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PII: S0034-5288(22)00166-7

DOI: https://doi.org/10.1016/j.rvsc.2022.05.008

Reference: YRVSC 4634

To appear in: *Research in Veterinary Science*

Received date: 25 October 2021

Revised date: 20 April 2022

Accepted date: 2 May 2022

Please cite this article as: S. De Vos, K. Demeyere, H. De Cock, et al., Comparison of serum tryptase as a diagnostic oncological marker in canine versus human mast cell neoplasms, *Research in Veterinary Science* (2022), https://doi.org/10.1016/j.rvsc.2022.05.008

Comparison of serum tryptase as a diagnostic oncological marker in canine versus human mast cell neoplasms

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Abstract

Canine mast cell tumors (MCTs) are a promising translational model for human mast cell neoplasms with striking similarities such as the Darier's sign and mutations in the KIT gene. Whereas mast cell neoplasms are rare in humans, MCTs are the most frequent malignant neoplasms of the skin in dogs. In human systemic mastocytosis, serum tryptase is an important diagnostic criterion. Surprisingly, serum tryptase levels were not yet investigated in dogs with MCTs. Therefore, the aim of this study was to investigate whether serum tryptase levels in dogs with cutaneous MCTs were elevated compared to those of a non-MCT control group. As a secondary aim, it was investigated whether surgical manipulation caused an increase in serum tryptase in canine MCT patients. A total of 48 serum samples were collected from dogs with different grades of cutaneous MCTs (n=24) and non-MCT controls (n=24). In dogs with cutaneous MCTs, blood was collected prior to and within 1 hour after surgery. Serum tryptase levels were measured using a commercially available canine-specific ELISA kit. No significant difference in serum tryptase levels was found between cutaneous MCT patients and non-MCT controls, nor in these levels before versus after surgery. Our findings in canine cutaneous MCTs are in accordance with human cutaneous mastocytosis, where serum tryptase levels tend to remain within the normal range. However, despite various similarities between aggressive mast cell tumors in dogs and humans, serum tryptase cannot be considered a diagnostic biomarker in dogs with cutaneous MCTs as part of a comparative oncologic strategy.

Keywords: comparative oncology; oncological marker; mastocytosis; canine mast cell tumor; serum tryptase

Introduction

In human and veterinary immunology, mast cells (MCs) play an important role in processes such as IgE-mediated (type I) hypersensitivity reactions and immune responses related to helminth infections (Finkelman et al., 2004; Moon et al., 2014; Galli et al., 2020; Tontini et al., 2021). Mast cells are also important in oncology and mast cell neoplasms occur in both human and canine patients (Rowell et al., 2011; Sultan et al. 2018). Dogs with mast cell tumors (MCTs) may serve as a model for comparative oncology due to the high incidence, as well as similarity to human genes, environmental factors, and treatment response (Rowell et al., 2011; Ranieri et al., 2015; Sultan et al., 2018; Willmann et al., 2019).

Human mastocytosis is a rare neoplastic condition with heterogenous clinical manifestations, including systemic mastocytosis (SM), cutaneous mastocytosis (CM) and mast cell sarcoma (Vaes et al., 2017; Valent et al., 2017; Wagner et al., 2018). Lesions may be highly aggressive or regress spontaneously (Valent et al., 2017; Wagner et al., 2018; Wilcock et al., 2019). In dogs, cutaneous MCTs are the most frequently diagnosed malignant skin tumors (London et al., 2003). Clinical behavior varies from low grade with excellent prognosis to very aggressive malignancies with very poor outcome (Patnaik et al., 1984; Kiupel et al., 2011). Histopathological grading is based on the Patnaik grading system that divides cutaneous MCT in three grades (Patnaik et al., 1984) or the more recent two-tier Kiupel grading system (Kiupel et al., 2011). High-grade cutaneous MCTs have a malignant behavior and may metastasize through the bloodstream and lymph nodes to internal organs such as liver and spleen (O'Keefe et al., 1987). In both species the Darier's sign is pathognomonic for MC disorders and is characterized by localized swelling, erythema, and wheal formation, caused by MC degranulation as a response to manipulation (Tams et al., 1981; Schwartz et al., 1987; London et al., 2003; Ranieri et al., 2015; Matito et al., 2018; Sultan et al., 2018). Likewise, mutations in the KIT gene, triggering uncontrolled proliferation of MCs, have been discovered in some cases of human (Longley et al., 1996; Longley et al., 1999) and canine (London et al., 1999; Mochizuki et al., 1999; Zemke et al., 2002; Webster et al., 2006; Takeuchi et al., 2013; Thompson et al., 2016; Horta et al., 2018; Thamm et al., 2019; Vozdova et al., 2020) MC neoplasms.

In humans, the serum tryptase level serves as a very important hematological marker in the diagnosis of MC neoplasms; over 90% of the patients with SM have levels above 20 ng/mL compared to a basal level of <15 ng/mL in normal individuals (Schwartz et al.,

1994; Horny et al., 2008; Valent et al., 2014; Vaes et al., 2017; Valent et al., 2017; Wilcock et al., 2019). Whereas patients with CM may have a normal basal serum tryptase level, elevation of serum tryptase serves as an important marker in SM (Schwartz et al., 1994; Horny et al., 2008; Valent et al., 2014; Vaes et al., 2017; Valent et al., 2017; Wilcock et al., 2019). So far, serum tryptase levels in dogs have not yet been described. In healthy canine tissues, tryptase has been investigated by ELISA and high levels per mg wet weight were measured in the intestine (Myles et al., 1995). In addition, immunohistochemistry (IHC) of tryptase revealed protein expression in canine cutaneous MCTs (Ozaki et al., 2002; Kiupel et al., 2004; Fernandez et al., 2005; Mederle et al., 2010; Pazdzior-Czapula et al., 2019). Only one study investigated the link between the IHC tryptase staining pattern and the survival time of the dogs with MCT did not find any association based on this qualitative analytical approach (Kiupel et al., 2004).

In the current study, we mainly assessed whether serum tryptase levels may also serve as a hematological biomarker in the diagnosis and prognostication in dogs with cutaneous MCTs. Additionally, these levels were compared before and after surgical excision of the MCT to investigate the effect of tumor manipulation.

Materials and methods

Dogs included in this study

A total of 48 dogs (24 dogs with cutaneous MCTs and an equal number of breed- and age-matched controls) were enrolled after an ethical committee approval and informed consent of the owners (EC2018/57 and EC2019/20). All dogs with cutaneous MCTs were presented for surgical excision of the tumor. Pre-operative screening for metastatic disease was carried out in 20 out of 24 dogs (83%) using abdominal ultrasound and FNA of liver and spleen. Two dogs with cutaneous MCTs received antihistaminic drugs. One dog received diphenhydramine hydrochloride (DPH) (Nustasium®, Vemedia Manufacturing B.V.) orally at a dose of 3 mg/kg 2 hours before surgery. Another dog received chlorpheniramine at a dose of 0.06 mg/kg and hydroxyzine at a dose of 1.75 mg/kg (Histacalmine®, Virbac) orally during 3 consecutive days before surgery. After surgery, the diagnosis of a MCT was histologically confirmed by a board-certified veterinary pathologist and all MCTs were classified following Patnaik and Kiupel grading systems (Patnaik et al., 19984; Kiupel et al., 2011). Serum samples of the MCT group consisted of 48 samples from 24 dogs. Approximately 3 mL of blood was drawn at 2 time points: prior to manipulation of the tumor and within 1 hour after

surgical removal of the MCT. Blood was collected in serum tubes, cooled in the fridge while allowed to clot and centrifuged at 2000 G (Biofuge primo R, Thermo scientific) for 5 minutes upon which 500 µl was stored at -80°C until use. For the non-MCT controls, dogs with severe illness, hematological abnormalities, and oncologic disease were excluded. In this control group, serum samples consisted of 24 cooled leftovers that were stored at -80°C until use.

Analysis of serum tryptase

A commercially quantitative sandwich canine specific ELISA was used to analyze all serum samples for the presence of tryptase (Canine Tryptase (TPS) ELISA kit, MyBioSource). The sensitivity of this kit was 0.1 ng/mL with a detection range between 0.5 ng/mL and 16 ng/mL. All serum samples were analyzed in 1 batch to prevent inter-lot-variation. Prior to analysis, all samples were naturally thawed to room temperature as suggested in the manual. To perform the ELISA, serum was diluted 1:2 in sample diluent buffer. After in-house validation of the ELISA, recovery of diluted samples (1:2) was higher compared to undiluted samples, both spiked with a known concentration (2,286 ng/mL) of canine tryptase standard (85.7% vs. 59.9% respectively; Table S3). Company validation of the ELISA showed a mean recovery of 82% (Table S3). Assay procedures were followed as described in the manual. Optical density was read at a wavelength of 450 nm by a plate reader (Multiskan GO, Thermo Fisher Scientific). All tryptase values were calculated in ng/mL.

Statistical analysis

Statistical analysis was performed with the statistical program "R" and packages included "base" (version 4.0.3), "ggplot2" (version 3.3.2), "ggpubr" (version 0.4.0) and "stats" (version 4.0.3). The Mann-Whitney test was used to compare the age between the controls and MCT group. The Wilcoxon signed-rank test was used to compare serum levels before and after surgical removal of the tumor in dogs with MCTs, irrespective of the histopathological grade. The same test was performed separately for each grade according to both grading systems (Patnaik et al., 1994; Kiupel et al., 2011). To investigate if serum tryptase levels were significantly different between controls and dogs with MCTs (pre-operatively), the Mann-Whitney test was used. To investigate if serum tryptase levels were significantly different between controls and MCTs according to both grading systems (Patnaik et al., 1984; Kiupel et al., 2011) the Kruskal-Wallis rank sum test was used. Finally, to investigate the correlation between the presence of

the Darier's sign in dogs with cutaneous MCTs and the serum tryptase level, the Mann-Whitney test was used.

Results

The age of all dogs included was comparable (Tables 1, 2, S1 and S2). Based on the abdominal ultrasound and FNA, there were no indication for metastatic disease in the liver nor spleen in any of the 20 dogs that were screened (Table S1). However, in 3 out of 9 dogs (33%) that had surgical resection of their (sentinel) lymph node(s), metastatic disease was confirmed on histopathology (Table S1). Pre-operative staging was not performed in the remaining 4 dogs (Table S1). Based on histopathology, clean surgical margins were obtained in all dogs but one with a Patnaik grade III, Kiupel high grade MCT (Table S1). Based on the Patnaik grading system (Patnaik et al., 1984), an equal number of grade I and III cutaneous MCTs were included (Tables 1 and S1). Patnaik grade II (Patnaik et al., 1984) MCTs were represented the most, which was to be expected as this tumor grade occurs more frequently (Tables 1 and S1). According to the Kiupel two-tier system (Kiupel et al., 2011), more MCTs were classified as low-grade compared to high-grade MCTs (Tables 1 and S1).

Based on the recombinant full-length dog tryptase standards (UniProt ID: P15944) of the ELISA, a linear calibration curve was obtained with a high goodness of fit (coefficient of determination R^2 = 0.992; Figure S1). All serum tryptase concentrations of the control group (n=24) were within the detection range (Table S2) and only in 2 out of 24 (8.3 %) dogs of the MCT group measurements were below the detection limit (Tables 3 and S1). Median serum tryptase levels of the control and MCT group before and after surgery are presented in Tables 3 and S1. Taking histopathological grade (Patnaik et al., 1984; Kiupel et al., 2011) into account, serum tryptase levels before and after surgery can also be found in Tables 3 and S1.

Regardless of histopathological grade, no significant difference was found between serum tryptase levels before and after surgery in canine MCTs (Table 3). When assessed separately within each grade category (Patnaik et al., 1984; Kiupel et al., 2011) no significant difference was found between serum tryptase levels before and after surgery (Table 3, Figures 1 and 2). The pre-and post- operative tryptase levels of dogs with MCTs did not significantly differ from those of the controls (Table 2). Finally, when comparing controls and dogs with MCTs separated by histopathological grade (Patnaik et al., 1984; Kiupel et al., 2011) again no significant differences were detected (Table 2). Furthermore, no significant difference was measured between the presence of

the Darier's sign in dogs with cutaneous MCTs and the serum tryptase levels in these dogs (Table 2).

Discussion

To investigate whether serum tryptase levels may serve as a diagnostic biomarker in the diagnosis in dogs with canine cutaneous MCTs in analogy with human SM patients, we compared serum tryptase levels in dogs with cutaneous MCTs versus non-MCT controls using a canine-specific and partially in-house validated commercial ELISA kit. Our results show that serum tryptase levels are comparable in dogs with cutaneous MCTs versus control dogs, irrespective of biological behavior, indicating that this biomarker cannot be used in canine MCT diagnosis. Therefore, despite various remarkable similarities between human SM and canine high-grade cutaneous MCTs, serum tryptase cannot be used as a diagnostic marker in dogs. This negative finding is important background knowledge for forthcoming comparative oncology research studies.

In humans, serum tryptase levels reflect the total MC burden (Schwartz et al., 1987; Schwartz et al., 1994; Kanthawatana et al., 1999; Akin et al., 2000; Schwartz et al., 2000; Sperr et al., 2002). Basal serum tryptase levels are below 15 ng/mL in 99% of healthy people (Schwartz et al., 1994; Sperr et al., 2002a; Sperr et al., 2002b; Sperr et al., 2009; Valent et al., 2017). In human SM, the serum tryptase level serves as a diagnostic criterion and is often elevated to >20 ng/mL (Schwartz et al., 1994; Payne et al., 2004; Vitte et al., 2015; Valent et al., 2017). Tryptase levels in human mastocytosis patients without bone marrow (BM) involvement, as is the case in CM, often remain within normal range (Valent et al., 2017; Sperr et al., 2002a; Sperr et al., 2002b; Exposito-Serrano et al., 2018). Our results also show low serum tryptase levels in dogs with cutaneous MCTs, which may be explained by the absence of BM infiltration. Although it is a limitation that no BM aspirations were performed in this study, it can be considered unlikely that BM involvement would have occurred. Indeed, in a study evaluating 157 dogs with cutaneous MCTs and in which BM aspirations were performed, an incidence of only 2.8% was observed (Endicott et al., 2007). Abnormal hematological parameters are considered risk factors for BM involvement (Endicott et al., 2007). Such hematological abnormalities were not present in dogs in our study (data not shown).

Tissue tryptase expression in canine MCTs has been qualitatively investigated by IHC (Ozaki et al., 2002; Kiupel et al., 2004; Fernandez et al., 2005; Mederle et al., 2010; Pazdzior-Czapula et al., 2019). In a study including 98 dogs, the IHC tryptase pattern

was not linked to histological grade nor survival time (Kiupel et al., 2004). In humans, positive IHC staining for tryptase has repeatedly been described in both SM and CM (Hu et al., 2002; Ribatti et al., 2009; Tran et al., 2009; Marrero et al., 2017; Lachapelle et al., 2021; Tirado et al., 2021). Importantly, in human CM, serum tryptase levels are not always elevated despite the positive tryptase expression on IHC stained lesions (Marrero et al., 2017; Lachapelle et al., 2021; Tirado et al., 2021).

Both dogs that received antihistaminic drugs (DPH and hydroxyzine) before surgery had tryptase levels like the other 22 dogs (Table S1). These drugs are inverse agonists, which are competitive for the histamine-receptor and do not influence the co-release of histamine and tryptase from MC granules (Renz et al., 1998; Brunet et al., 1990; Sanchez et al., 2017). In human patients with anaphylaxis, the plasma tryptase levels were independent of the administration of DPH or a placebo (Renz et al., 1998).

Although our sample size was relatively small (n= 24 for both groups), we consider our data generalizable because serum tryptase levels did not differ significantly either between individual dogs or in time within each MCT dog. Whether or not serum tryptase levels are elevated in dogs with MCTs with BM infiltration remains to be investigated.

As a secondary aim, we also compared serum tryptase levels before and within 1 hour after surgery to investigate the role of MCT manipulation but again did not observe differences. In humans with allergic disease, the half-life of tryptase is 90 to 120 minutes (Schwartz et al., 1989; Schwartz et al., 1994) with a maximum level within 15 to 120 minutes after the reaction onset (Schwartz et al., 1989; Fisher et al., 1994; Schwartz et al., 1994; Renz et al., 1998). As our sample prelevation fell within this range, it is not likely that the lack of increase in postoperative serum tryptase levels would be due to inappropriate timing. The lack of postoperative increase in serum tryptase contrasts with a study investigating histamine release in 16 dogs with cutaneous MCTs, where the plasma histamine concentration was higher (albeit not significantly) after tumor manipulation (Sanchez et al., 2017). A recent study investigated the baseline plasma histamine level prior to surgery in 10 dogs with resectable MCTs (Curley et al., 2021). In all dogs, they ranged from 0 to 1.0 ng/mL with a median level of 0.4 ng/mL (Curley et al., 2021). Since no information on histological grade nor metastatic status of the dogs included was provided, the histamine level could not be linked to biological behavior of the tumor. Two older studies did show significantly higher plasma histamine concentrations in dogs with MCTs compared to controls (Fox et al., 1990;

Ishiguro et al., 2003). These levels were not related to tumoral grade nor stage, renouncing plasma histamine as a biomarker in canine MCTs (Fox et al., 1990; Ishiguro et al., 2003). Similarly, due to its low sensitivity, plasma histamine is not reliable as a screening test in human mastocytosis (Koide et al., 2002).

The difference between histamine and tryptase levels after manipulation may be due to the lower molecular weight of histamine facilitating its rapid diffusion into the tissues (Koide et al., 2002), although tryptase is released together with histamine from MCs (Schwartz et al., 1987a; Schwartz et al., 1987b; Schwartz et al., 2000; Veien et al., 2000). In both species, MC degranulation can result in localized swelling, erythema and wheal formation, the so-called Darier's sign (Schwartz et al., 1987a; Tams et al., 1981; Sultan et al., 2018; Vaes et al., 2017; London et al., 2003; Matito et al., 2018). The presence or absence of the Darier's sign does not seem to be correlated with the serum tryptase levels in human CM (Marrero et al., 2017; Exposito-Serrano et al., 2018; Lachapelle et al., 2021). In 6 out of the 24 (25%) dogs, the owner reported that the size of the MCT changed throughout time. In one of these dogs, wheal-and-flare of the mass was observed immediately after manipulation at the time of the physical examination. However, none of them had an increased serum tryptase level. Furthermore, our preliminary data show that the presence of lymphatic metastatic disease does not seem to have an influence on serum tryptase levels in dogs as the 3 dogs that had confirmed metastatic disease in the lymph nodes at time of surgery did not have increased tryptase levels (Table S1). Unfortunately (sentinel) lymph nodes were removed during surgery in only 9 dogs (38%). Therefore, some additional dogs included may already have had lymphatic metastatic disease at time of surgery. Either way, our results show that, even in patients with high-grade MCTs or confirmed lymphatic disease, serum tryptase levels remain low in dogs.

In various studies, a variation among pathologists when grading canine MCTs has been observed (Northrup et al., 2005; Kiupel et al., 2011; Sabattini et al., 2015). Therefore, a consensus on the use of both the Patnaik (Patnaik et al., 1984) and Kiupel (Kiupel et al., 2011) grading systems is recently published (Berlato et al., 2021). In our study, all MCT samples were graded following both grading systems by only one board-certified veterinary pathologist. This can be considered a limitation to our study, due to the possible discrepancy in the assessment of tumor grade. However, such potential interobserver variability would not have any impact since our results show low serum

tryptase levels in all dogs regardless the tumor grade or the presence of metastatic disease.

To conclude, our preliminary findings on serum tryptase levels in dogs with cutaneous MCTs, regardless their grade, are in accordance with data on human CM, where serum tryptase levels tend to remain within the normal range. Despite its diagnostic relevance in human SM, we disclaim the role of serum tryptase as a diagnostic biomarker for canine cutaneous MCTs. Our results provide insights that are important for future comparative oncology strategies.

Conflict of interest

We have no conflicts of interest to disclose.

Acknowledgements

The authors would like to thank all the owners and their dogs that have participated in this study, Dr. Sophie Vandenabeele, prof. Koen Chiers, Yentl van den Bergh, Bart De Pauw and MyBioSource for the technical support.

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Table 1. General overview of all dogs included.

Characteristics	**Non-mast cell tumor control group**	**Mast cell tumor group**
Total (n)	24	24
Age (median)	7 years and 1 month	7 years and 9 months
Age (range)	6 months – 11 years and 6 months	6 months – 12 years and 10 months
Sex	**n (%)**	**n (%)**
Female		
Intact	1 (4.17)	5 (20.8)
Neutered	6 (26.1)	9 (37.5)
Male		
Intact	8 (33.3)	4 (16.7)
Neutered	9 (39.1)	6 (25.0)
Patnaik grading MCTs[†]	**n (%)**	**n (%)**
Grade I	N/A	6 (25.0)
Grade II	N/A	12 (50.0)
Grade III	N/A	6 (25.0)
Kiupel grading MCTs[‡]		
Low-grade	N/A	15 (62.5)
High-grade	N/A	9 (37.5)

[†]Patnaik grading system (1984). [‡]Kiupel grading system (2011)

Table 2. Statistical methods used to compare age and serum tryptase levels between non-MCT controls versus dogs with cutaneous MCTs.

Comparison	Statistical method	P-value
Age between the non-MCT controls vs. dogs with MCTs	Mann-Whitney test	0.509
Serum tryptase levels in non-MCT controls vs. all MCTs (pre-operatively)	Mann-Whitney test	0.564
Serum tryptase levels in non-MCT controls, grade I, II and III MCTs[†]	Kruskal-Wallis rank sum test	0.189
Serum tryptase levels in non-MCT controls, low- and high-grade MCTs[‡]	Kruskal-Wallis rank sum test	0.323
Presence of Darier's sign on clinical examination and serum tryptase level	Mann-Whitney test	1.000

[†]Patnaik grading system (1984), [‡]Kiupel grading system (2011), N/A: Not applicable

Table 3. Serum tryptase levels in non-MCT controls versus dogs with MCTs before and after surgery.

Histopathological grading	Median (range) tryptase level before surgery (ng/mL)	Median (range) tryptase level after surgery (ng/mL)	Difference before and after surgery†: P-value
Non-MCT control dogs	1.054 (0.537 – 1.752)	N/A	N/A
Dogs with MCTs	1.268 (<0.5– 1.655)	1.118 (<0.5 – 2.006)	P = 0.874
Patnaik grading MCTs	0.816 (<0.5 – 1.582)	0.917 (<0.5 – 1.669)	P = 0.438
Grade I			
Grade II	1.297 (0.732 – 1.655)	1.337 (0.837 – 2.006)	P = 0.791
Grade III	1.316 (<0.5 – 1.396)	1.013 (<0.5 – 1.225)	P = 0.125
Kiupel grading MCTs			
Low-grade	1.297 (<0.5 – 1.655)	1.279 (<0.5 – 2.006)	P = 0.743
High-grade	1.007 (<0.5 – 1.396)	0.947 (<0.5 – 1.311)	P = 0.903

†Wilcoxon signed-rank test. N/A: Not applicable.

Table S1. Overview of the characteristics of all dogs with cutaneous MCTs and their serum tryptase levels at the individual dog level.

Dog	MCT Patnaik grade (Kiupel grade)	Breed	Sex	Age	Tryptase concentration pre-operative (ng/mL)	Tryptase concentration post-operative (ng/mL)	Tumor characteristics	Metastatic disease
1	Grade I (low)	American Staffordshire terrier	Female neutered	9 years 3 months	*<0.5*	*<0.5*	Shrinkage and swelling	No
2	Grade I (low)†	Beagle	Female neutered	8 years 10 months	0.816	0.917	No change in diameter	No
3	Grade I (low)	Bull terrier	Female neutered	3 years 3 months	1.813	1.040	Tumor growth	Unknown
4	Grade I (low)	Labrador retriever	Male neutered	6 years 5 months	1.582	1.669	No change in diameter	Unknown
5	Grade I (low)	Rhodesian ridgeback	Male neutered	5 years 3 months	0.921	0.793	No change in diameter§	Unknown
6	Grade I (low)	West Highland white terrier	Female neutered	12 years 1 month	0.526	0.528	No change in diameter	No
7	Grade II (low)	American Staffordshire terrier	Female neutered	8 years 3 months	1.064	0.974	Shrinkage and swelling	No
8	Grade II (low)	Boxer	Male neutered	5 years 5 months	1.482	1.408	Tumor growth	No
9	Grade II (low)	Golden retriever	Male intact	3 years 9 months	1.655	1.590	No change in diameter	No
10	Grade II (low)	Golden retriever	Female intact	6 years 8 months	1.418	1.026	Swelling after manipulation	No
11	Grade II (low)	Labrador retriever	Male neutered	3 years 8 months	1.487	1.363	No change in diameter	No
12	Grade II (low)	Labrador retriever	Male intact	3 years 9 months	1.303	1.195	No change in diameter	Unknown
13	Grade II (low)	Labrador retriever	Female neutered	11 years 11 months	1.291	1.405	Tumor growth	No
14	Grade II (low)	Rhodesian ridgeback	Male intact	7 years 3 months	1.244	1.397	No change in diameter	No
15	Grade II (low)	Siberian husky	Female neutered	9 years 4 months	1.619	2.006	Shrinkage	No
16	Grade II (high)†	Beagle	Female neutered	8 years 2 months	0.732	0.837	Shrinkage and swelling‡	No
17	Grade II (high)	French bulldog	Female intact	0 years 6 months	1.052	1.311	No change in diameter	No
18	Grade II (high)	Labrador retriever	Male neutered	11 years 4 months	0.842	0.847	No change in diameter	No

19	Grade (high)°	III	Bernese mountain dog	Female intact	9 years 7 months	1.325	1.225	Tumor growth	Retropharyngeal lymph nodes
20	Grade (high)	III	Boxer	Male intact	5 years 3 months	0.700	0.741	Tumor growth	Submandular lymph nodes
21	Grade (high)	III	Crossbreed	Female intact	8 years 3 months	1.316	1.013	Tumor growth	No
22	Grade (high)	III	Crossbreed	Female neutered	12 years 10 months	*<0.5*	*<0.5*	Shrinkage and swelling	No
23	Grade (high)	III	Golden retriever	Female intact	9 years 6 months	1.396	1.204	Shrinkage and swelling	No
24	Grade (high)	III	Shar-Pei	Male neutered	6 years 0 months	0.963	0.880	Tumor growth	Submandibular lymph nodes

†Dog presented twice with a cutaneous MCT. ‡Dog received diphenhydramine hydrochloride (DPH) orally at a dose of 3 mg/kg 2 hours prior to surgery. §Dog received chlorpheniramine at a dose of 0.06 mg/kg and hydroxyzine at a dose of 1.75 mg/kg orally during 3 consecutive days prior to surgery. °Lateral and deep margins not free. N/A: Not applicable.

Table S2. Overview of the characteristics of all non-MCT control dogs and their serum tryptase levels at the individual dog level.

Dog	Breed	Sex	Age	Tryptase concentration (ng/mL)	Service to which the dog was presented
1	Beagle	Male intact	2 years 3 months	0.996	Nutrition
2	Beagle	Female neutered	2 years 3 months	1.000	Nutrition
3	Beagle	Male intact	6 years 5 months	1.247	Dermatology
4	Beagle	Male neutered	6 years 6 months	1.065	Nutrition
5	Bouvier des Flanders	Female neutered	7 years 4 months	1.267	Internal medicine
6	Boxer	Male neutered	7 years 0 months	0.923	Orthopedics
7	Crossbreed	Male neutered	0 years 11 months	1.099	Internal medicine
8	Crossbreed	Male neutered	9 years 4 months	0.677	Surgery
9	Dalmatian	Male intact	11 years 6 months	1.001	Internal medicine
10	Dobermann	Male neutered	5 years 6 months	0.945	Cardiology
11	English bulldog	Male intact	1 year 10 months	1.399	Neurology
12	English bulldog	Male neutered	3 years 5 months	0.724	Orthopedics
13	Golden Retriever	Male intact	9 years 0 months	1.147	Internal medicine
14	Golden retriever	Male intact	10 years 5 months	0.697	Orthopedic
15	Belgian shepherd	Male neutered	10 years 9 months	1.752	Surgery
16	Italian sighthound	Female neutered	8 years 4 months	0.537	Surgery
17	Labrador retriever	Female neutered	8 years 4 months	1.234	Internal medicine
18	Leonberger	Male intact	7 years 2 months	1.043	Orthopedics
19	Lhasa apso	Male neutered	9 years 5 months	0.856	Stomatology
20	Maltese dog	Female neutered	11 years 5 months	0.860	Stomatology
21	Petit Brabançon	Female intact	0 years 6 months	1.544	Surgery
22	Pinscher	Male neutered	5 years 4 months	1.433	Orthopedics
23	Dachshund	Female neutered	8 years 1 month	1.359	Stomatology
24	West Highland white terrier	Male intact	4 years 5 months	1.397	Dermatology

Table S3. Partial in-house and company validation of the commercial ELISA kit.

In-house validation	**Recovery (%)**
Linearity check	
1 non-MCT control serum sample (undiluted vs. 1:2 diluted)	80.4
1 MCT grade III† (high-grade‡) serum sample (undiluted vs. 1:2 diluted)	86.1
Spike	
Spike + undiluted serum (pool of 4 MCT patients)	59.9
Spike + diluted (1:2) serum (pool of 4 MCT patients)	85.7
Company validation	
Spike	
3 levels canine tryptase into 5 healthy canine serum samples	82

†Patnaik grading system (1984). ‡Kiupel grading system (2011).

Figure legends

Figure 1. Boxplot of serum tryptase levels (ng/mL) in non-MCT control dogs and dogs with cutaneous MCTs (grading based on Patnaik et al., 1984)[10]. In the MCT group, serum samples were taken before (pre) and after (post) surgical removal of the tumor to investigate the effect of tumor manipulation on serum tryptase levels.

Figure 2. Boxplot of serum tryptase levels (ng/mL) in non-MCT control dogs and dogs with cutaneous MCTs (grading based on Kiupel et al., 2011)[11]. In the MCT group, serum samples were taken before (pre) and after (post) surgical removal of the tumor to investigate the effect of tumor manipulation on serum tryptase levels.

Figure S1. Calibration curve obtained with the ELISA kit (Canine Tryptase (TPS) ELISA kit, MyBioSource). Standard Concentration Gradients (S6 to S1): 16, 8, 4, 2, 1, 0.5 ng/mL.

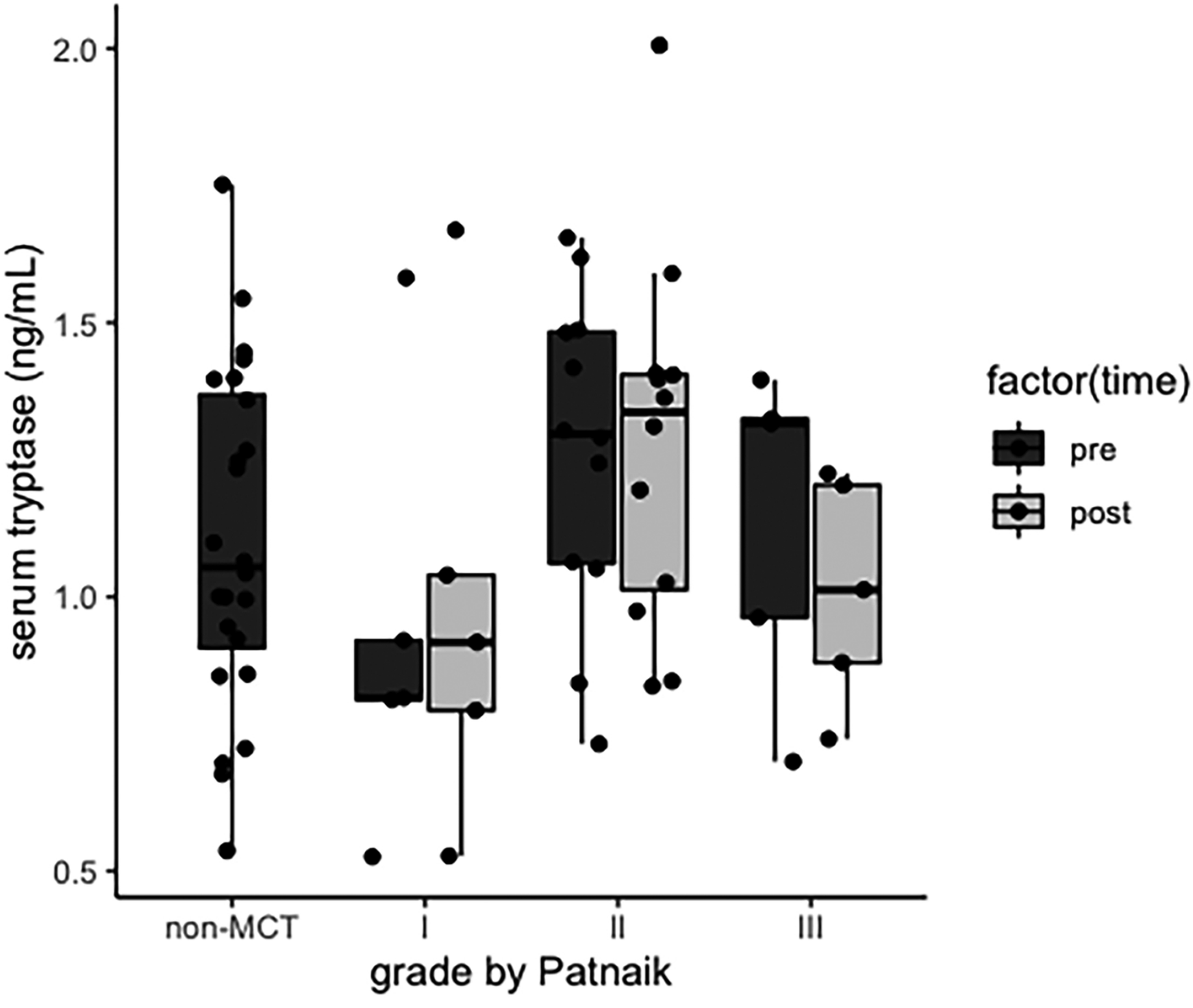
2.0
1.5
1.0
0.5
serum tryptase (ng/mL)
non-MCT
I
II
III
grade by Patnaik
factor(time)
pre
post

Figure 1

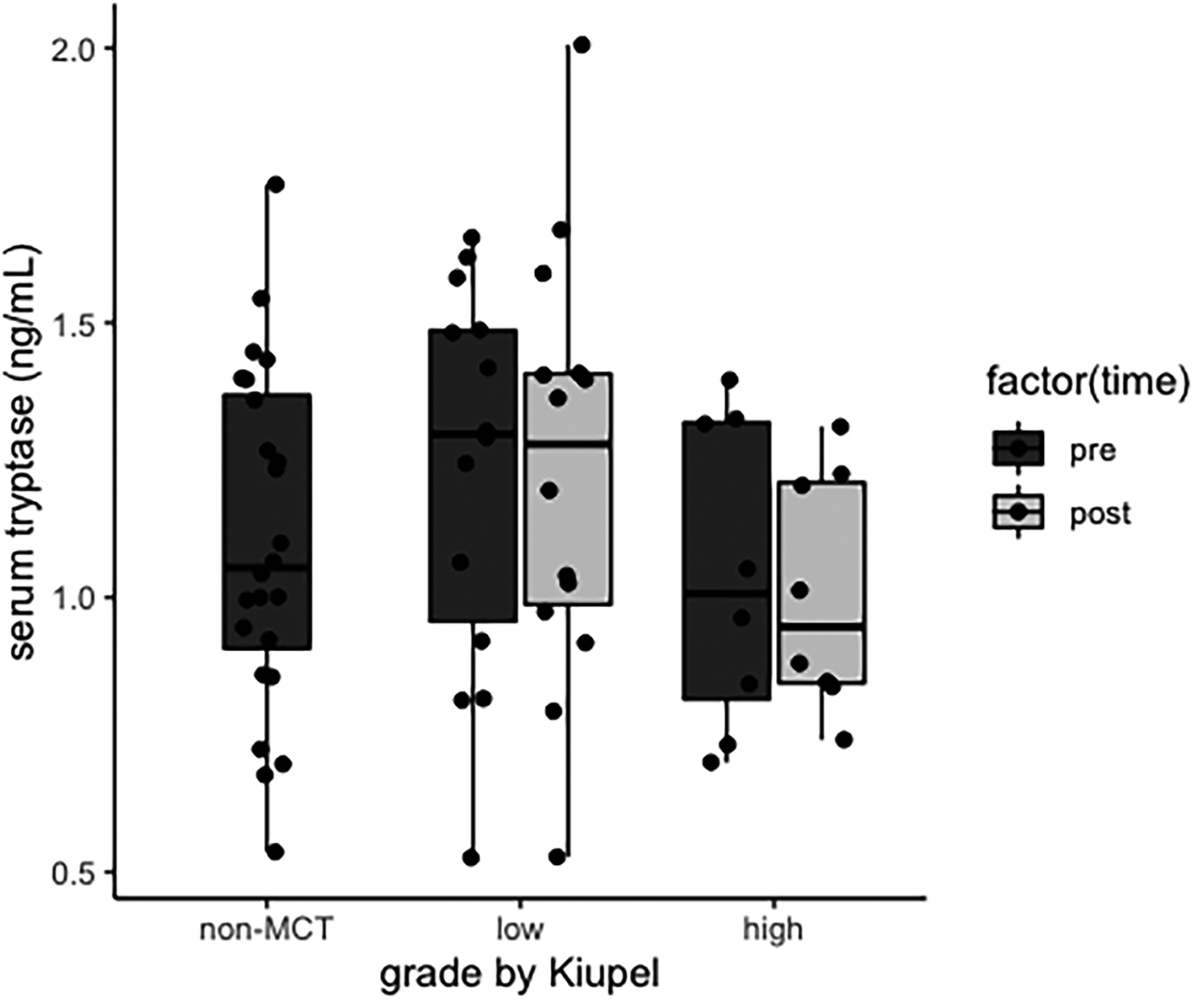
2.0
1.5
1.0
0.5
serum tryptase (ng/mL)
non-MCT
low
high
grade by Kiupel
factor(time)
pre
post

Figure 2