Aus dem Department für Biologische Wissenschaften und Pathobiologie

der Veterinärmedizinischen Universität Wien

Institut für Pathologie

(Leiter: Univ.-Prof. Dr.med.vet. Herbert Weissenböck, Dipl.ECPHM)

The relevance of prognostic factors for canine gastrointestinal soft tissue tumors

Diplomarbeit

Veterinärmedizinische Universität Wien

vorgelegt von

Asta Theodora Victoria Proksch

Wien, im Juli, 2025

Betreuer: Ass. Prof. Dr. med. vet. Christof Bertram, PhD, Dipl.ACVP

Institut für Pathologie

Department für Biologische Wissenschaften und Pathobiologie

Veterinärmedizinische Universität Wien

Begutachterin: Dr^{in} med. vet. Brigitte Degasperi, Dipl. ECVS

Klinisches Zentrum für Kleintiere

Veterinärmedizinische Universität Wien

Eigenständigkeitserklärung

Hiermit erkläre ich, dass ich die vorgelegte Arbeit selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe. Alle übernommenen Textstellen aus fremden Quellen wurden kenntlich gemacht.

Ich habe die entscheidenden Arbeiten selbst durchgeführt und alle zuarbeitend Tätigen mit ihrem Beitrag zur Arbeit angeführt. An einigen Stellen meines selbst verfassten englischen Textes habe ich zur Sprachkorrektur ChatGPT (OpenAI, 2022) genutzt.

Die vorliegende Arbeit wurde nicht an anderer Stelle eingereicht oder veröffentlicht.

Wien, den 22.07.205 Asta Theodora Victoria Proksch

Acknowledgements

First, I would like to express my sincere gratitude to my supervisor, Ass. Prof. Dr. med. vet. Christof Bertram, PhD, Dipl.ACVP for his invaluable support and guidance throughout this project. I am also grateful to Dr^{in} med. vet. Brigitte Degasperi, Dipl. ECVS for kindly agreeing to review my thesis.

Furthermore, I would like to thank Dr. Klemens Alton and Mag. med. vet. Verena Strauss from InHisto Tierpathologie and Taryn Donovan, DVM, DACVP from The Animal Medical Center for the provision of samples.

Finally, I would like to thank all those involved in the immunohistochemical staining and scanning of the histological slides at the Institute of Pathology of the Vetmeduni, as well as the veterinarians for providing clinical information on their respective patients.

Abstract (Englisch)

Non-angiogenic, non-lymphogenic intestinal mesenchymal tumors (NIMTs) are a diverse group of gastrointestinal neoplasms in dogs, the most common being leiomyosarcomas (LMSAs) and gastrointestinal stromal tumors (GISTs). Advances in immunohistochemical (IHC) techniques, particularly the use of markers like CD117 (c-Kit) and Discovered-on-GIST-1 (DOG-1), have led to more accurate diagnoses and a rise in GIST identification. This study evaluated histopathological and immunohistochemical parameters of 18 canine NIMT using Whole-Slide Imaging, and comparing the findings with clinical outcomes. Although the small sample size limited statistical significance, several results were consistent with previous research. A key finding was the reclassification of seven tumors from LMSAs to GISTs, which underscores the essential role of IHC in differential diagnosis. Additionally, the study found that GISTs had higher mitotic counts (MC) than LMSAs, aligning with earlier studies linking elevated MC with increased metastatic risk and reduced survival. While a threshold of MC ≥2 was highly sensitive and specific for predicting tumor-related death within one year, statistical significance was not reached. Overall, the study reinforces the importance of IHC and MC in diagnosing and assessing prognosis in canine NIMTs, while noting that classification and treatment strategies remain areas of ongoing research.

Abstract (Deutsch)

Nicht-angiogene, nicht-lymphogene intestinale mesenchymale Tumoren (NIMTs) sind eine vielfältige Gruppe gastrointestinaler Neoplasmen bei Hunden, von denen Leiomyosarkome (LMSAs) und gastrointestinale Stromatumoren (GISTs) die häufigsten sind. Fortschritte in der Immunhistochemie (IHC), insbesondere die Verwendung von Markern wie CD117 (c-Kit) und Discovered-on-GIST-1 (DOG-1), haben zu genaueren Diagnosen und vermehrt zur Identifizierung von GIST geführt. In dieser Studie wurden histopathologische und immunhistochemische Parameter von 18 kaninen NIMT mittels Whole-Slide-Imaging und die Ergebnisse mit dem klinischen Ergebnis verglichen. Obwohl die geringe Stichprobengröße die statistische Aussagekraft einschränkte, stimmten mehrere Ergebnisse mit früheren Untersuchungen überein. Ein zentrales Ergebnis war die Reklassifizierung von sieben Tumoren von LMSAs zu GISTs, was die wesentliche Rolle der IHC bei der Differentialdiagnose unterstreicht. Darüber hinaus ergab die Studie, dass GIST eine höhere Mitosenzahl (MC) aufweisen als LMSA, was mit früheren Studien übereinstimmt, die eine erhöhte MC mit einem erhöhten Metastasierungsrisiko und einer geringeren Überlebensrate in Verbindung bringen. Obwohl ein Schwellenwert von MC ≥2 eine hohe Sensitivität und Spezifität für die Vorhersage des tumorbedingten Todes innerhalb eines Jahres aufwies, wurde keine statistische Signifikanz erreicht. Insgesamt unterstreicht die Studie die Bedeutung von IHC und MC bei der Diagnose und Prognosebeurteilung von NIMTs bei Hunden und weist gleichzeitig darauf hin, dass Klassifizierungs- und Behandlungsstrategien weiterhin Gegenstand der Forschung sind.

Table of content

1 Introduction

The term non-angiogenic, non-lymphogenic intestinal mesenchymal tumors (NIMTs) describes a diverse group of neoplasms arising in the stomach, small and large intestine and includes fibrosarcomas, fibromas, leiomyosarcomas (LMSAs), leiomyomas, gastrointestinal stromal tumors (GISTs), as well as tumors of neuronal origin such as peripheral nerve sheath tumors (PNSTs) (1). Among these, GISTs and LMSAs are the most frequently identified in canine patients, accounting for about 10 to 30% of all gastrointestinal neoplasms (1). In NIMTs, one study with 40 cases found GISTs and LMSAs to make up 45% and 30% of the cases, respectively, with the remaining 25% being classified as non-GIST/nonleiomyosarcomas (1). A more recent study with 47 cases found these percentages to be at 68% (n = 32), 30% (n = 14) and 2% (n = 1) (2). Notably, the diagnosis of GISTs has become more prevalent in recent years, mostly due to advances in diagnostic techniques, especially in the use of immunohistochemical markers, that allow an accurate identification of NIMTs (2, 3).

1.1 Leiomyosarcomas and Gastrointestinal Stromal Tumors

LMSAs originate from smooth muscle cells and are commonly located in the gastrointestinal tract, the female genital tract and less often in soft tissue (4). Despite their locally invasive behavior, these tumors generally exhibit a slow growth pattern (4). When diagnosed in the gastrointestinal tract, the small intestine was identified as the most common tumor site (1, 2, 5). In one study on LMSAs, metastases were observed in approximately 50% of affected dogs, with the most frequent sites being the mesenteric lymph nodes, peritoneum, and liver (6). Nevertheless, canine patients often have a long median survival time, and in many cases die of causes unrelated to the tumor (6).

GISTs arise from interstitial cells of Cajal, which are specialized cells found within the submucosal and myenteric plexuses of the gastrointestinal tract, in which they function as pacemaker cells. Contrary to LMSAs, GISTs express c-KIT (CD117) (5). CD117 codes for the tyrosine kinase receptor KIT, is considered a growth receptor (proto-oncogene), that may become oncogenic through activating mutations. These mutations are most commonly found in exon 11 in GISTs, similar to canine mast cell tumors, and drive uncontrolled cell proliferation and therefore oncogenesis (1). Besides the important role in tumorigenesis, immunohistochemistry (IHC) for CD117 is commonly used as a diagnostic criterion to differentiate GISTs from LMSA (see section 1.2).

In humans, GISTs most commonly occur in the stomach, followed by the small intestine. In contrast, one study in dogs reported the small intestine as the predominant site, with the cecum as the second most frequent location (2). Two additional studies on canine gastrointestinal sarcomas found that all cecal tumors examined were classified as GISTs, of which some had been diagnosed as LMSAs before the usage of immunohistochemistry (IHC) (1, 5). Among the three studies, only one identified the stomach as a tumor site, with 2 out of 28 GIST cases located in that region (1, 2, 5). In human GISTs, four histological patterns have been identified: spindle-shaped, epthelioid, myxoid, and fascicular. The spindle-shaped pattern is the most common, followed by the epithelioid type, with some tumors exhibiting a mixture of both (1). In canine GISTs, however, thus far only the two patterns spindloid/storiform and epithelioid have been described (1). In humans, GISTs have the highest prevalence of all mesenchymal gastrointestinal tumors and can be located throughout the whole gastrointestinal tract (3). There have also been reports of GISTs in other primates, guinea pigs as well as equids (3, 7, 8).

One study comparing biopsy and clinical characteristics of LMSAs and GISTs in dogs with their respective clinical outcome came to the conclusion that, while these two types of sarcomas share several characteristics in their biological behavior, anatomic location differed between the two and mitotic count was statistically higher in GISTs. In both tumor tyoes, the mitotic count and the completeness of surgical margins were shown to be of use as prognostic factors, as a higher mitotic count as well as incomplete surgical margins correlated positively with a shorter median survival time (MST). Furthermore, dogs with GISTs with weaker immunohistochemical staining of CD117 were found to have a shorter MST (2). In terms of biological behavior, GISTs demonstrate a wide spectrum ranging from indolent to aggressive forms, including metastatic potential (9). Thus, the metastatic rate ranges from 7.14% (two out of 28 cases) to 32.14% (nine out of 28 cases) (5, 9, 1, 3, 2). Nonetheless, the overall median survival time as well as the moderate incidence of metastasis does not differ statistically between GISTs and LMSAs (1, 2). Canine patients with gastrointestinal sarcomas had a median age of 10,6 to 11,2 years (1–3). They showed unspecific gastrointestinal clinical signs such as anorexia, lethargy, vomiting, weight loss, diarrhea and in some acute cases obstruction or perforation of the intestine. No predisposition in any breed or sex could be identified (6, 3).

1.2 Diagnosing NIMTs

Since a definitive differentiation of LMSAs and GISTs based solely on clinical signs or imaging modalities such as ultrasonography is not possible, a biopsy or surgical excision of the tumor

is required, followed by histopathological examination and immunohistochemical analysis for accurate classification (1). Histological differentiation of these tumors solely based on routine hematoxylin and eosin (HE) stain is challenging, which has led to an inaccurate understanding of NIMTs in older literature (1). The recent development of new immunohistochemical staining techniques has partially resulted in a reclassification of some NIMTs (2).

As mentioned above, immunohistochemistry plays a significant role in the differentiation of gastrointestinal sarcomas, with CD117 (c-Kit) being the most commonly used marker for GISTs. Smooth muscle actin (SMA) serves as a useful marker for identifying leiomyosarcomas (LMSAs) or leiomyomas. However, as approximately 20% to 30% of canine GISTs may also be SMA positive, it should not be used as the only IHC marker for tumor classification. The S100 protein as well as PGP 9.5, markers for cells of the nervous system, can aid in the identification of tumors of neuronal origin. While such tumors are relatively rare in the canine gastrointestinal tract, S100 staining can be valuable in cases where both SMA and c-Kit are negative, helping to guide differential diagnosis (1, 5). Recently, the protein DOG-1 (Discovered On GIST-1) has been recognized as a more sensitive IHC marker for GISTs. In human studies, DOG-1 was found to have a higher sensitivity than CD117 in GISTs located in the stomach, although CD117 was more sensitive in intestinal GISTs (10). Similar studies regarding the sensitivity of the IHC markers based on the anatomic location are currently lacking.

Following complete surgical excision of gastrointestinal sarcomas, most dogs generally exhibit a long overall median survival time, even without receiving additional therapy. Nonetheless, adjuvant treatment options are available. In cases of GISTs, where oncogenesis is driven by c-Kit mutations, tyrosine kinase inhibitors, such as toceranib, have demonstrated therapeutic benefit (11). LMSAs, on the other hand, have been treated using cytotoxic chemotherapeutic drugs including doxorubicin, cyclophosphamide, and vincristine, either individually or in combination protocols (6).

1.3 Prognostic factors in NIMTs

When comparing tumor characteristics with clinical outcome, previous studies evaluated various parameters as potential prognostic factors. These included patient-related parameters such as breed, sex and age, as well as tumor-related parameters, including diagnosis, size, location, mitotic count, necrosis and metastasis (2, 3, 12).

When quantifying mitotic figures in a tumor, mitotic count (MC) or mitotic index (MI) can be used. MC is a way of assessing cell proliferation by counting mitotic figures within a defined area. A correlation between MC and survival time was observed in 72 out of 109 studies on various tumor types (13). Alternatively, some studies employed the mitotic index, which is defined as the number of mitotic figures relative to the number of tumor cells, but this approach was only evaluated in few studies and did not always demonstrate a significant association with survival time (13).

An alternative for measuring cell proliferation is the immunohistochemical staining of Ki67. Ki67 is a protein expressed in the nucleus during all active phases of the cell cycle (G1, S, G2, and M phases), making it more abundantly detectable than mitotic figures, which are only present in the M phase (9). Ki67 is usually assessed by counting up to 500 or 1000 cells and calculating the percentage of cells stained positively (14). Thus far, this has mostly been done manually, although automatic image analyses using scanned histological slides are on the rise (14). While a correlation between Ki67 count and the presence of mitotic figures (MC, as well as MI) has been shown, several studies in dogs described a moderate or missing correlation between Ki67 count and MI for various tumor types (15–17, 9). Nonetheless, Ki67 has been reported to be associated with shorter survival periods and has even been described as being a better prognostic marker than MC in some tumor types (17, 14, 18). In NIMTs, Ki67 and MI correlated positively with survival, as both were higher in GISTs located in the small intestine than in the large intestine (9).

Another parameter commonly used for prognostication of veterinary tumors is necrosis (19). Necrosis is categorized either as present or absent, or divided into groups based on the percentage of necrotic tissue observed on the slide (2, 3). In NIMTs, necrosis has been reported to correlate positively with tumor size, but not median survival time (9).

The degree of differentiation provides insight into nuclear morphometry, i.e. the variability of size, shape and appearance of cells in a defined area, which is also often used when grading various tumors in veterinary medicine (20).

Lymphovascular invasion (LVI) is commonly noted in tumor descriptions, yet the specific histological criteria for identifying it remain poorly defined in veterinary pathology and has only been systematically analyzed in very few types of tumors in dogs and cats (21, 22).

Tumor size and the presence of metastases are used as parameters for staging GISTs in human patients (10). In canine gastrointestinal tumors, the completeness of surgical margins

has been identified as a significant prognostic factor for median survival time. In cases of GISTs, additional factors such as the intensity of CD117 immunostaining, Ki67 count and the mitotic index also play a crucial role (2).

1.4 Research Objective

This study aimed to investigate prognostic factors in canine NIMT biopsies, including mitotic count, nuclear pleomorphism, necrosis, lymphovascular invasion, Ki-67 positivity, and CD117 staining intensity, using Whole Slide Imaging. These histopathological features were then compared to corresponding clinical outcomes to assess their prognostic value. Additionally, the study sought to validate and replicate findings from previous research, contributing to a better understanding of diagnostic markers and their relevance in predicting disease progression.

2 Materials and Methods

2.1 Case selection

The diagnostic archive between 2007 to 2022 from InHisto Tierpathologie, Veterinary University of Vienna and The Schwarzman Animal Medical Center New York were searched for dogs with NIMTs. Inclusion criteria was any tumor type of this group (fibrosarcomas, fibromas, LMSAs, leiomyomas, GISTs, PNSTs), primary location within the stomach or intestine, canine patient, submission of a biopsy sample (with potential follow up of the patient) and availability of formalin-fixed paraffin blocks of the tumor. Thereby 38 cases were identified. Ethical approval was not required for this study, as all tissue samples were originally collected for routine diagnostic purposes. No additional procedures or interventions were performed on the animals specifically for the purposes of this research.

Clinical data was collected from internal medical records and, in the case of biopsies from InHisto Tierpathologie, via a survey sent to the corresponding clinics. The standardized survey and general communication with the referring clinics were sent via email. The survey contained the following information (see annex):

- Case number
- Name of the dog
- Name of the owner
- Breed
- Date of birth
- Sex
- Diagnosis of the tumor
- Age at diagnosis
- Location of the tumor
- Choice of therapy: date of tumor excision, further therapy
- Presence of metastases, their location and way of diagnosis
- Date of loss of follow-up or death
- Cause of death (spontaneous vs. euthanasia), suspected reason for death (tumor-related vs. other reason)

The same information was collected from internal records for biopsies from Veterinary University of Vienna and The Animal Medical Center. Age at loss of follow-up or death was calculated using these informations.

From these, 9 cases with a post-operative survival period of 3 days or less were excluded, as their survival time after the removal of the respective tumor was shortened due to peri- or post-operative complications, which does not necessarily reflect the tumor behavior. Due to lack of information on clinical outcome of the patients, another 11 cases had to be excluded, leaving 18 cases.

2.2 Histochemical and immunohistochemical staining

For each case, a single representative formalin-fixed paraffin-embedded tissue block of the tumor was selected from the archive. Firstly, the blocks were routinely sectioned and stained following the standard Hematoxylin and Eosin (HE) protocol.

After these 18 slides had been stained with HE, an immunohistochemical stain was done for c-Kit (CD117) and Ki-67. For this, 2 µm thin sections of formalin-fixed paraffin-embedded (FFPE) tissue were cut with a microtome, mounted on a coated slide (Superfrost™Plus Adhesion Microscope Slides, Epredia, USA) and dried for 30 minutes at 60°C in a heating cabinet to prevent detachment from the slide. After deparaffinization (2x 5min NeoClear) and rehydration (2x 5min 100% alcohol, 1x 5min 96% alcohol, 1x 5min 70% alcohol and 2x 5min aqua distillata), the antigen of interest was unmasked by heating in the PT Module Tank (97°C for 20min) in citrate buffer pH6 for Ki67 and EDTA buffer pH8 for CD117. Automated immunohistochemistry was performed using the Lab Vision Autostainer 360 (Thermo Fisher Scientific Inc., Waltham, MA, USA). Endogenous peroxidase activity was blocked by incubating (5min.) the sections in a 3% hydrogen peroxide solution. Interfering background staining was reduced by using a 1.5% goat serum (10min), which prevents the non-specific binding of antibodies to various tissue components. An antibody specific to the epitope of interest was then applied (30min), these being Monoclonal Mouse Anti-Human Ki-67 Antigen ((Immunologic, Duiven, Netherlands) for Ki67 and Polyclonal Rabbit Anti-Human CD117 (Immunologic, Duiven, Netherlands) for CD117.

After application of the primary antibody, the BrightVision reagent was used to detect a specific mouse IgG or rabbit IgG antibody. For Ki67 the corresponding reagent was BrightVision Poly-HRP-Anti-mouse IgG (Immunologic, Duiven, Netherlands) and for CD117 BrightVision Poly-HRP-Anti-rabbit IgG (Immunologic, Duiven, Netherlands). The specific primary antibody was localized by an enzyme-labelled polymer conjugated secondary antibody (30min), which recognizes mouse or rabbit immunoglobulins. The enzyme with which the secondary antibody was labelled was horseradish peroxidase (HRP). The resulting complex was visualized by

adding a chromogen (DAB, 8min). For orientation and contrast, counterstaining with hematoxylin (1min) was performed. Finally, the sections were dehydrated (2x 5min aqua distillata, 1x 5min 70% alcohol, 1x 5min 96% alcohol, 2x 5min 100% alcohol and 2x 5min NeoClear) and mounted (NeoMount).

After staining, the slides were scanned using the digital slide scanner Pannoramic Scan (3DHISTECH Ltd., Budapest, Hungary) with the scan settings 40x magnification and resolution of 0.25 µm/pixel in single focal plane (22).

2.3 Collection of histopathological data and immunohistochemical analysis

The digital slides were viewed and analysed using the software Sliderunner (Version 2.0.0) (23). The included cases were evaluated on following parameters by the author and reviewed by an experienced pathologist:

Tumor diagnosis: Biopsies staining positively for CD117 were diagnosed as GISTs. Tissue staining negatively for CD117 was diagnosed as a LMSA, when infiltrative growth was present or a leiomyoma, when not.

Mitotic Count: Firstly, the whole tumor on the slide was searched for an area with the presumed highest density of mitotic figures. This region was selected as a rectangular (3:4) region with the size of 2,37mm^2, the equivalent of 10 standard-sized high-power fields (HPFs), as previously described by Bertram et al. (24). If no mitotic figures could be found, a random area of the same size was chosen (25). In this region all mitotic figures, as described by Donovan et al., were annotated (26).

Nuclear Pleormorphism: Secondly, for each case, at least 100 neoplastic nuclei were annotated by deliniating their borders within a randomly chosen rectangular area of ¼HPF to ½HPF within the tumor, depending on the cell density. Then, for each case, the mean nuclear size and standard deviation of the mean size were calculated, as done in the study by Haghofer et al. (27).

Necrosis: Necrotic areas on the slide were outlined and their combined area measured. Afterwards, the percentage of necrosis, when compared to the measured area of the tumor included in the biopsy, was calculated (19).

Lymphovascular invasion: Then, the whole slide images were analyzed at medium magnification, looking for LVI in the tumor as well as the surrounding tissue. LVI, if present,

were marked as either “LVI” or “possible LVI”, depending on whether only the strict or soft criteria, according to Moore et al., were fulfilled (21).

Ki67 count: In the immunohistochemical stained slides a random rectangular area of 1HPF (0,237mm^2) was chosen, in which all Ki67 positive cells were counted. In two cases, the immunohistochemical sections could not be produced as the tissue was washed away despite several attempts, which is why Ki67 staining, wherefor count was not possible.

CD117: In case of positive staining, the intensity was defined as weak, moderate or strong, leaning on the paper by Alcazar et al (2). Moreover, the staining pattern was described as either patchy or diffuse.

2.4 Statistical analysis

Tumor-related death (TRD) was defined as either spontaneous death or euthanasia because of the tumor. For better comparison of the histopathological parameters in tumors with and without TRD, they were visualized using scatterplots. Based on these, the lowest value of all cases with TRD was chosen as the prognostic threshold for each parameter. Using these thresholds, sensitivity, specificity and precision were calculated. While sensitivity measures how effectively a test identifies true positives, specificity shows how accurately it identifies true negatives. Precision is the percentage of true positives among all positive results, showing how reliable a positive test outcome is (28). Afterwards, using IBM SPSS Statistics (Version 30.0.0.0 (172); IBM Corporation, Armonk, NY, USA), sensitivity and specificity were visualized using ROC-curves and calculating the area under the ROC-curve (AUC). The AUC ranges from 0, indicating an inverse prognostic value, to 1, indicating perfect prognostic accuracy, with a value of 0.5 representing no predictive ability beyond random chance.

Moreover, a Kaplan-Meier analysis was done for each parameter, comparing the survival time between cases with values above and cases with values below the chosen thresholds. All cases, in which the dogs did not die because of the tumor, were censored. Finally, the hazard ratio was calculated using z-standardization for a better comparison between the parameters.

3 Results

3.1 Clinical data

Of the 18 cases, there were four dogs of a mixed breed, two beagles, two labradors, and one case each of American Water spaniel, Bernese Mountain Dog, Chihuahua, German Shorthaired Pointer, Golden Retriever, Magyar Viszla, Pitbull, Schnauzer, Miniature Schnauzer and Shar-Pei. Ten dogs were female, eight of these spayed, and of the eight male dogs three were castrated. The age of the animals at the time of the surgical excision of the tumor ranged from 6 years 1 month to 15 years 7 months, with a median age of 9 years.

Originally, only one tumor had been diagnosed as a GIST, but after re-evaluation using CD117 IHC, seven further cases were diagnosed as GISTs. These biopsies had never been stained using CD117 before and their former diagnoses had been leiomyosarcoma (LMSA) (n = 4) and in one case each leiomyoma, fibroma and soft tissue tumor. Seven cases were diagnosed as leiomyosarcomas and three cases as leiomyomas. There was no tumor with histological criteria suggestive of neurogenic origin.

The most common tumor locations were the stomach (n = 4), with two cases each being diagnoses as GISTs and LMSAs, followed by the rectum (n = 4), with one diagnosis of GIST and three LMSAs. The three tumors found in the cecum were diagnosed as GISTs. Added to that, the location of one GIST was described as the mesentery and not further specified. The three leiomyomas were located in the duodenum (n = 2) and the colon (n = 1). Of the 15 cases, in which diagnostic imaging was used, two dogs were suspected to have metastases in the liver and the abdomen (not further specified), based upon ultrasonographical examination. No additional diagnostics were performed on these suspected metastases, as the dogs were euthanized.

Five of the six dogs with a known death date were euthanized. Of these deaths the abovementioned two cases with suspected metastases were tumor-related, the other four dogs were euthanized or died due to unrelated reasons, these being orthopedic problems (n = 2), chronic pancreatitis (n = 1) and suspected intoxication (n = 1). The remaining twelve dogs were lost to follow-up. The time from surgical excision to loss of follow-up ranged from 1 week to 5 years 1 month, with a median of 10 months. The two cases with TRD survived for 10 months 2 weeks and 9 months 3 weeks (median 10 months). The median survival time for dogs diagnosed with GISTs was 313 days and for dogs with LMSAs 427 days.

3.2 Histopathological data

The overall median of MC was 0. When only including the eight cases with a MC of more than 0 in the calculation, it ranged from 1 to 14 with a median of 2.5. The mean size of the neoplastic nuclei per case ranged from 12.21 to 65.65μm^2 (median: 34.85μm^2) and the standard deviation was between 4.58μm^2 and 27.62μm^2 (median: 12.91μm^2). Ten cases had necrotic areas of up to 45.60% and no lymphovascular invasion could be seen in any of the slides. The eight cases, which were stained positive for CD117, were all ranked as having a strong intensity, with the staining pattern differing between cases, being diffuse (n = 4) or patchy (n = 4). The Ki67 count ranged from 0 to 158, with a median of 38.

Figure 1 displays scatterplots comparing the values between cases with TRD in less than one year (group 1) and cases with loss of follow-up or death due to other causes of more than one year (group 2), along with their respective diagnoses. As can be seen, MC was higher in GISTs than in LMSAs and leiomyomas. Moreover, mean nuclear size as well as standard deviation for nuclear size was the highest in cases of LMSA, while necrosis was the highest in cases diagnosed as leiomyomas.

The beforementioned thresholds, as well as the calculated sensitivity, specificity and precision can be found in Table 1. Because the lowest value for necrosis in group 1 was 0%, the next smallest value was chosen, which was 4%. Due to the way these limits were chosen, sensitivity is 100% for all parameters except necrosis. The variable with the highest specificity was MC, with a specificity of 88% and a sensitivity of 100%.

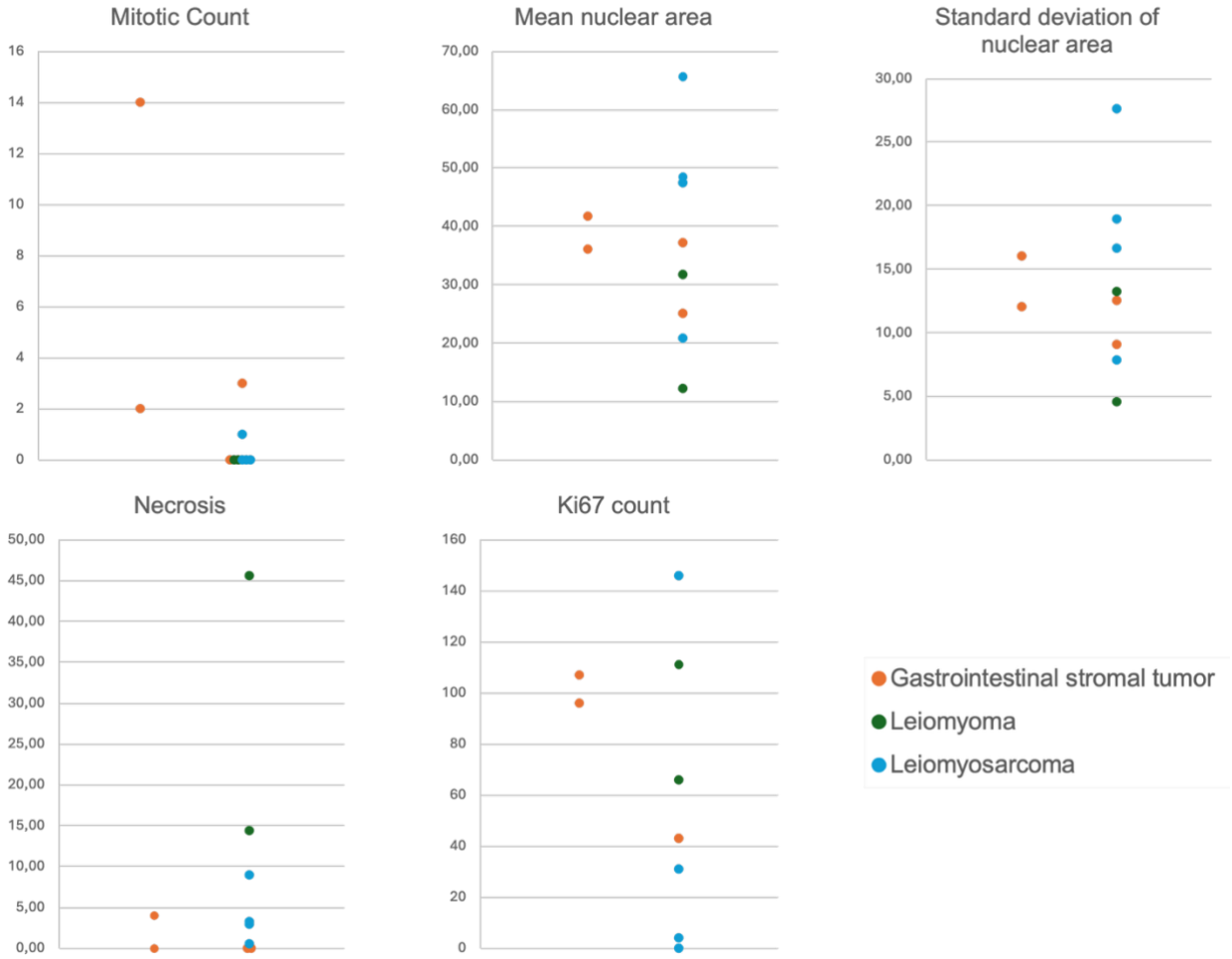

Fig. 1. Scatterplots showing the values of each parameter for each case. The dots in the left collums represent the cases with TRD, the right collum the remaining cases.

Parameter	Threshold value	Se	Sp	Pre
Mitotic count	≥ 2	1.00	0.88	0.67
Mean nuclear area	≥ 36	1.00	0.50	0.33
Standard deviation nuclear area	≥ 12	1.00	0.38	0.29
Necrosis	≥ 4%	0.50	0.75	0.25
Ki67	≥ 95	1.00	0.71	0.50

Tbl. 1. Histopathologic parameters with their corresponding thresholds, sensitivity (Se), specificity (Sp) and precision (Pre) values.

Figure 2 and Table 2 show the ROC-curves and corresponding AUC values. Among the parameters evaluated, only the AUC for MC demonstrated statistical significance, with a value of 93.8% (95% CI: 77.3% to 110.2%) and a p-value of < 0.001. Due to technical failure that prevented successful IHC staining of Ki67 in two of the included cases, its ROC curve is displayed separately.

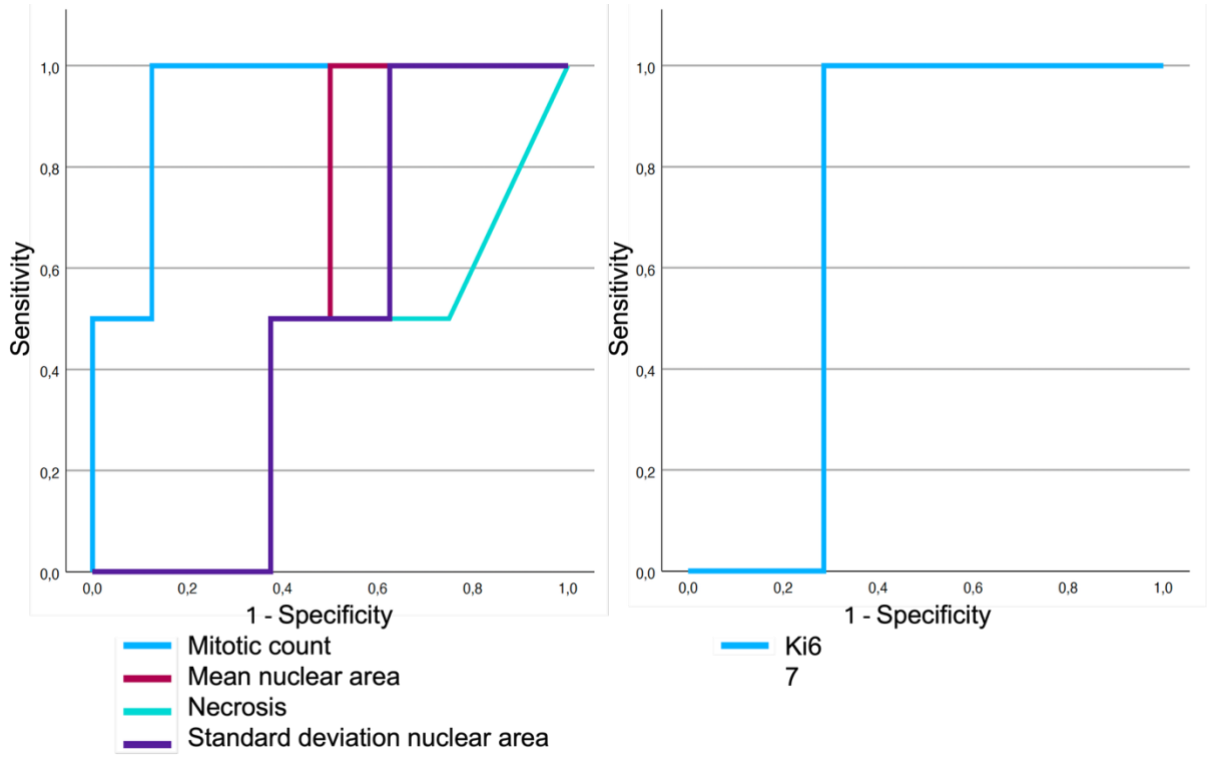

Fig. 2. ROC-curves for each metric parameter. The corresponding AUC values are presented in Table 3. Due to technical limitations that prevented successful IHC staining of Ki67 in one of the included cases, its ROC curve is displayed separately.

Parameter	AUC	95% CI	p-value
Mitotic count	0.938	0.773-1.102	< 0.001
Mean nuclear area	0.563	0.220-0.905	0.72
Standard deviation of nuclear area	0.500	0.138-0.862	1.00
Necrosis	0.375	-0.060-0.810	0.57
Ki67 count	0.714	0.380-1.049	0.21

Tbl. 2. AUC values, the corresponding confidence intervals and their *p*-values.

The hazard ratios are provided in Table 3. Although none reached statistical significance, the highest hazard ratio was also observed for the parameter MC with 1,898 (p = 0,09).

Figure 3 depicts the Kaplan-Meier curves for the respective parameters, with all deaths not related to the tumors and cases, which were lost to follow-up, censored. Given the definition of the thresholds (except for necrosis), it is expected that the curves representing cases exceeding the threshold, of which two had TRD, lie below those corresponding to cases with values above the selected limit, of which none died related to the tumor.

Parameter	**Hazard ratio**	**95% CI**	**p-value**
Mitotic count	1.898	0.911-3.955	0.09
Mean nuclear area	1.255	0.362-4.350	0.72
Standard deviation nuclear area	1.119	0.336-3.728	0.86
Necrosis	0.204	0.002-18.973	0.49
Ki67 count	1.593	0.404-6.277	0.51

Tbl. 3. Calculated hazard ratios, confidence intervalls and p-values for each parameter.

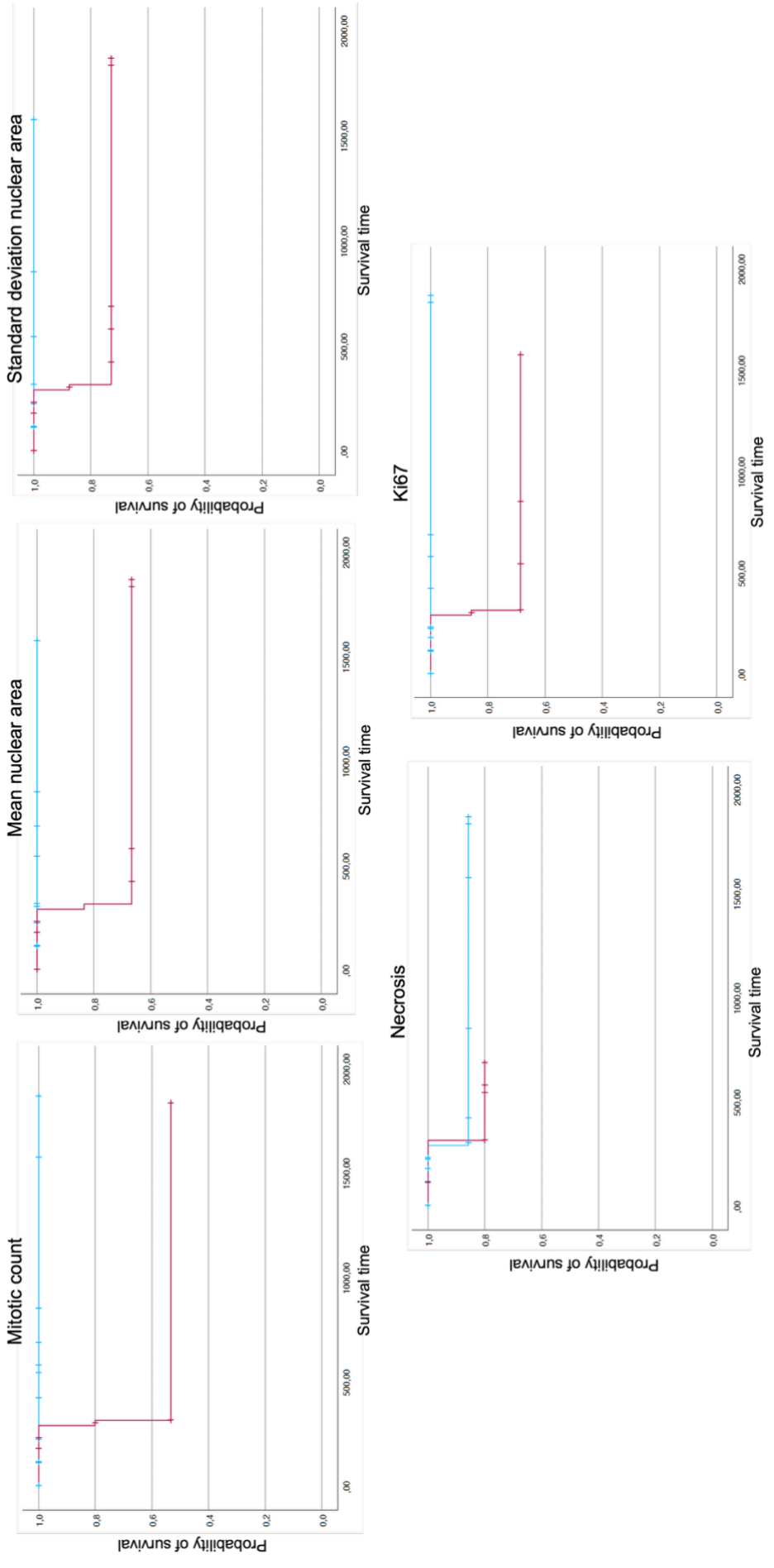

Fig. 3. Kaplan Meier curves for each metric parameter, survival time is shown in days. Cases with values above chosen thresholds in blue, cases with values below chosen thresholds in red. Censored cases (lost to follow-up or tumor-unrelated death) are represented as tick marks.

4 Discussion

The objective of this study was to evaluate selected histopathological parameters in canine gastrointestinal mesenchymal tumors in relation to clinical outcome. The parameters assessed included MC, nuclear pleomorphism (as determined by nuclear morphometry), necrosis, lymphovascular invasion, Ki67 count and the intensity of CD117 immunohistochemical staining. Although the sample size in this study was limited, which likely contributed to the lack of statistical significance in many analyses, some findings consistent with previous studies could nonetheless be reproduced.

Among the various findings, the reclassification of seven tumors from LMSAs to GISTs stood out the most as it underlines the importance of the usage of IHC when diagnosing gastrointestinal mesenchymal tumors. While Del Alcazar et al. reported a reclassification from LMSAs to GISTs of 6,4% (3 out of 47 cases), the percentage was much higher in this study with 38,8% (7 out of 18 cases) (2). The main reason for this difference is probably the fact that IHC was not performed routinely in all cases of gastrointestinal sarcomas, as the high prevalence of GISTs in dogs was not as known at the time of diagnosis. In addition to CD117, which is commonly used to distinguish between GISTs and other mesenchymal tumors, other markers have been proposed as valuable diagnostic tools, such as smooth muscle actin (SMA) and DOG1, which has been found to be more sensitive, as it is also positive in some GISTs, which are not CD117-positive (29, 30). Added to that, a moderate or strong intensity of CD117 staining had previously been reported to correlate with a median survival time of 1418 days (2). This could not be reproduced as all eight cases of GISTs were stained with a strong intensity and only two of these survived more than 1418 days (1579 days and 1838 days), while the remaining dogs survived or were not lost to follow-up for a maximum of 321 days.

(11)(11)This differentiation between GISTs and other gastrointestinal mesenchymal tumors is especially of importance when chemotherapy is considered as an additional treatment to surgical removal. As mentioned above, GISTs express c-Kit, a tyrosine kinase receptor, which is why tyrosine kinase inhibitors have become the standard treatment option for GISTs in humans . Similarly, toceranib phosphate (Palladia®), which functions as a tyrosine kinase inhibitor and is used in the treatment of dogs, has been shown to lead to clinical benefit in canine GISTs (11). The only dog included in this study which received chemotherapy had been initially diagnosed as a LMSA and was treated with Lomustine, a cytostatic drug most commonly used to treat tumors of the central nervous system or lymphomas (31). In this study,

this particular tumor stained positive for CD117, leading to a reclassification from LMSA to GIST. This diagnosis was made prior to the routine use of CD117 and before the true prevalence of canine GISTs was well established, highlighting the critical role of immunohistochemistry in guiding treatment decisions.

Another finding consistent with existing literature is that the MC was higher in GISTs than LMSAs. A higher MC has also been reported to correlate with a higher risk for metastasis and a shorter median survival time. One study found a significant association between a MC of 9 or above and an increased risk of metastasis. This number stems from a grading system of soft tissue sarcomas in dogs and was not specifically adapted to GIST (2, 32). While there was only one case with a MC above 9 in this study, it was one of the two cases in which metastasis was suspected during ultrasonographical examination. While using a threshold of ≥ 2 or above for the MC as a criteria for tumor-related death in less than one year had a calculated sensitivity of 100% and a specificity of 88% in this study. As mentioned above, although none of the calculated hazard ratios were statistically significant, the value for MC was 1.898 ($p = 0.09$), which suggests a higher MC in cases with tumor-related death.

An additional parameter for quantifying cell proliferation in this study was Ki67 staining, which appeared to show only a weak correlation with the respective MC. This might be due to Ki67 being expressed in more phases of the cell cycle and the area chosen for analysis, which was not the same for Ki67 and MC.

Besides MC, a further factor included in the beforementioned grading system for soft-tissue sarcomas is necrosis, which has been found to correlate with mean survival time and tumor metastasis in soft tissue sarcomas (19). In this study, the cases with the most amount of necrosis were diagnosed as leiomyomas, which might seem counterintuitive. However, when diagnosing soft-tissue sarcomas, the degree of differentiation as well as the presence of mitotic figures are also taken into account besides the presence of necrosis. In this study, the diagnosis leiomyoma was made, when the biopsies stained negative for CD117 and no invasive growth could be seen. Moreover, the missing correlation between the amount of necrosis and clinical outcome has been reported in a previous study, for both GISTs and gastrointestinal LMSAs (2).

Previous studies reported that all tumors located in the cecum were (re-) diagnosed as GISTs, with one study suggesting that many tumors which have been classified as LMSA might have been GISTs and proposing GISTs as the most common tumor of the cecum (5, 1). All cecal

tumors in this study were also found to be GISTs, two of which had been diagnosed as LMSAs before being immunohistochemically stained negative for CD117.

The primary limiting factor of this study was the small sample size, which could be the reason for many of the evaluated prognostic parameters being statistically insignificant. As previously mentioned, nine cases were excluded due to perioperative or postoperative death, and eleven were omitted due to incomplete clinical data. Although the latter cases may have contributed valuable information to certain analyses, including them selectively would have made it difficult to maintain a clear overview across statistical evaluations. Added to that, information on the completeness on surgical excision as well as a more definitive diagnosis on the suspected metastases could have benefitted interpretation of the study results, had they been available. Furthermore, it remains uncertain whether the patients would have died as a direct result of the tumor had they not been euthanized following the detection of these suspected metastases. Moreover, 12 out of 18 dogs (66,7%) were lost to follow-up, as most of the surgical excisions were done by large clinics instead of the primary care veterinarian and the majority of patients did not return to the clinic after the wound of the incision had healed, wherefore the overall median survival time cannot be calculated more precisely. Additionally, the limited number of cases included in this study is not representative of a larger population. Given that only two dogs experienced tumor-related death, the thresholds applied in the statistical analysis were appropriate for the available data but may not be generalizable to a larger population.

Finally, given that this was a retrospective study and the data were obtained from tissue biopsies, which only represent a part of the entire tumor, it is possible that the sampled tissue may not accurately reflect the overall tumor characteristics. Added to that, there might have been cases with undetected metastases, as no extensive screening was done on all dogs and no necropsies were included in this study.

Despite these limitations, this thesis reinforces the findings of previous research, particularly highlighting the critical role of immunohistochemical staining and the prognostic significance of MC. While recent advancements have been made in the understanding of intestinal mesenchymal tumors, the classification and differential diagnosis of gastrointestinal sarcomas continue to remain a subject of ongoing research, particularly due to their implications for the selection of treatment.

5 References

1. Hayes S, Yuzbasiyan-Gurkan V, Gregory-Bryson E, Kiupel M. Classification of canine nonangiogenic, nonlymphogenic, gastrointestinal sarcomas based on microscopic, immunohistochemical, and molecular characteristics. Vet Pathol 2013; 50(5):779–88.

2. Del Alcazar CM, Mahoney JA, Dittrich K, Stefanovski D, Church ME. Outcome, prognostic factors and histological characterization of canine gastrointestinal sarcomas. Vet Comp Oncol 2021; 19(3):578–86.

3. D. Frost, J. Lasota, and M. Miettinen. Gastrointestinal Stromal Tumors and Leiomyomas in the Dog: A Histopathologic, Immunohistochemical, and Molecular Genetic Study of 50 Cases.

4. Avallone G, Pellegrino V, Muscatello LV, Roccabianca P, Castellani G, Sala C et al. Canine smooth muscle tumors: A clinicopathological study. Vet Pathol 2022; 59(2):244–55.

5. Russell KN, Mehler SJ, Skorupski KA, Baez JL, Shofer FS, Goldschmidt MH. Clinical and immunohistochemical differentiation of gastrointestinal stromal tumors from leiomyosarcomas in dogs: 42 cases (1990-2003). J Am Vet Med Assoc 2007; 230(9):1329–33.

6. Cohen M, Post GS, Wright JC. Gastrointestinal Leiomyosarcoma in 14 Dogs. Veterinary Internal Medicne 2003; 17(1):107–10.

7. Bertram CA, Donovan TA, Bertram B, Sabara J, Klopfleisch R. Neoplasia in pet guinea pigs: a retrospective analysis of 2,474 autopsy examinations. J Vet Diagn Invest 2025; 37(1):94–103.

8. Del Piero F, Summers BA, Cummings JF, Mandelli G, Blomme EA. Gastrointestinal stromal tumors in equids. Vet Pathol 2001; 38(6):689–97.

9. Gillespie V, Baer K, Farrelly J, Craft D, Luong R. Canine gastrointestinal stromal tumors: immunohistochemical expression of CD34 and examination of prognostic indicators including proliferation markers Ki67 and AgNOR. Vet Pathol 2011; 48(1):283–91.

10. Parab TM, DeRogatis MJ, Boaz AM, Grasso SA, Issack PS, Duarte DA et al. Gastrointestinal stromal tumors: a comprehensive review. J Gastrointest Oncol 2019; 10(1):144–54.

11. Berger EP, Johannes CM, Jergens AE, Allenspach K, Powers BE, Du Y et al. Retrospective evaluation of toceranib phosphate (Palladia®) use in the treatment of gastrointestinal stromal tumors of dogs. J Vet Intern Med 2018; 32(6):2045–53.

12. Bettini G, Morini M, Marcato PS. Gastrointestinal spindle cell tumours of the dog: histological and immunohistochemical study. J Comp Pathol 2003; 129(4):283–93.

13. Bertram CA, Donovan TA, Bartel A. Mitotic activity: A systematic literature review of the assessment methodology and prognostic value in canine tumors. Vet Pathol 2024; 61(5):752–64.

14. Bacci B, Brunetti B, Avallone G, Morisi A, Martinoli G, Bacon NJ. Proliferation scores in canine anal sac adenocarcinomas: Ki67 global score is superior to Ki67 hotspot indices and mitotic count for prognosis. Vet Pathol 2025:3009858251338855.

15. van Lelyveld S, Warland J, Miller R, Maw H, Foale R, Goodfellow M 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):312–9.

16. Griffey, S. M., Kraegel, S.A., Madewell, B.R. Proliferation Indices in Spontaneous Canine \lung Cancer: Proliferating Cell Nuclear Antigen (PCNA), Ki-67 (MIB1) and Mitotic Counts.

17. Sierra Matiz OR, Santilli J, Anai LA, Da Silva MCL, Sueiro FA, Sequeira JL et al. Prognostic significance of Ki67 and its correlation with mitotic index in dogs with diffuse large B-cell lymphoma treated with 19-week CHOP-based protocol. J Vet Diagn Invest 2017; 30(2):263–7.

18. Maglennon GA, Murphy S, Adams V, Miller J, Smith K, Blunden A et al. Association of Ki67 index with prognosis for intermediate-grade canine cutaneous mast cell tumours. Vet Comp Oncol 2008; 6(4):268–74.

19. Corbin EM et al. Tumor Necrosis Guideline, version 2.0: Veterinary Cancer Guidelines and Protocols [cited 2025 May 2]. Available from: URL: http://vetcancerprotocols.org.

20. Avallone G, Rasotto R, Chambers JK, Miller AD, Behling-Kelly E, Monti P et al. Review of Histological Grading Systems in Veterinary Medicine. Vet Pathol 2021; 58(5):809–28.

21. Moore FM et al. Lymphovascular Invasion Guideline, version 1.0: Veterinary Cancer Guidelines and Protocolsv [cited 2025 May 2]. Available from: URL: http://vetcancerprotocols.org.

22. Ehrensing G, Craig LE. Intravascular neoplastic cells in canine cutaneous plasmacytomas. J Vet Diagn Invest 2018; 30(2):329–32.

23. Aubreville M, Bertram C, Klopfleisch R, Maier A. SlideRunner - A Tool for Massive Cell Annotations in Whole Slide Images 2018:309–14. Available from: URL: http://arxiv.org/pdf/1802.02347v1.

24. Bertram CA, Aubreville M, Donovan TA, Bartel A, Wilm F, Marzahl C et al. Computer-assisted mitotic count using a deep learning-based algorithm improves interobserver reproducibility and accuracy. Vet Pathol 2022; 59(2):211–26.

25. Meuten DJea. Mitotic Count Guideline, version 1.0: Veterinary Cancer Guidelines and Protocols [cited 2025 May 2]. Available from: URL: http://vetcancerprotocols.org.

26. Donovan TA, Moore FM, Bertram CA, Luong R, Bolfa P, Klopfleisch R et al. Mitotic Figures-Normal, Atypical, and Imposters: A Guide to Identification. Vet Pathol 2021; 58(2):243–57.

27. Haghofer A, Parlak E, Bartel A, Donovan TA, Assenmacher C-A, Bolfa P et al. Nuclear pleomorphism in canine cutaneous mast cell tumors: Comparison of reproducibility and

prognostic relevance between estimates, manual morphometry, and algorithmic morphometry. Vet Pathol 2025; 62(2):161–77.

28. Parikh R, Mathai A, Parikh S, Chandra Sekhar G, Thomas R. Understanding and using sensitivity, specificity and predictive values. Indian J Ophthalmol 2008; 56(1):45.

29. Dailey DD, Ehrhart EJ, Duval DL, Bass T, Powers BE. DOG1 is a sensitive and specific immunohistochemical marker for diagnosis of canine gastrointestinal stromal tumors. J Vet Diagn Invest 2015; 27(3):268–77.

30. Miettinen M, Wang Z-F, Lasota J. DOG1 antibody in the differential diagnosis of gastrointestinal stromal tumors: a study of 1840 cases. Am J Surg Pathol 2009; 33(9):1401–8.

31. Brooks W. Lomustine [cited 2025 Jun 23]. Available from: URL: https://veterinarypartner.vin.com/default.aspx?pId=19239&catId=102896&id=8186473&ind=1611&objTypeID=10.

32. Kuntz CA, Dernell WS, Powers BE, Devitt C, Straw RC, Withrow SJ. Prognostic factors for surgical treatment of soft-tissue sarcomas in dogs: 75 cases (1986-1996). J Am Vet Med Assoc 1997; 211(9):1147–51.

6 Annex

The following pages depict the survey, which was sent to the corresponding clinics and was used to collect clinical data on the canine patients included in this study.

Praxis für Tierpathologie GesbR
Dr. Starzerstr. 15, 2100 Korneuburg

vetmeduni vienna

Fallnummer:

interne Fallnummer:

Tiername:

Besitzername:

Rasse:

Geburtsdatum: (TT/MM/JJJJ)

Geschlecht: bitte auswählen

Pathologische Diagnose:

Alter zum Diagnosezeitpunkt: (in Jahren & Monaten)

Lokalisation des Tumors:
- ◯ Magen
- ◯ Dünndarm
  - ☐ Duodenum
  - ☐ Jejunum
  - ☐ Ileum
- ◯ Dickdarm
  - ☐ Caecum
  - ☐ Colon
  - ☐ Rectum

Therapie:
- ☐ initiale Chirurgie am: (TT/MM/JJJJ)
- ☐ Weitere Therapien (erneute Chirurgie, Chemotherapie, etc.)

Waren Metastasen des beschriebenen Tumors vorhanden?
- ◯ Nein
- ◯ Verdächtig/ Ja,

und zwar an folgender Lokalisation/ folgenden Lokalisationen:

Praxis für Tierpathologie GesbR
Dr. Starzerstr. 15, 2100 Korneuburg

vetmeduni vienna

Die Diagnostik der Metastasen erfolgte mittels:
- ☐ Bildgebung
- ☐ Zytologie
- ☐ Histologie
- ☐ Anderes: _____

Das Tier ist ...
- ○ lebendig zum letzten Untersuchungszeitpunkt am ...
- ○ verstorben am ... _____ (TT/MM/JJJJ)

Bitte nur im Todesfall ausfüllen:

Das Tier ist ...
- ○ spontan verstorben
- ○ euthanasiert worden

Die Todesursache war ...
- ◉ Tumor-assoziiert, nämlich:
- ○ eine andere Ursache, nämlich: _____