (GIST) that affect the intestines of dogs, evaluated from June 2014 to December 2020 at the Veterinary Clinical Analysis Laboratory - VetPat (Campinas, SP, Brazil)

Dog	Diagnosis	Ki-67	CD117	DOG-1	S100	1A4	HHF35	Desmin	Other markers (+)	Other markers (-)
1	GIST	8%	+	nv	nv	+	+	-	COX-2	nv
2	GIST	5%	+	+	+	-	-	-	nv	nv
3	GIST	20%	-	+	+	-	-	-	MyoD1	AE1AE3/CD99
4	GIST	5%	+	+	+	+	+	-	nv	nv
5	GIST	8%	+	nv	+	+	+	-	Vimentin	nv
6	GIST	10%	-	+	+	+	+	-	nv	nv
7	GIST	10%	+	+	+	+	+	-	nv	nv
8	GIST	nv	+	nv	+	+	+	-	Vimentin	AE1AE3
9	GIST	8%	-	+	+	-	-	-	nv	nv
10	GIST	nv	+	nv	+	+	-	-	Vimentin	MyoD1
11	GIST	5%	+	nv	+	+	-	-	nv	GFAP
12	GIST	10%	+	nv	-	+	+	nv	nv	GFAP
13	GIST	15%	-	+	+	-	-	+	nv	nv
14	GIST	nv	+	nv	+	+	-	-	nv	MyoD1/AE1AE3
15	GIST	nv	+	nv	-	+	+	-	Vimentin	nv
16	GIST	8%	-	+	-	+	+	+	nv	AE1AE3
17	GIST	15%	+	+	+	-	-	-	nv	nv
18	GIST	12%	-	+	-	+	+	-	nv	nv
19	GIST	15%	+	+	+	+	-	-	nv	nv
20	GIST	8%	-	+	+	-	+	-	nv	nv
21	GIST	12%	+	nv	+	-	-	-	nv	GFAP
22	GIST	nv	+	nv	-	+	+	-	Vimentin	AE1AE3
23	GIST	nv	+	nv	-	+	+	nv	Vimentin/MyoD1	nv
24	GIST	nv	+	nv	+	-	-	-	Vimentin	AE1AE3

+: positive; -: negative; nv: not valued

Table 3. Immunohistochemical panels used to define other sarcomas that affect the intestines of dogs, evaluated from June 2014 to December 2020 at the Veterinary Clinical Analysis Laboratory - VetPat (Campinas, SP, Brazil)

Dog	Diagnosis	Ki-67	CD117	DOG-1	S100	1A4	HHF35	Desmin	Other markers (+)	Other markers (-)
1	CHONDROSARCOMA	nv	-	nv	+	-	-	-	Vimentin	AE1AE3
2	FIBROSARCOMA	45%	nv	-	+	-	-	-	nv	nv
3	FIBROSARCOMA	10%	-	nv	+	-	-	-	nv	GFAP
4	FIBROSARCOMA	15%	nv	-	+	-	-	-	nv	nv
5	FIBROSARCOMA	60%	-	nv	+	-	-	-	Vimentin	p53
6	HEMANGIOSARCOMA	12%	-	-	-	-	-	-	CD31	nv
7	LEIOMYOSARCOMA	nv	-	nv	-	+	+	+	nv	MyoD1
8	LEIOMYOSARCOMA	nv	-	nv	+	+	+	-	Vimentin	AE1AE3
9	MYXOID LIPOSARCOMA	nv	-	nv	+	-	-	-	Vimentin	nv
10	MYOFIBROSARCOMA	nv	-	nv	-	+	+	-	Vimentin	nv
11	NEUROFIBROSARCOMA	nv	-	nv	+	-	-	-	Vimentin	AE1AE3
12	NEUROFIBROSARCOMA	nv	-	nv	+	-	-	-	Vimentin	AE1AE3

+: positive; -: negative; nv: not valued

In the studied population, mixed breed dogs, Dachshunds, Poodle and Golden Retrievers were the most diagnosed with GIST, coinciding with those described by Berger et al.[7]; in contrast, some studies mentioned that other breeds can be affected, showing no predisposition to its development[29]. In the other intestinal sarcomas, the highest prevalence was in the mixed breed dogs and Dachshunds, differing from Patnaik et al.[30], who cited Collies and German Shepherds as the most affected.

Studies have shown a higher frequency of intestinal neoplasms in middle-aged to senile dogs, mainly males[11,31,32], partially corroborating the results of this study, since that the most affected were over 9.5 years old, however females were the most diagnosed.

The intestinal tumor locations of GIST were not determined in all reports, however, they were similar to those described by Alcazar et al.[9], Russel et al.[14] and Dailey et al.[26], with the cecum being the most affected. Regarding the other intestinal sarcomas, the most affected region was the small intestine, disagreeing in parts with the literature data that cited jejunum and cecum[14]. According to Maas et al.[33], the proximal locations of tumors, as in the small intestine, cause impairment in the functioning of the gastrointestinal tract, culminating in evident clinical signs and, therefore, early diagnosis, favoring the prognosis; unlike the other, such as in the cecum, where few changes can be noticed, because of its indirect position the passage of intestinal contents, resulting in tumors of larger diameters, chances of ulcerations and infections.

In the present study, most dogs with GIST and other intestinal sarcomas showed invasion only of the muscular intestinal layer, which may influence the prognosis, because according to Lee et al.[18], neoplasms that compromise the serosa may reduce the survival of those affected. In addiction, Leandro and Sá[1] and Hayes et al.[24] showed that GIST can affect mainly the submucosa and muscular, and in some cases, all intestinal layers, corroborating with the current study, since many animals showed invasion of the layers described.

Regarding the immunohistochemical markers used for the diagnosis of GIST, studies revealed that most tumors showed mutations in the KIT gene[36]; however, a discrete percentage of these tumors may express mutations only in the PDGFRA gene, not reacting or reacting weakly against KIT antibodies, not being diagnosed as GIST[17], as observed in the present study. In this sense, the antiserum against a GIST-specific gene, responsible for encoding the DOG-1 protein, was used, since it seems to be expressed in typical GIST (mutations in KIT and PDGFRA), and in GIST with negative mutations in the KIT gene, aiding in their diagnosis[17], confirming the data from this survey. Still on this theme, according to Dailey et al.[26], the DOG-1 immunoexpression presents higher sensitivity and specificity when compared to CD117, and may be considered an important marker in suspected cases of GIST, since its expression seems to be independent of CD117[7], reinforcing the acuity of its inclusion in this study.

Rios-Moreno et al.[16] evaluated the immunoexpression of PKC and DOG-1 in 99 human GIST and observed 91% DOG-1 expression, with positivity in two of the five negative for CD117. Although the present study did not investigate PKC expression and was performed in the canine species, it was noted that all GIST were positive for DOG-1, including tumors negative for CD117. In this sense, in view of the literature and the immunohistochemical results of the present survey, DOG-1 and CD117 are indispensable antibodies for defining the diagnosis of GIST, not being expressed in other gastrointestinal smooth muscle neoplasms[37].

GIST can still frequently express positivity for markers used in the diagnosis of other intestinal sarcomas, and structural and immunohistochemical analyses are important tools for defining the distinct classifications of GIST based on their origin[1]. Russel et al.[14] highlighted that the positivity for SMA in GIST is related to the location of Cajal interstitial cells along the intestinal muscle layer, demonstrating the muscle cell origin of these tumors, as observed in this study, regarding the tumors evaluated through the markers HHF35 and IA4, respectively. Also, according to Kisluk et al.[38], this immunoexpression may be related to the presence of smooth muscle cells in the intestinal muscular or mucosal layer, generating a positive interpretation for the markers when evaluated by immunohistochemistry.

Immunoreactivity for desmin was observed in a small percentage (8.3%) of GIST cases diagnosed in the present research, corroborating the results of Hirota[36], in which almost all of these tumors were negative for this marker, as it is considered a specific protein of smooth muscle cells. The data from this study also coincided with those obtained by Miettinen and Lasota[39], that approximately 5-10% of GIST can express positivity for this marker, also revealing their myogenic origin.

In this study, a considerable percentage of GIST were positive for S100, a marker characteristic of lesions originating from the myenteric nerve plexus, supporting the data described by Frost et al.[31] and Mettinen and Lasota[39] and evidencing the neurogenic origin of these tumors; in contrast, it differed from the descriptions of Hirota[36], that most GIST were negative for S100. According to Kisluk et al.[38], expression of this marker is considered rare in some GIST locations, but frequently expressed in tumors in the small intestine. As mentioned earlier, although the tumor locations were not determined in all dogs in the present survey, statements in front of the marker and the location of the neoplasms become limited.

Hayes et al.[24], Maas et al.[33] and Leandro et al.[40] highlighted that almost all GIST are positive for vimentin and negative for epithelial cell markers (cytokeratin AE1/AE3), hematopoietic markers (CD3, CD20 and CD79a) and for glial fibrillary acidic protein (GFAP), but in the present study it was noted that less than half of the GIST were positive for vimentin and none positive for the other markers, resembling in parts with the mentioned studies. According to Sarlomo-Rikala et al.[41], the absence of labeling for GFAP and AE1/AE3 assists in confirming the diagnosis of GIST, since these markers are commonly found in intestinal Schwannomas and tumors of epithelial origin, respectively.

Data from the scientific literature related to the determination of the neoplastic proliferative index,

measured by Ki-67 immunostaining, are not yet fully elucidated in dogs and humans; however, this tool can be used to determine the potential for malignancy(42). The study carried out by Zhao et al.(43) to evaluate the survival of 418 humans with GIST, demonstrated that proliferation rates above 8% can confer an unfavorable prognosis, influencing tumor progression, the development of metastases and the response to adjuvant treatment.

5. Conclusions

Based on histomorphology, GIST and other intestinal sarcomas present similar structural and phenotypic aspects in dogs, which makes the definitive diagnosis difficult and, consequently, the establishment of an adequate therapeutic protocol. Therefore, the association of histopathology and immunohistochemistry is essential for the classification of these neoplasms, which may provide a better prognosis for affected dogs. GISTs often express markers such as CD117 and DOG-1, unlike other intestinal sarcomas; however, CD117-negative GISTs can be found, being misdiagnosed as other tumors when not analyzed for DOG-1.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Author contributions

Conceptualization: M.L. Costa, F.A.R. Sueiro, P.C. Jark and F.G.G. Dias. *Formal analysis*: M.L. Costa and F.A.R. Sueiro. *Acquisition of financing*: F.A.R. Sueiro. *Investigation*: M.L. Costa, L.L. Carvalho, M.A. Rodrigues and F.G.G. Dias. *Methodology*: M.L. Costa, F.A.R. Sueiro, P.C. Jark, L.L. Carvalho and F.G.G. Dias. *Project management*: M.L. Costa, F.A.R. Sueiro and P.C. Jark. *Supervision*: M.A. Rodrigues and F.G.G. Dias. *Writing (review & editing)*: M.L. Costa, M.A. Rodrigues, V.T.S. Almeida and F.G.G. Dias.

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