

UNIVERSIDADE DE LISBOA
FACULDADE DE MEDICINA VETERINÁRIA

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CHARACTERIZATION OF PROGNOSIS INDICATORS IN A POPULATION OF DOGS WITH
CUTANEOUS MAST CELL TUMORS TREATED AT AN ONCOLOGY REFERRAL CENTER IN
PORTUGAL

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2025

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Ano de conclusão (indicar o da data da realização das provas públicas): 2025

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Acknowledgements

E assim se passaram 6 anos na FMV e o maior sonho da Teresinha pequenina tornou-se realidade - ser médica veterinária! A mesma Teresinha que desde sempre se agarra a todos os animais que vê na rua para dar festas e pegar ao colo, que trazia os restos dos restaurantes para dar ao cão que estava à porta e que implorava aos pais para trazer qualquer animal para casa. A felicidade é tão grande que não existem palavras que a descrevam! No entanto, há muitas pessoas a que agradecer por chegar até aqui.

Em primeiro lugar, quero agradecer à Professora Doutora Berta São Braz, tanto por ter aceitado este desafio, como por ser uma grande inspiração. Uma professora que adora ensinar e nos motiva a cada dia, que nos faz pensar e é sempre um ombro amigo quando precisamos!

Ao Professor Doutor Joaquim Henriques, pela sua orientação, disponibilidade e paciência mesmo quando eu enviava 20 mensagens de seguida e aparecia no hospital para mais uma reunião não agendada.

A toda a equipa do Anicura Atlântico Hospital Veterinário, por me receberem de braços abertos durante o estágio e me fazerem sentir em casa desde o primeiro dia. Um agradecimento especial às médicas Catarina, Sofia, Adriana e Inês por tornarem todos os turnos mais leves e as noites mais divertidas. À Rita, por me esclarecer sempre as dúvidas e puxar pela minha autonomia. Ao Dr. João Caiano, Dra. Sónia Fonseca e Dra. Daniela Ferreira que me fizeram apaixonar pela ecografia e se preocupavam sempre em explicar e perguntar se percebia o que estávamos a ver. Ao Dr. José Martins, por ajudar em toda a colheita de casos e dar as melhores dicas para a tese. À Enf. Sílvia e Enf. Joana Ferreira pelas horas passadas em cirurgia e explicarem todos os passos da anestesia. À Enf. Ana Rita Charrua, Enf. Carolina Rebimba e Enf. Joana Alves pela paciência no internamento, desde incentivarem a tentar outra vez quando não conseguia à primeira, a explicarem como se fazia as análises mais uma vez. À Aux. Sónia Beirão por animar todos os turnos. Por último, mas não menos importante, aos estagiários que entraram nesta aventura comigo e, em especial, ao Seruca, Adriana, Mariana e Francisco. Em particular, agradecer ao Seruca, a maior surpresa deste último ano e um grande apoio ao longo de todo o estágio.

Ao Queen's Veterinary School Hospital, da Universidade de Cambridge, pela experiência que me proporcionaram. Nunca vou esquecer estes 2 meses e tudo o que aprendi. Quero agradecer especialmente à Susana por tornar este sonho possível, pela disponibilidade, boa disposição e conversas na cozinha a beber chá. À Inês, André Lopes e André Dias por viverem comigo esta aventura e serem os melhores housemates que podia pedir.

À minha família, que sempre acreditou em mim e que este sonho era possível, por todas as vezes que me desejaram boa sorte para um exame e perguntarem como tinha corrido a seguir. Agradecer principalmente aos meus pais e aos meus irmãos por lidarem com o meu humor particularmente desafiante durante as épocas de exames e me lembrarem de parar quando estudava todas aquelas horas seguidas, me incentivaram a sair da minha zona de conforto ao ir viver 1 ano para fora e festejaram comigo quando soube da entrada em Cambridge. Fizeram de mim a pessoa que sou hoje.

Ao Duarte, por celebrar comigo todas as minhas conquistas, acreditar que sou capaz de tudo, por me motivar e lembrar que não é preciso estar sempre perfeito. A melhor companhia de qualquer pausa de estudo, mesmo que implicasse conduzir 40 minutos para um plano de meia hora.

Aos amigos que me viram crescer, Catarina, Margarida, Ana Catarina, Miguel, Diogo, amigos da Ericeira, do CVG e da JENGA, pela amizade e apoio incondicional, por estudarem comigo e perceberem quando eu não conseguia, mais uma vez, estar presente por estar em casa a estudar.

Aos amigos de Erasmus, a Bea, Manuel, Gonçalo, Martim e Camila, por viverem comigo o melhor e mais desafiante ano da minha vida.

Aos amigos da FMV, Zé, Gonçalo, Nana, Mafalda, Sara, Sofia e Daniel, que são a prova viva que o curso não se faz sozinho. Muitas sessões de estudo, festas e jantares que nunca serão esquecidos.

À minha madrinha, Charraz, por todas as mensagens e chamadas quando eu tinha crises existenciais e achava que não ia passar num exame, pelos brunchs e jantares com horas de conversa e, acima de tudo, por descomplicar o curso e ser o pilar que foi para chegar até aqui. À minha afilhada, Sara, por me ter escolhido como madrinha, confiar em mim para a ajudar neste percurso, fazer-me lembrar todas as cadeiras e o que era importante saber para cada uma.

E por último, aos grandes amores da minha vida, que fizeram com que cada hora de estudo valesse a pena - o Alfie, a Mia e a Nury - e aos que já só estão no meu coração - a Kika, Pantufa, Stark e Goma.

A todos os amigos, familiares, professores, médicos, enfermeiros e auxiliares que se cruzaram no meu caminho e me ajudaram a chegar até aqui, um obrigado do fundo do coração!

Tenho a certeza que a Teresinha de 6 anos estaria muito orgulhosa.

CARACTERIZAÇÃO DOS FATORES DE PROGNÓSTICO DE UMA POPULAÇÃO DE CÃES COM MASTOCITOMA CUTÂNEO TRATADOS NUM CENTRO DE REFERÊNCIA ONCOLÓGICA EM PORTUGAL

Resumo

Os mastocitomas cutâneos (cMCTs) são uma das neoplasias cutâneas malignas mais frequentes em cães, correspondendo a 7-21% de todos os tumores cutâneos diagnosticados. A heterogeneidade e comportamento biológico variável destes tumores tornam o prognóstico e plano terapêutico elementos fundamentais na abordagem clínica destes casos.

O presente estudo teve como objetivo principal caracterizar uma amostra populacional de cães diagnosticados com cMCT e avaliar a influência de fatores epidemiológicos, clínicos e histológicos no tempo de sobrevivência (ST), de forma a identificar fatores de prognóstico significativos. Um objetivo adicional foi analisar o impacto da vinblastina (VBL) em contexto neoadjuvante na redução da dimensão do tumor, uma vez que 10 animais desta amostra tinham sido submetidos a este tratamento.

O estudo retrospectivo foi realizado com base em 65 casos de mastocitoma cutâneo, recolhidos do Centro de Oncologia de Referência do Anicura Atlântico Hospital Veterinário (HVA). Os casos foram caracterizados de acordo com o sexo, idade, estado reprodutivo, raça, presença de comorbilidades, referência, tipo de lesão e localização, método de diagnóstico e tratamento, sendo ainda distribuídos em grau histológico, estadiamento clínico e marcadores de proliferação celular. Na análise de sobrevivência, verificou-se que o grau de estadiamento clínico ($p=0.039$), a dimensão do tumor ($p=0.028$), o índice de Ki-67 ($p=0.024$) e estado reprodutivo ($p=0.001$) influenciaram significativamente o ST. A cirurgia demonstrou ser curativa na maioria dos casos, com uma taxa de recidiva reduzida. Relativamente ao tratamento com VBL neoadjuvante, observou-se uma redução significativa do tamanho tumoral ($p=0.011$), sendo comprovado o seu efeito citoreductor, com uma diminuição média de aproximadamente 26% após tratamento com VBL. Na associação entre animais tratados e não tratados com VBL neoadjuvante e o ST, período livre de doença (DFI) e recidiva verificou-se que, apesar das diferenças não serem significativas, os animais não sujeitos ao tratamento apresentavam ST e DFI mais longos, assim como menor probabilidade de recidiva.

Este estudo retrospectivo permitiu identificar fatores de prognóstico significativos, tal como o estado reprodutivo fértil, descrito pela primeira vez como potencial fator protetor na sobrevivência, e uma melhor compreensão do papel da vinblastina em contexto neoadjuvante no tratamento do cMCT.

Palavras-chave: Mastocitoma Cutâneo, prognóstico, sobrevivência, vinblastina, fertilidade

CHARACTERIZATION OF PROGNOSTIC INDICATORS IN A POPULATION OF DOGS WITH CUTANEOUS MAST CELL TUMORS TREATED AT AN ONCOLOGY REFERRAL CENTER IN PORTUGAL

Abstract

Canine cutaneous mast cell tumors (cMCTs) are among the most frequent malignant skin neoplasms in dogs, accounting for 7-21% of all diagnosed cutaneous tumors. The heterogeneity and variable biological behavior of these tumors make prognosis and therapeutic planning crucial elements in their clinical management.

The main objective of this study was to characterize a population of dogs diagnosed with cMCT and to evaluate the influence of epidemiological, clinical and histological factors on survival time (ST), in order to identify significant prognostic factors. An additional objective was to investigate the impact of vinblastine (VBL) used in a neoadjuvant approach on tumor size reduction, as 10 animals in the present study had received this treatment.

This retrospective study was based on 65 cases of cMCT collected at the Oncology Referral Center of the Anicura Atlântico Veterinary Hospital (HVA). Cases were characterized according to sex, age, reproductive status, breed, presence of comorbidities, referral source, lesion type and location, diagnostic method and treatment. They were further classified by histological grade, clinical stage and cell proliferation markers. Survival analysis showed that clinical stage ($p = 0.039$), tumor size ($p = 0.028$), Ki-67 index ($p = 0.024$) and reproductive status ($p = 0.001$) significantly influenced ST. Surgery showed to be curative in most cases, with a low recurrence rate.

Regarding neoadjuvant VBL treatment, a significant tumor size reduction was observed ($p = 0.011$), confirming its cytoreductive potential, with an average decrease of approximately 26% following VBL administration. When comparing animals treated and not treated with neoadjuvant VBL in relation to ST, disease-free interval (DFI) and recurrence, although differences were not statistically significant, animals without neoadjuvant VBL treatment showed longer ST and DFI, as well as lower probabilities of recurrence.

This retrospective study allowed the identification of significant prognostic factors, in particular reproductive status intact as a possible protective factor on survival and contributed to a better understanding of the role of vinblastine as a neoadjuvant therapy in the treatment of cMCT.

Keywords: Cutaneous Mast Cell Tumor, prognosis, survival, vinblastine, intact dogs

CARACTERIZAÇÃO DOS FATORES DE PROGNÓSTICO DE UMA POPULAÇÃO DE CÃES COM MASTOCITOMA CUTÂNEO TRATADOS NUM CENTRO DE REFERÊNCIA ONCOLÓGICA EM PORTUGAL

Resumo alargado

Os mastocitomas cutâneos (cMCTs) são uma das neoplasias cutâneas mais frequentes em cães, correspondendo a 7-21% de todos os tumores cutâneos diagnosticados. São mais comuns em cães sem raça definida e, de entre as raças puras, apresentam maior risco de incidência no Boxer, Boston Terrier, Bulldog, Pug, Labrador e Golden Retriever, Cocker Spaniel, Schnauzer, Beagle, Weimaraner e Shar-Pei Chinês. Pelo contrário, raças como Pastor Alemão, Chihuahua, Poodle e Yorkshire Terrier parecem ter menor risco de desenvolvimento. Os MCTs podem surgir em qualquer idade, sendo diagnosticados com maior frequência em cães adultos a idosos, entre os 7 e os 10 anos. A maioria dos estudos não reporta uma associação entre o sexo e a ocorrência de MCT, no entanto, alguns autores referem que machos apresentam um risco 25% superior para o desenvolvimento de mastocitomas de alto grau.

Relativamente à etiologia, o desenvolvimento dos cMCTs continua pouco esclarecido, sendo descrito como, provavelmente, multifatorial. Entre possíveis causas, as alterações moleculares revelam um papel importante, em particular as mutações no proto-oncogene c-KIT que representam a anomalia mais associada a esta neoplasia. Em condições normais, o KIT regula a proliferação, diferenciação e sobrevivência dos mastócitos através da ligação ao ligando *Stem Cell Factor* (SCF). No entanto, mutações no c-KIT levam à ativação independente da ligação ao SCF, que resulta numa proliferação descontrolada dos mastócitos e conseqüente desenvolvimento tumoral. Estas alterações podem ainda ser acompanhadas por outras anomalias cromossómicas e possivelmente por fatores adicionais ainda não identificados.

Do ponto de vista fisiológico, os mastócitos constituem células do sistema imunitário localizadas sobretudo nas interfaces ambientais, como a pele, pulmões e trato gastrointestinal. Contêm grânulos citoplasmáticos ricos em mediadores bioativos como histamina, citocinas, heparina e proteases, fundamentais na resposta inflamatória e imunitária que desempenham. No entanto, no contexto neoplásico, a desregulação destas células pode originar sinais clínicos sistémicos, incluindo prurido, eritema, vômito, ulceração gástrica, diarreia e, em casos mais graves, choque anafilático.

O comportamento biológico é altamente variável, desde pequenas massas elevadas até extremamente agressivas, com ulceração e necrose. O tronco, períneo e região inguino-genital são os locais mais comuns, com apresentação solitária a múltiplos nódulos confinados à derme ou com infiltração nos tecidos adjacentes.

Relativamente ao diagnóstico de MCT, este é normalmente feito, numa fase inicial, por citologia, com o recurso à punção aspirativa de agulha fina (PAF). Apesar de fornecer um diagnóstico preciso em aproximadamente 92-96% dos casos, por citologia apenas não é possível definir o grau do MCT e prognóstico, sendo necessário correlacionar com a análise histopatológica. A histopatologia é considerada o *gold-standard* para diagnóstico, fornecendo informações importantes relativamente ao grau tumoral, prognóstico e qual a melhor abordagem terapêutica a seguir. Os dois sistemas de graduação mais conhecidos e amplamente utilizados são o Sistema de Kiupel e o Sistema de Patnaik, sendo atualmente aconselhada a utilização em simultâneo de ambos, de forma a obter uma melhor compreensão do comportamento biológico do tumor. Independentemente do grau histológico, todos os tumores devem ser sujeitos a um estadiamento clínico, sendo o mais consensual o definido pela Organização Mundial de Saúde, que definiu 4 estadios, do I-IV. O grau I inclui MCT solitários confinados à derme e sem envolvimento do linfonodo regional e o IV mastocitomas com metástases distantes. Um estadiamento completo, apesar de não ser necessário em todos os casos, deve incluir um exame físico detalhado, análises hematológicas e bioquímicas, citologia dos linfonodos regionais, ecografia abdominal, com ou sem aspiração de agulha fina do baço e fígado, e em casos específicos FNA da medula óssea e radiografias torácicas.

A heterogeneidade destas neoplasias torna o prognóstico desafiante e dependente de múltiplos fatores, incluindo o grau histológico e avaliação de margens cirúrgicas, estadiamento, localização anatómica e dimensão da neoplasia, marcadores de proliferação celular, fatores epidemiológicos, presença de sinais clínicos, recidiva e presença de metástases. Entre estes, o grau histológico é o mais consensual, com correlação direta com o ST, DFI e potencial metastático, sendo que os MCT de baixo-grau/Grau I apresentam um bom prognóstico, com taxas de sobrevivência mais longas e baixo potencial metastático quando comparados a MCT de alto grau. Dentro dos marcadores de proliferação celular, os mais utilizados são o índice mitótico, com um *cut-off* de 5/10 HPF e o Ki-67 com um *cut-off* de 1.8%.

Em termos de tratamento, a cirurgia constitui normalmente a primeira linha de tratamento, podendo, sobretudo em tumores de baixo grau e margens limpas, ser curativa em muitos casos. No entanto, em tumores de maiores dimensões, localização complexa ou presença de metástases, a cirurgia isolada pode não ser suficiente, sendo necessário recorrer a quimioterapia ou terapias locais. Entre os compostos quimioterápicos, a vinblastina é um dos fármacos mais utilizados quer em regime adjuvante, quer neoadjuvante. A utilização neoadjuvante visa reduzir a dimensão da massa antes da cirurgia, de forma a facilitar a obtenção de margens adequadas e a diminuir o risco de recidiva.

Este trabalho teve como objetivo principal caracterizar uma amostra populacional de cães diagnosticados com cMCT e avaliar a influência de fatores epidemiológicos, clínicos e histológicos no tempo de sobrevivência (ST), de forma a identificar fatores de prognóstico significativos. Um objetivo adicional foi analisar o impacto da vinblastina (VBL) em contexto neoadjuvante na redução da dimensão do tumor, uma vez que 10 animais desta amostra tinham sido submetidos a este tratamento.

O estudo retrospectivo foi realizado com base em 65 casos de mastocitoma cutâneo, recolhidos do Centro de Oncologia de Referência do Anicura Atlântico Hospital Veterinário (HVA). Para a sua seleção, os animais necessitavam de um diagnóstico definitivo de mastocitoma estabelecido entre Janeiro de 2023 e Dezembro de 2024 e apresentados para consulta no HVA. Seriam excluídos animais com ausência de diagnóstico, informação incompleta relevante para o estudo, desde idade, raça e historial médico e resultados de exames complementares, ou com presença de outras neoplasias em tratamento. Os casos selecionados foram caracterizados de acordo com o sexo, idade, estado reprodutivo, raça, presença de comorbilidades, referência, tipo de lesão e localização, método de diagnóstico e tratamento, sendo ainda distribuídos em grau histológico e margens, estadiamento clínico, marcadores de proliferação celular e expressão do padrão KIT. Observou-se que, tal como descrito na literatura, os cães sem raça definida representavam a maioria da amostra, com uma média de idades de 9 anos e sem predisposição sexual bem definida, sendo apenas ligeiramente mais predominante nos machos (60%). Assim como mencionado noutros estudos, a manifestação clínica mais comum foram os nódulos solitários (80%) e localizados no tronco, períneo e região inguino-genital. Em relação ao grau histológico, as proporções observadas foram semelhantes a estudos anteriores, com a maioria dos tumores de baixo grau no sistema de Kiupel e de grau intermédio no sistema de Patnaik. Quando efetuada, a cirurgia demonstrou ser curativa na maioria dos casos, com uma taxa de recidiva reduzida.

Sendo o principal objetivo avaliar a influência de diferentes fatores epidemiológicos, clínicos e histológicos na sobrevivência, foi realizada uma análise de sobrevivência que verificou que o grau de estadiamento clínico ($p=0.039$), a dimensão do tumor ($p=0.028$), o índice de Ki-67 ($p=0.024$) e o estado reprodutivo ($p=0.001$) influenciaram significativamente o ST. Estádios clínicos mais avançados e índices de Ki-67 demonstraram tempos de sobrevivência mais curtos. Na dimensão do tumor não se observou apenas que tumores de maiores dimensões apresentaram menores tempos de sobrevivência, como também os de maiores dimensões eram maioritariamente de alto-grau. O estado reprodutivo destacou-se por, pela primeira vez, a fertilidade ser descrita como potencial fator protetor da mortalidade, visto que além de animais férteis obterem melhores tempos de sobrevivência, demonstraram ainda 80% menor risco de mortalidade. Variáveis como sexo, idade, raça, localização do tumor, grau histológico, índice mitótico (MI) e expressão do padrão KIT não demonstraram ser

estatisticamente significativas. No entanto, foram observadas algumas tendências, como machos mais prováveis de apresentar tumores de alto grau, índice mitótico com um tempo de sobrevivência menor quando MI >1, padrões de expressão KIT 2 e 3 com tempos de sobrevivência inferior quando comparados ao padrão 1, assim como uma taxa de sobrevivência de 20% aos 407 dias em pacientes com apresentação de sinais clínicos sistêmicos.

Relativamente ao tratamento com VBL neoadjuvante, observou-se uma redução significativa do tamanho tumoral ($p= 0.011$), sendo comprovado o seu efeito citoreductor, com uma diminuição média de aproximadamente 26% após tratamento com VBL. Na associação entre animais tratados e não tratados com VBL neoadjuvante e o ST, período livre de doença (DFI) e recidiva verificou-se que, apesar das diferenças não serem significativas, os animais não sujeitos ao tratamento apresentavam ST e DFI mais longos, assim como menor probabilidade de recidiva.

Este estudo permitiu identificar fatores de prognóstico significativos, como o grau de estadiamento clínico, dimensão do tumor, índice de Ki-67 e o estado reprodutivo, sendo o estado reprodutivo fértil descrito pela primeira vez como possível fator protetor na sobrevivência. Este estudo reforça ainda que a vinblastina pode reduzir eficazmente o tamanho dos MCTs em contexto neoadjuvante, o que realça o seu potencial e benefício clínico apesar do número reduzido de casos tratados.

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List of Abbreviations

AgNOR - Argyrophilic nucleolar organizing regions

CLPs - Common Lymphoid Progenitors

CMMIA - Intervention and Minimally Invasive Surgery Center

cMCT - Cutaneous Mast Cell Tumor

CT - Computed Tomography

DFI - Disease-Free Interval

FNA – Fine-needle Aspiration

GMPs - Granulocyte-monocyte Progenitors

HPF - High-power field

HSCs - Pluripotent Hematopoietic Stem Cells

HVA - Anicura Atlântico Veterinary Hospital

MCT - Mast Cell Tumor

MI - Mitotic Index

MPPs - Multipotent Progenitors

NGF - Nerve Growth Factor

NK - Natural Killer

OOH - Out-of-hours

PAF - Fine-needle Aspiration Punction

PCNA - Proliferating Cell Nuclear Antigen

SCF - Stem Cell Factor

ST - Survival time

VBL - Vinblastine

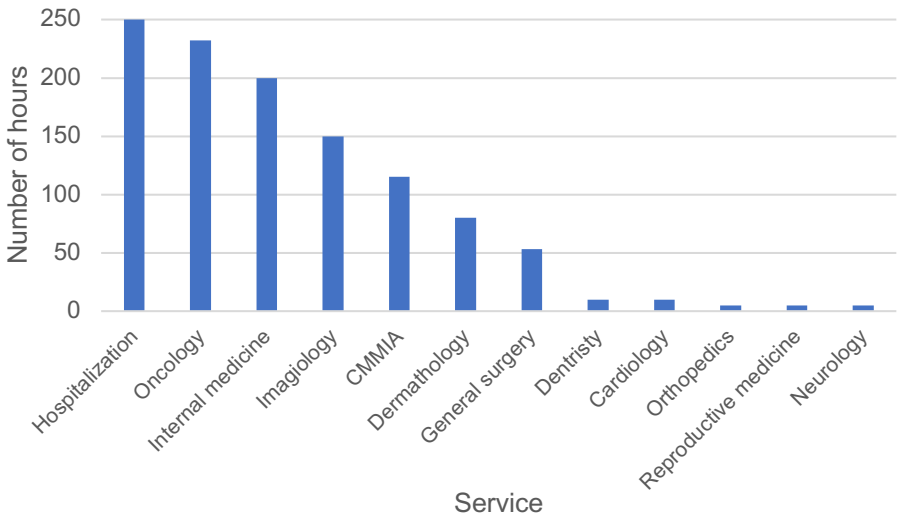
SECTION 1 - TRAINEESHIP REPORT

The curricular traineeship was divided between two institutions: Anicura Atlântico Veterinary Hospital (HVA) and The Queen’s Veterinary School Hospital of the University of Cambridge.

1. Anicura Atlantico Veterinary Hospital

The first traineeship took place in Mafra, at Anicura Atlantico Veterinary Hospital (HVA), under the supervision of Dr. Joaquim Henriques. The externship lasted approximately seven months, from September 16th 2024, to March 30th 2025, corresponding to around 1120 hours (Graphic 1).

Graphic 1: Distribution of hours spent per service at HVA traineeship



The hospital offers a wide range of Small Animal services, including medicine, surgery and diagnostic imaging. The Medicine Service encompasses internal medicine, preventive and reproductive medicine, hospitalization, dermatology, cardiology, neurology, dentistry, orthopedics and ophthalmology. The Imaging Service includes ultrasonography, computed tomography (CT), radiology and fluoroscopy, while the surgical service covers general surgery, orthopedic, laparoscopic and endoscopic surgery. HVA also has two referral centers: the Interventional and Minimally Invasive Surgery Centre (CIMMIA) and the Advanced Oncology Center.

During the traineeship, students rotated between services, being assigned to a clinician for two-week periods. Both day and night shifts could be assigned, with approximately 2 night shifts per month.

In the internal medicine rotation, there was the opportunity to follow case histories and clinical signs, actively participating in the formulation of differential diagnoses and engaging in discussions of diagnostic and therapeutic plans with veterinarians and nurses.

In oncology, the activities included the observation of first and second-opinion consultations, with the chance of performing cytological evaluation under the microscope, staging and interpretation of laboratory tests, as well as observing and discussing treatments, and innovative surgeries such as pulmonary and hepatic lobectomy, lobectomy, nephrectomy and several adrenalectomies.

Regarding hospitalization, the unit was divided into canine, feline and infectious disease wards. Daily responsibilities involved patient rounds, case handovers and clinical examinations, as well as procedures such as placing intravenous catheters, wound care, urine collection by cystocentesis, placement of feeding tubes and urinary catheterization. Additional experience was gained in emergency situations, including CPR, rapid drug preparation and administration, placement of endotracheal tubes, along with performing and interpreting AFAST and TFAST scans.

In diagnostic imaging, experience was acquired in abdominal and thoracic ultrasound, CT scans and radiographies, including the interpretation of the different complementary exams, with the opportunity of performing procedures such as cystocentesis, thoracocentesis, abdominocentesis and ultrasound-guided Fine-Needle Aspiration (FNAs). Assistance was also provided in patient preparation for CT scans, including endotracheal intubation, positioning and anesthetic monitoring.

The surgical rotation, with emphasis on minimally invasive techniques, allowed observation and participation in advanced procedures including intra and extrahepatic portosystemic shunts correction, cavotomy, ureterostomy and cystolithotomy. At the interventional center, the unique opportunity to witness the first V-Clamp procedure performed in Portugal.

Overall, this experience strengthened both clinical and professional skills, such as communication with animal owners and the interpretation of diagnostic tests, including hematology, biochemistry, endocrinology, cytology and fluid analysis. In addition, it provided the opportunity to deepen knowledge and confidence in various clinical specialties beyond general medicine.

2. The Queen's Veterinary School Hospital of the University of Cambridge

The traineeship at the University of Cambridge took place at The Queen's Veterinary School Hospital, with a duration of two months, from May 1st to June 29th, 2025, under the supervision of Dr. Susana Monforte Monteiro, Senior Neurologist. The program consisted of 8 weeks of clinical rotations: two weeks in Internal Medicine, two weeks in Oncology, two weeks

in Neurology and two weeks in the Out-of-hours (OOH) service, which included both night and weekend shifts.

Each rotation began with daily rounds, during which inpatients diagnostic and treatment plans were reviewed and upcoming consultations discussed. Externs were invited to attend appointments and for new cases were often given the opportunity to collect the clinical history from the owners, which was then reviewed with senior clinicians, followed by the formulation of differential diagnoses and diagnostic and therapeutic plans. At the end of the day, another set of rounds took place, to discuss again the inpatients and how the day appointments were handled.

Whenever the cases required any procedures, it was possible to observe the interventions, from endoscopy, computed tomography, ultrasound and magnetic resonance imaging (MRI) to treatments such as chemotherapy and radiotherapy (the latter not currently available in Portugal).

The traineeship also offered the opportunity to shadow world-class specialists, including Prof. Jane Dobson in Oncology, Prof. Paul Freeman in Neurology and Drs. Katie McCullen and Barbara Skelly in Internal Medicine. A hallmark of the experience was the strong multidisciplinary involvement in case management, with specialists from different fields discussing cases closely. In addition, participation in the weekly journal club further enriched the training by encouraging critical thinking of current literature and evidence-based decision making.

SECTION 2 - CHARACTERIZATION OF A POPULATION OF DOGS WITH CUTANEOUS MAST CELL TUMORS TREATED AT AN ONCOLOGY REFERRAL CENTER IN PORTUGAL

1. INTRODUCTION

Cutaneous mast cell tumors (cMCTs) are among the most frequent malignant skin tumors in dogs. Their significance comes both from their high frequency and heterogeneity, with a varied biological behavior that can range from small, slow-growing nodules to aggressive tumors with metastases. This diversity complicates prognosis and treatment planning.

Several prognostic indicators have been investigated, including histology grade, clinical stage, tumor size, location, proliferation and immunohistochemical markers. Genetic abnormalities, particularly in the c-KIT gene, have also been associated to more aggressive tumors. However, results are not always consistent across research and there is still a debate on the impact of some of the prognostic factors, for example, epidemiological parameters such as sex and reproductive status.

Surgery is typically the first-line therapy and can be curative in small, low-grade tumors with clear margins. In larger or more complex instances, complementary therapy is frequently required. Vinblastine is one of the most used chemotherapeutic drugs, either before (neoadjuvant) or after (adjuvant) surgery. Its cytoreductive effect has been described, but only a few studies have evaluated this specifically and the impact on long-term survival and recurrence remains uncertain.

This study aims to characterize a population of dogs with cMCTs, evaluate the impact of different prognostic factors and assess the efficacy of neoadjuvant vinblastine in tumor size reduction and outcome. The findings in this study are expected to provide knowledge that may support clinical decisions and contribute to future research on this disease.

2. CANINE CUTANEOUS MAST CELL TUMORS – LITERATURE REVIEW

2.1. Epidemiology

Cutaneous mast cell tumors (cMCTs) are among the most common skin neoplasms in dogs, representing between 7% and 21% of all cutaneous tumors diagnosed (Berlato et al., 2021; M.Vail et al., 2020; Ribeiro et al., 2022; Shoop et al., 2015; Villamil et al., 2011; Webster et al., 2008). These are considered the most frequent malignant skin tumors and the third most common tumor subtype in dogs (De Nardi et al., 2022; Villamil et al., 2011).

Mast Cell Tumors (MCTs) are also one of the most frequent malignant cutaneous tumors in cats, although less common than in dogs, and rare in other species (Kiupel, 2017; Oliveira et al., 2020).

2.1.1. Etiology

The etiology of MCTs in dogs remains largely unknown. Although chronic inflammation and exposure to irritants have been proposed as potential factors, no epidemiologic evidence supports the involvement of topical carcinogens in MCT development (De Nardi et al., 2022; M.Vail et al., 2020). Viral causes or horizontal transmission have not been demonstrated. Genetic factors likely play a significant role, with changes in the p53 tumor suppressor pathway identified in some cases, though true mutations are rare (M.Vail et al., 2020).

The most consistently described molecular abnormality in canine MCTs involves mutations in the c-kit proto-oncogene, which encodes the receptor tyrosine kinase KIT (De Nardi et al., 2022; Kiupel, 2017; M.Vail et al., 2020). KIT normally regulates mast cell proliferation, differentiation and survival by binding its ligand, stem cell factor (SCF). Mutations in c-kit result in ligand-independent activation of KIT, leading to uncontrolled mast cell proliferation and tumor development (De Nardi et al., 2022; Kiupel, 2017). These mutations are found in up to 45% of canine MCTs and are strongly associated with more aggressive tumor behavior, higher histologic grade, decreased survival times and increased rates of tumor recurrence (De Nardi et al., 2022; Shoop et al., 2015).

In addition to c-kit mutations, other cytogenetic abnormalities such as chromosomal copy number variations, including losses of chromosome 5 and gains of chromosome 31, have been linked to more aggressive tumor phenotypes (De Nardi et al., 2022).

2.1.2. Breed

MCTs occur most often in mixed-breed dogs (De Nardi et al., 2022). However, several pure breeds are at increased risk, including Boxers, Boston terriers, Bulldogs, Pugs, Labrador and Golden Retrievers, Cocker Spaniels, Schnauzers, Beagles, Weimaraners and Shar-peis (De Nardi et al., 2022; Mochizuki et al., 2017; M.Vail et al., 2020; Oliveira et al., 2020). Conversely, breeds such as German Shepherds, Chihuahuas, Poodles, and Yorkshire Terriers appear to have a lower risk of MCT development (De Nardi et al., 2022; Mochizuki et al., 2017).

2.1.3. Sex

Most studies report no gender predisposition for the development of cutaneous MCTs (De Nardi et al., 2022; Kiupel, 2017; Patnaik et al., 1984; Shoop et al., 2015). A few studies suggest an increased risk in male and intact dogs, while others report a higher incidence in females, particularly neutered females (Oliveira et al., 2020; Shoop et al., 2015).

2.1.4. Age

MCTs are more common in adult to older dogs, typically presenting between 7.5 and 10.5 years of age (De Nardi et al., 2022; O'Connell & Thomson, 2013; Oliveira et al., 2020; Patnaik et al., 1984; Shoop et al., 2015).

The risk increases with age, with dogs over 10 years having 41 times greater odds of developing MCT compared with those under 2 years (Bellamy & Berlato, 2021). Although rare, a case of spontaneous regression was described in a 3-week-old puppy, with lesions resolving over 28 weeks (Kiupel, 2017; M.Vail et al., 2020).

2.2. Clinical behavior

2.2.1. Morphology

Mast cell tumors exhibit significant variation in presentation and biological behavior, ranging from benign to highly aggressive forms (Mochizuki et al., 2017; Ong et al., 2025).

In dogs, MCTs most commonly appear as solitary nodules in the skin or subcutis (Escoda Llorens et al., 2025; Kiupel, 2017; M.Vail et al., 2020). Multiple tumors can also develop, particularly in breeds such as Boxers, Boston terriers, Golden Retrievers and Pugs. One study reported that 56% of pugs had between 2 and 7 tumors at distinct sites. Subcutaneous (scMCT) forms differ biologically and should be distinguished accordingly (Kiupel, 2017).

The clinical differences between cMCT and scMCT are tumor size, color change and hair loss, with hair loss being the most distinctive feature in cMCT (Itoh et al., 2024). This can be explained by the fact that cMCTs initially proliferate within the dermis (Itoh et al., 2024; Willmann et al., 2021), leading to early involvement of hair follicles and subsequent hair loss, whereas scMCTs are typically confined to the subcutaneous tissue (Itoh et al., 2024; Willmann et al., 2021), preserving the dermal structure (Itoh et al., 2024).

The gross appearance of Cutaneous MCTs is highly variable, ranging from small, raised skin masses to larger, ulcerated growths (Jaturakan, 2024). cMCTs may appear delimited, elevated, firm, soft or pruritic, and can have erythematous areas, subcutaneous tissue invasion or ulceration in up to 30% of cases (De Nardi et al., 2022). Well-circumscribed solitary tumors tend to grow slowly and may be present for months, while ulcerated and poorly circumscribed lesions are often rapidly expanding (Kiupel, 2017; M.Vail et al., 2020). With this extremely varied range of clinical appearances, they are sometimes inadvertently mistaken for nonneoplastic lesions (M.Vail et al., 2020).

2.2.2. Location

The trunk, perineum and inguino-genital regions are the most common sites of cMCTs in dogs ($\pm 50\%$), followed by the limbs ($\pm 40\%$) and the head and neck ($\pm 10\%$) (Bellamy & Berlato, 2021; De Nardi et al., 2022; M.Vail et al., 2020; Welle et al., 2008).

Although less common, MCTs can also develop in extracutaneous sites such as the conjunctiva, oral cavity, nasopharynx or spine (Kiupel, 2017; M.Vail et al., 2020). Visceral forms are rare, often associated with advanced or aggressive disease and have been documented in the gastrointestinal tract, liver and spleen (Oliveira et al., 2020).

When MCTs are found in multiple tissues, they most often represent metastases from a primary cutaneous lesion (Kiupel, 2017). Metastatic spread typically affects regional lymph nodes, spleen, liver, bone marrow and more rarely the lungs (De Nardi et al., 2022; Escoda Llorens et al., 2025). Disseminated MCTs and mast cell (MC) leukemia remain extremely rare in dogs (Kiupel, 2017).

2.2.3. Paraneoplastic Syndrome

Paraneoplastic syndromes can be described as the indirect effects of tumors, typically due to tumor synthesis and release of biologically active substances such as hormones, growth factors and cytokines (J. Elliott, 2014). In MCTs these effects are caused by the release of histamine, heparin, eosinophil chemotactic factor and proteolytic enzymes from mast cell granules (Jaturakan, 2024; Ong et al., 2025; Patnaik et al., 1984). These mediators can cause local and systemic signs including Darier's sign, gastrointestinal ulceration, coagulopathy, eosinophilia, hypotension and collapse (De Nardi et al., 2022; Ledur et al., 2023; Ong et al., 2025). Darier's sign is a clinical manifestation observed when mechanical manipulation of a MCT leads to degranulation of the mast cells, resulting in local erythema, wheal formation and swelling at the site (De Nardi et al., 2022; Macy, 1985; M.Vail et al., 2020).

2.3. Diagnosis

The diagnostic evaluation of dogs suspected of having MCTs aims to reach a definitive diagnosis through cytopathological and histopathological examination, define a complete clinical staging, identify concomitant paraneoplastic syndromes (De Nardi et al., 2022; Rogers, 1996; Welle et al., 2008) and identify relevant prognostic factors (Welle et al., 2008).

Although the diagnosis of MCTs by FNA cytology or histopathology is straightforward in the majority of cases (Welle et al., 2008), MCTs can have highly variable biological behavior and pathologists focus more of their efforts on accurately identifying more aggressive cases (Kiupel & Camus, 2019; Sledge et al., 2016).

2.3.1. Cytology

FNA cytology is the preferred and most frequently used method for the initial diagnosis of cutaneous and subcutaneous MCTs (Blackwood et al., 2012; Bostock, 1986; De Nardi et al., 2022; M.Vail et al., 2020), as it provides an accurate diagnosis in approximately 92-96% of cases (Blackwood et al., 2012; De Nardi et al., 2022; Sledge et al., 2016; Welle et al., 2008).

Most neoplastic mast cells appear as individualized, small to medium-sized round cells with abundant cytoplasmic granules that stain purplish red (metachromatic) (Bostock, 1986; Macy, 1985; M.Vail et al., 2020).

Cytological characteristics such as granulation, mitotic figures and nuclear features can suggest degree of differentiation (Macy, 1985; Welle et al., 2008). In anaplastic tumors with poor granulation, diagnosis may be more challenging (Kiupel & Camus, 2019; Macy, 1985; Rogers, 1996). Cytological grading systems based on the two-tier histological grading system have been proposed (Camus et al., 2016; De Nardi et al., 2022; Sledge et al., 2016). High-grade MCTs are identified by poor granulation or by the presence of two or more of the following: mitotic figures, anisokaryosis >50%, binucleation or multinucleation and nuclear pleomorphism (Bellamy & Berlato, 2022; Camus et al., 2016; De Nardi et al., 2022). MCTs are considered low grade when these characteristics are missing (Bellamy & Berlato, 2022; Camus et al., 2016; De Nardi et al., 2022). This system shows sensitivity of 85-88% and specificity of 95-97% compared to histopathology (Bellamy & Berlato, 2022; Camus et al., 2016; Hergt et al., 2016; Scarpa et al., 2016). However, approximately one third of MCTs identified as high grade cytologically are downgraded histologically (De Nardi et al., 2022; Sledge et al., 2016).

Even in poorly differentiated MCTs, some granules can be observed in cytologic preparations (Kiupel, 2017; Kiupel & Camus, 2019). However, granules occasionally do not stain with Diff-Quik, making Wright-Giemsa or toluidine blue preferred alternatives (Gieger et al., 2005; Kiupel et al., 2004; Macy, 1985; M.Vail et al., 2020). In highly cellular samples, free granules may obscure nuclei, making cytologic grading difficult (Kiupel, 2017).

Large numbers of eosinophils, due to the release of eosinophil chemotactic substances, are commonly seen in cytologic samples (Kiupel & Camus, 2019; Macy, 1985). The release of proteolytic enzymes, especially tryptase, stimulate fibroblasts proliferation and neovascularization, which may mislead interpretation by mimicking mesenchymal tumors (Kiupel & Camus, 2019).

Cytology of all cutaneous and subcutaneous masses is recommended before excision to ensure adequate surgical planning, as MCTs can resemble lipomas or other round cell tumors (Gieger et al., 2005; Rogers, 1996). Manipulation of the mass should be minimized prior to aspiration to avoid diagnostic confusion and degranulation (Kiupel & Camus, 2019).

2.3.2. Histopathology

Histopathology is considered the gold standard for grading and diagnosing MCTs in dogs (De Nardi et al., 2022; Gieger et al., 2005; Sledge et al., 2016; Stefanello et al., 2015; Willmann et al., 2021), providing useful information for treatment planning and prognosis (Blackwood et al., 2012; De Nardi et al., 2022; Oliveira et al., 2020; Sledge et al., 2016). Although cytology is applicable for initial diagnosis, histopathology after tumor excision is required to determine tumor grade and further direct complementary diagnostic exams and therapeutics (De Nardi et al., 2022; Dobson & Scase, 2007; Gieger et al., 2005; M.Vail et al., 2020; Thompson et al., 2011). The tissue samples for histopathological evaluation are typically obtained through excisional biopsies, however, when complete excision is not feasible, incisional biopsies may be used (De Nardi et al., 2022). The histopathology technique involves fixation of the neoplastic tissue in formalin, followed by routine processing and staining, usually with hematoxylin and eosin and occasionally with special stains such as toluidine blue, in order to identify the mast cell granules (Kiupel & Camus, 2019; Patnaik et al., 1984; Welle et al., 2008).

2.3.2.1. Histological grading systems

Several histologic grading schemes have been developed to grade MCTs based upon their differentiation and biologic behavior (Bardales et al., 2025; Camus et al., 2016; Kiupel, 2017; Martins et al., 2022; M.Vail et al., 2020). Among the 3 existing systems, 2 are in common use: the Patnaik system and the Kiupel system (Bardales et al., 2025; Camus et al., 2016).

The Patnaik grading system, developed in 1984, divides MCTs into three grades: Grade I (well-differentiated), Grade II (intermediately differentiated) and Grade III (poorly differentiated) (Catarino et al., 2025; Donnelly et al., 2015; Kiupel, 2017; M.Vail et al., 2020; Patnaik et al., 1984; Sledge et al., 2016; Welle et al., 2008; Willmann et al., 2021). (Table 1)

Table 1: Patnaik histological grading system for Mast Cell Tumors. Adapted from Patnaik et al., 1984

Grade I	MCTs are confined to the superficial dermis, separated by collagen bundles and located in interfollicular spaces and are composed of distinct, round, monomorphic neoplastic MCs that have a round nucleus and no nucleolus. Edema or necrosis are absent or minimal and mitoses are rare (< 2/10 HPF);
Grade II	MCTs are located in the superficial or deep dermis, may also infiltrate the subcutis and subjacent skeletal muscle and are more cellular than grade 1 MCTs. The neoplastic cells are often pleomorphic, less basophilic and have indented nuclei, with a single nucleolus. Diffuse edema and necrosis are common and mitoses are rare (0-2/HPF);

Grade III	MCTs are more cellular than grade 1 and 2 and are composed of packed populations of pleomorphic cells that have irregularly shaped nuclei and multiple nucleoli. Multinucleate and bizarre cells are common. Mitotic figures are frequent (3-6/HPF);
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The Bostock grading system, the first created and introduced in 1973, is similar to the Patnaik one. However, it applies grades in reverse order compared to Patnaik, making it less intuitive (Bostock, 1986; Sledge et al., 2016): Grade I tumors are poorly differentiated, Grade II intermediately differentiated and Grade III well-differentiated tumors (Bostock, 1986).

While the Patnaik system is widely used (Gieger et al., 2005; Kiupel, 2017; Sledge et al., 2016), it has been criticized as subjective, particularly when grading intermediate tumors, leading to inconsistent application between pathologists (Kiupel, 2017; M.Vail et al., 2020). Some studies have reported a high inter-observer variability in grading MCTs using the Patnaik system (Kiupel, 2017; Kiupel et al., 2011; Kiupel & Camus, 2019; M.Vail et al., 2020; Northrup et al., 2005a, 2005b). For example, one of the studies demonstrated that when each pathologist graded the material according to their own set of histologic criteria, there was 50.3% agreement among them (Northrup et al., 2005b) and when the Patnaik grading system was used, the total agreement only increased to 62.1% (Northrup et al., 2005b).

In order to overcome these limitations, a two-tier grading system (Table 2) was proposed by Kiupel et al. 2011. This system groups MCTs into low and high grades based on histological features such as mitotic count, multinucleation, bizarre nuclei and karyomegaly (Bardales et al., 2025; Berlato et al., 2021; Kiupel, 2017; Kiupel et al., 2011).

Table 2: Kiupel histological grading system for Mast Cell Tumors. Adapted from Kiupel et al., 2011

High-grade	The diagnosis of a high-grade MCT is based on the presence of any of the following criteria: (1) seven or more mitotic figures in 10 high-power fields (HPF); (2) three or more multinucleated cells that have three or more nuclei in 10 HPF; (3) three or more bizarre nuclei in 10 HPF; (4) karyomegaly and anisokaryosis, as defined by nuclear diameters of at least 10% of neoplastic cells varying by at least two times;
Low-grade	Absence of any of the above criteria;

The Kiupel system is preferred for its decreased inter-observer variation and providing 96.8% interobserver consistency (Camus et al., 2016; Kiupel, 2017; Kiupel & Camus, 2019; Sledge et al., 2016), while demonstrating strong correlations with overall survival, risk of metastasis and MCT-associated mortality (Bellamy & Berlato, 2022; Kiupel, 2017; Kiupel et al., 2011; Kiupel & Camus, 2019; M.Vail et al., 2020; Oliveira et al., 2020; Sabbatini et al., 2017; Sledge et al., 2016). Approximately 90% of MCTs are classified as low-grade under the Kiupel system (Kiupel, 2017; Kiupel et al., 2011) and around 15% of these low-grade MCTs may have

a more aggressive biological behavior (Kiupel et al., 2011; Stefanello et al., 2015; Willmann et al., 2021). By eliminating the intermediate-grade category and focusing on clearly defined features, the Kiupel system offers more objective criteria and higher reproducibility (Bellamy & Berlato, 2022; Berlato et al., 2021; Kiupel, 2017; Kiupel et al., 2011; Willmann et al., 2021).

Both grading systems play an important role in the clinical management of MCTs. While the Patnaik system remains the "gold standard" in some regions (De Nardi et al., 2022), studies suggest that the Kiupel system is more reliable for predicting aggressive tumor behavior and outcomes (Bellamy & Berlato, 2022; Kiupel, 2017; Sledge et al., 2016; Willmann et al., 2021).

Today, it is common practice for pathologists to provide a grade using both the Patnaik and Kiupel systems as a clinical consensus of which is the most useful has not yet been reached (Bardales et al., 2025; Sledge et al., 2016).

2.3.2.2. Surgical margins

Evaluation of tumor margins is an important part of assessing excised cutaneous MCTs, however it can be challenging and may be of limited prognostic significance (Sledge et al., 2016). Numerous low-grade cutaneous MCTs are not encapsulated, but the margins of the tumors are well demarcated and easy to identify, however, in more aggressive MCTs, neoplastic cells invade surrounding tissue, complicating margin evaluation (Kiupel, 2017). Many cutaneous MCTs have a reactive halo composed of edema, inflammatory cells, mast cells and reactive stromal cells surrounding newly formed capillaries. This halo can be several centimeters thick, making it difficult to determine tumor margins grossly (Kiupel, 2017; Kiupel & Camus, 2019). While some mast cells in the halo might be neoplastic cells, others are "inflammatory" mast cells attracted by chemokines (Kiupel, 2017). Until reliable markers are available, satellites (clusters of five or more mast cells) are assumed to be neoplastic, whereas single, well-differentiated mast cells are considered "inflammatory" (Kiupel, 2017; Kiupel & Camus, 2019). Secondary inflammation, ulceration and necrosis further complicate margin evaluation (Kiupel & Camus, 2019).

Ideally, surgical margins should be inked and submitted for complete margin evaluation (Kiupel, 2017; Kiupel & Camus, 2019; Sledge et al., 2016). Margins must be reported clearly, including if surrounding tissues were trimmed during surgery, to ensure accurate margin assessment (Kiupel, 2017).

The recommended histopathological method includes a combination of tangential sections to assess margin cleanliness and radial sections to measure the distance of neoplastic cells to these margins (Kiupel, 2017; Ong et al., 2025). Margins are typically defined as "clean" if 10 mm or more of normal tissue is present between tumor and margin, and "close" if the distance is 1 to 9 mm (Gieger et al., 2005). Another classification defines margins as complete resection (no tumor cells within 1mm), complete but close (mast cells within 1mm), or

incomplete (mast cells at the surgical margin) (Blackwood et al., 2012). Numeric margins on radial sections should be reported as M1 = margin infiltrated; M2 = margin is close, within 1-2 mm; M3 = margin is clean 2-5 mm; M4 = margin is clean >5 mm. This method will ensure consistency and provide the most accurate information for further therapeutic intervention and also allow for comparison of data between future studies (Kiupel, 2017). The pathologist might also define the margins by the length alone, in millimeters, without a specific classification, for the oncologist or clinician to choose the classification that suits better their clinical setting.

2.4. Staging

Clinical staging and histopathological grading are the main tools for estimating biological behavior and disease extent, both are essential for therapeutic decision-making and prognostication (Giantin et al., 2025). Staging is done by the oncologist using clinical criteria, histopathology and cytology (Kiupel, 2017) and means to assess the extent of its local and systemic spread. The exercise of staging a MCT is valuable in making therapeutic decisions about whether local therapies such as surgery and radiation are likely to be curative or whether systemic therapy is indicated (Rogers, 1996). Once the diagnosis of MCT has been made, appropriate staging should be carried out (Bellamy & Berlato, 2022; Blackwood et al., 2012).

2.4.1. Staging Systems

Independently of the histopathological grading, all MCTs are also staged based on the clinical staging system of the World Health Organization (WHO) published by Owen et al. in 1980. Table 3 resumes the WHO staging system.

Table 3: World Health Organization Clinical Staging System for Mast Cell Tumors. Adapted from Owen et al., 1980

STAGE	DESCRIPTION
I	Solitary tumor confined to the dermis without regional lymph node involvement. Ia. Without systemic signs Ib. With systemic signs
II	Solitary tumor confined to the dermis with regional lymph node involvement. IIa. Without systemic signs IIb. With systemic signs
III	Multiple dermal tumors or large, infiltrating tumors, with or without regional lymph node involvement. IIIa. Without systemic signs IIIb. With systemic signs

IV	Any tumor presenting with distant metastasis, including blood or bone marrow involvement.
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The WHO staging system is vague in the definition of stage III and combines “multiple cutaneous nodules” with “large infiltrating tumor” (Dobson & Scase, 2007) and several studies suggest that no difference in outcome exists between patients with single MCTs and those with multiple MCTs of the same histological grade (Mullins et al., 2006; Thamm et al., 2006; Welle et al., 2008). A consensus panel of the European Society of Veterinary Oncologists and the Veterinary Cancer Society recommended, therefore, that the criteria between stages 2 and 3 should be switched, since lymph node spread carried a worse prognosis than simple detection of multiple cutaneous MCTs (Kiupel, 2017). Therefore, an adjusted staging system has recently been proposed where an additional stage with disseminated/multiple cutaneous MCTs (≥ 3 cutaneous MCTs) without lymph node or other organ involvement is included (De Nardi et al., 2022; J. W. Elliott et al., 2016; Willmann et al., 2021).

2.4.2. Lymph Node and Metastasis

Mast cell tumors frequently metastasize to lymph nodes (LN) first and then to the spleen, liver and other visceral organs (Giantin et al., 2025; Gross et al., 2005; M.Vail et al., 2020; O’Keefe, 1990; Ribeiro et al., 2022; Welle et al., 2008), while lung involvement is very rare (Giantin et al., 2025; M.Vail et al., 2020; Ribeiro et al., 2022; Valent et al., 2017; Welle et al., 2008). Presence or absence of MCTs in the draining LN is one of the key components of staging cutaneous MCTs (Kiupel, 2017). Histological analysis and classification of LN metastasis according to Weishaar et al. (2014) is considered a standard approach for staging and evaluate the prognosis of dogs with MCT (Stefanello et al., 2024).

Accurate determination of stage 2 MCTs requires proper identification of the draining lymph node (Marconato et al., 2020; Sledge et al., 2016). Sentinel lymph node (SLN) mapping has been shown to be important for staging in dogs with MCTs (Giantin et al., 2025) and are not necessarily the closest anatomic regional LN (Crescio et al., 2022; Giantin et al., 2025; Graf et al., 2018; Marconato et al., 2020). Approximately 40% of draining nodes would be missed if only the anatomically closest node were sampled (Kiupel & Camus, 2019).

Solitary mast cells are commonly observed within lymph nodes, especially with special stains, but may represent inflammatory rather than neoplastic cells (Rogers, 1996; Sledge et al., 2016). Therefore, an occasional solitary MC is not indicative of metastasis, clustering and aggregates are more concerning (Krick et al., 2009; M.Vail et al., 2020).

A classification scheme for MC infiltrates has been proposed for lymph node FNAs and has been shown to correlate with survival (Kiupel, 2017; Krick et al., 2009). LN with metastases (HN3) were identified by disruption of normal LN architecture with discrete foci, nodules, sheets

or masses of neoplastic MCs (Kiupel, 2017; Krick et al., 2009; Weishaar et al., 2014). Removal of a cytologically suspicious LN for histologic assessment may be necessary to determine if the MCs present truly represent metastatic disease (M.Vail et al., 2020).

2.4.3. Complementary Examinations for Staging

Not every dog requires full staging and examination of the lymph nodes is sufficient for most low-grade MCTs (Bellamy & Berlato, 2022; Kiupel, 2017). If lymph node metastasis is confirmed or the cutaneous tumor is graded as high grade, full staging and systemic therapy should be considered (De Nardi et al., 2022; M.Vail et al., 2020; Sledge et al., 2016; Willmann et al., 2021). A complete staging workup should ideally include a thorough physical examination, complete blood count and serum biochemistry, cytology of the regional lymph node (even if normal in size), abdominal ultrasound (with or without FNA of spleen and liver) and in selected cases FNA of the bone marrow and thoracic radiographs (Blackwood et al., 2012; M.Vail et al., 2020; Sledge et al., 2016; Welle et al., 2008; Willmann et al., 2021).

Abdominal imaging is strongly recommended in cases with clinical or histopathologic criteria indicative of an aggressive disease (Horta et al., 2018; Sledge et al., 2016; Willmann et al., 2021). Although routine aspiration of ultrasonographical normal spleen and liver in the staging of high-grade mast cell tumors might be controversial, cytologic evaluation by FNA of the spleen and liver is indicated regardless of ultrasonographic appearance, based on its prognostic association (Bellamy & Berlato, 2022; Book et al., 2011; De Nardi et al., 2022; Stefanello et al., 2009).

Although thoracic radiographs rarely demonstrate metastasis, it is reasonable to look for them before an invasive procedure to rule out occult cardiopulmonary disease (M.Vail et al., 2020; Rogers, 1996).

Buffy coat smears have historically been used to detect circulating MC in peripheral blood (Dobson & Scase, 2007; Macy, 1985; M.Vail et al., 2020; Sledge et al., 2016). However, this technique is now considered outdated due to its poor sensitivity and specificity, as circulating mast cells are rarely observed, even in dogs with advanced disease (Blackwood et al., 2012; Dobson & Scase, 2007; M.Vail et al., 2020; Sledge et al., 2016; Welle et al., 2008).

Bone marrow (BM) aspiration is no longer recommended as a routine staging since BM infiltration is rare in the absence of systemic signs or high-grade disease and the diagnostic value is low (M.Vail et al., 2020; Welle et al., 2008).

2.5. Prognosis

Prognosis evaluation of canine MCTs is highly variable, depending on multiple factors (Blackwood et al., 2012; Welle et al., 2008). Prognostic factors include histological grade, clinical stage, tumor size, anatomic location, mitotic index, recurrence history, breed, age, LN

involvement and metastasis existence (Ledur et al., 2023; Thompson et al., 2011; Welle et al., 2008). Molecular and immunohistochemical markers, as Ki-67, AgNOR, KIT expression pattern and C-Kit mutations, have improved prognostic accuracy, particularly in intermediate to high-grade cases (De Nardi et al., 2022; Kiupel & Camus, 2019; Webster et al., 2008).

No single factor can fully predict biological behavior or treatment outcomes (M.Vail et al., 2020; Ribeiro et al., 2022; Webster et al., 2008). The combination of several factors offers the most reliable approach to prognosis, guiding therapeutic decision-making and avoiding under or over treatment (M.Vail et al., 2020; Ong et al., 2025; Ribeiro et al., 2022).

2.5.1. Epidemiological factors

Some epidemiological factors, especially age, breed and sex, correlate with prognosis and tumor grade in canine MCTs (Blackwood et al., 2012; Catarino et al., 2025; Mochizuki et al., 2017; Oliveira et al., 2020; Shoop et al., 2015).

Regarding age, older dogs are more prone to develop high-grade tumors, with significant differences in mean age at diagnosis among grades: 7.6 years for grade I, 8.1 for grade II and 9.2 for grade III (Catarino et al., 2025). Dogs with more than 10 years old are 41 times more likely to develop MCTs compared to dogs under the age 2 (Catarino et al., 2025; Reynolds et al., 2019).

Breed also influences prognosis. Boxers and Pugs usually develop low to intermediate-grade tumors, while Labradors and Shar-Peis are more predisposed to aggressive, high-grade tumors (Blackwood et al., 2012; M.Vail et al., 2020; Oliveira et al., 2020; Welle et al., 2008).

Sex appears to have limited prognostic relevance. While some studies demonstrate a higher incidence of multiple MCTs in spayed females (Mullins et al., 2006; O'Connell & Thomson, 2013), the majority show no obvious connection with tumor grade or outcome (Catarino et al., 2025; Shoop et al., 2015). On the other hand, Bellamy & Berlato (2021) and Mochizuki et al. (2017) reported that male dogs are approximately 25% more likely to develop high-grade tumors, with this trend observed in both intact and neutered animals.

2.5.2. Clinical signs

Local signs suggestive of aggressive behavior such as ulceration, local inflammation, rapid growth, poor demarcation, satellite nodules and paraneoplastic signs, are linked with worse outcomes (Blackwood et al., 2012; Kiupel, 2017). Systemic signs including vomiting, anorexia, melena, diarrhea, widespread erythema and edema are associated to mast cell degranulation and indicate more extensive disease, frequently linked to a reserved prognosis (Ledur et al., 2023; M.Vail et al., 2020). GI signs, in particular, are recognized as negative prognostic factors for survival (Mullins et al., 2006).

2.5.3. Location

Tumor location is a documented prognostic factor, although findings are not always consistent (Catarino et al., 2025; Ribeiro et al., 2022; Willmann et al., 2021). MCTs in the inguinal, perineal, preputial, mucocutaneous, oral and nail bed regions have traditionally been linked with more aggressive biological behavior, higher metastatic potential and decreased survival time (ST) (J. M. Dobson & Scase, 2007; Gieger et al., 2005; M.Vail et al., 2020; Ribeiro et al., 2022; Welle et al., 2008). On the contrary, tumors on the limbs (excluding digits) are associated with longer disease-free intervals, reduced metastatic rates and better results to adjunctive therapies as radiation (Gieger et al., 2005; Welle et al., 2008).

2.5.4. Number of tumors

Most dogs present with solitary MCTs, however 10-21% may develop multiple tumors simultaneously or sequentially (Kiupel, 2017; Mullins et al., 2006). The prognosis of multiple tumors remains unclear. While some studies observed no difference in outcome compared to single MCTs (M.Vail et al., 2020), others indicated a poor prognosis in dogs with multiple synchronous MCTs (Kiupel & Camus, 2019). Each lesion should be assessed individually, with grade and other prognostic factors applied to each tumor (De Nardi et al., 2022).

2.5.5. Tumor size and Growth rate

The prognosis is also influenced by tumor growth and size. MCTs that remain localized and stable for longer periods of time (months or years) are typically benign (Kiupel, 2017; M.Vail et al., 2020; Welle et al., 2008). On the contrary, rapid tumor growth is associated with a worse prognosis. For example, only 25% of dogs with tumors present for less than 28 weeks survived more than 30 weeks, compared to 83% with tumors present for longer periods of time (M.Vail et al., 2020). Larger tumor sizes have also been associated with shorter disease-free intervals (DFI) and lower survival times (Rogers, 1996; Welle et al., 2008).

2.5.6. Histological grade

Histological grade is the most consistent and reliable prognostic factor (M.Vail et al., 2020; Ong et al., 2025). It has a direct correlation with survival time, disease-free interval and metastatic potential (Gieger et al., 2005).

The Patnaik 3-tier and Kiupel 2-tier grading systems are widely used and proven as independent prognostic tools (Berlato et al., 2021; Kiupel, 2017; Sabattini et al., 2015). When used together, cutaneous MCTs can be classified in 4 categories in order to provide a more precise prognosis: Grade I/low-grade, Grade II/low-grade, Grade II/high-grade, and Grade

III/high-grade, with prognosis ranging from excellent to poor (Berlato et al., 2021; Horta et al., 2018; Sabattini et al., 2015).

Grade I/low-grade MCTs generally have an excellent prognosis and are frequently cured by surgical excision alone, with long-term survival rates reaching more than 90% and low metastatic potential (<5%) (Berlato et al., 2021; Blackwood et al., 2012; Webster et al., 2007). Patnaik et al. (1984) reported that 93% of dogs with grade I MCTs survived for more than 1500 days following diagnosis.

Intermediate-grade (Patnaik grade II) MCTs remains the most challenging category, exhibiting highly variable behavior. While approximately 50% of patients with these tumors experience long-term survival, 5% to 22% develop metastases and approximately 44% might develop new MCTs after treatment (Book et al., 2011; Camus et al., 2016; Welle et al., 2008). Dogs with Grade II/low-grade tumors generally have a good prognosis, with studies demonstrating that only 3-17% of dogs died from MCT-related causes, while 94% were still alive after one year. In contrast, Grade II/high-grade tumors carry a more guarded prognosis, with 14-56% of dogs dying due to MCT-related causes, a median survival time (MST) ranging from 7.5 to 23.3 months and only 46% survival after one year (Berlato et al., 2021; Horta et al., 2018; Sabattini et al., 2015).

Grade III/high-grade MCTs, on the other hand, are associated with poor outcomes, aggressive behavior and high rates of recurrence and metastasis (55-96%) (Book et al., 2011; Ong et al., 2025). Compared to low-grade MCT, high-grade MCT are 50 times more likely to result in MCT-related death and the MST is usually less than 4 months (Mochizuki et al., 2017). Kiupel (2017) reported that 71% of dogs with high-grade MCTs died within one year, compared to only 6% in low-grade cases. A poor prognosis is further associated with histological features such as a high mitotic count (>4/10 HPF), multinucleation and specific KIT expression patterns (Escoda Llorens et al., 2025; Kiupel, 2017; Thompson et al., 2011).

Under the Kiupel System, dogs with low-grade tumors had a median survival time of more than 2 years, while those with high-grade tumors had median survival times of less than 4 months (Kiupel & Camus, 2019).

Although crucial, histologic grade must be interpreted in conjunction with other clinical and molecular parameters (De Nardi et al., 2022; Sledge et al., 2016). While some low-grade tumors can behave aggressively, certain high-grade tumors may exhibit less malignant behavior, especially when smaller and with low mitotic activity (De Nardi et al., 2022; Moore et al., 2020).

2.5.7. Clinical stage

Clinical stage is an important prognostic factor, often used in combination with histological grade to guide treatment decisions (Giantin et al., 2025; M.Vail et al., 2020). In

general, lower stages (0 and I) are associated with better outcomes (Welle et al., 2008), whereas the presence of regional or distant metastases significantly decreases survival time (ST) (O'Connell & Thomson, 2013; Welle et al., 2008).

Lymph node metastasis is a well-known negative prognostic indicator (Marconato et al., 2020; Stefanello et al., 2024; Weishaar et al., 2014). The histologic classification of LN, such as the HN0-HN3 system, provides useful prognostic information, with HN2 and HN3 being associated with significantly shorter disease-free interval and survival time than HN0-HN1 (Kiupel, 2017; Weishaar et al., 2014). Removal of metastatic LNs has been associated to better outcomes especially when combined with chemotherapy (Marconato et al., 2020; Stefanello et al., 2009). Metastasis in the spleen, liver or bone marrow are linked to a poor prognosis and decreased survival times (Book et al., 2011; De Nardi et al., 2022).

Despite some discrepancies in the prognostic impact of WHO staging (Gieger et al., 2005), clinical stage is still an independent outcome predictor (Mullins et al., 2006). Early diagnosis is crucial, especially for high-grade MCTs, since the presence of metastasis at initial presentation significantly limits the prognosis (Ong et al., 2025; Ribeiro et al., 2022).

2.5.8. Recurrence

Local recurrence (LR) is a major aspect in the management of MCTs, especially for high-grade tumors and is defined as a mass associated with or beneath the surgical scar (Donnelly et al., 2015). Ong et al. (2025) demonstrated that inadequate surgical margins in high-grade MCTs enhanced the probability of local regrowth, which occurred in 58% of dogs and even after complete excision, 26% of cases had recurrence. This is consistent with previous studies which showed recurrence in 36% of high-grade MCTs compared to only 4% in low-grade, despite complete histologic margins (Donnelly et al., 2015; Ong et al., 2025).

Donnelly et al. (2015) found that tumors that recurred were larger and had a higher mitotic index than those that did not. In the same study, the removal of MCTs with histologic clean margins, even if 3 mm, appeared adequate to prevent local recurrence in 96% of low-grade MCTs (Berlato et al., 2021; Donnelly et al., 2015).

Local recurrence is frequently a precursor of metastasis and poor outcomes, with MST of five to six months after recurrence (O'Connell & Thomson, 2013; Oliveira et al., 2020; Ong et al., 2025). LR and lymph node metastasis are the most common causes of treatment failure in high-grade MCTs (Ong et al., 2025). As a result, surgical revision or adjuvant radiation therapy should be discussed, even with complete excision (Donnelly et al., 2015).

Not all incompletely excised MCTs reappear. Recurrence is reported in only 10-30% of cases with proven incomplete margins (M.Vail et al., 2020). For low-grade MCTs with low proliferation rates recurrence is rare even after partial excision (Sledge et al., 2016).

The definition of recurrence should be precise and separate from new tumors at distant locations which might be de novo lesions than real recurrences (Kiupel, 2017).

2.5.9. Cell proliferation rate

Uncontrolled cellular proliferation is a hallmark of cancer and a significant prognostic factor in canine MCTs (Kiupel, 2017; Webster et al., 2007). Several proliferation markers have been studied to predict behavior, prognosis, recurrence, metastasis and survival time (Kiupel, 2017; Vascellari et al., 2013). Among these, the most widely used include mitotic index, Ki-67, argyrophilic nucleolar organizing regions (AgNOR) and proliferating cell nuclear antigen (PCNA) (Webster et al., 2007).

The mitotic index (MI), or mitotic count, is the most used and accessible proliferation marker. It counts the number of mitoses per 10 high-power fields (HPF) in hematoxylin and eosin-stained histological sections (Berlato et al., 2015, 2021; Romansik et al., 2007; Vascellari et al., 2013). MI has considerable predictive relevance, with studies showing that dogs with a $MI \leq 5$ have considerably longer MST, often exceeding 70 months, while dogs with $MI > 5$ exhibit MST between 2 to 5 months (Bellamy & Berlato, 2022; De Nardi et al., 2022; Romansik et al., 2007). Some researchers have demonstrated that a $MI > 7$ might be an even stronger prognostic indicator for poor outcome and recurrence, providing a potentially superior cut-off compared to the standard 5 mitoses per 10 HPF (Kiupel, 2017; Sledge et al., 2016).

Ki-67 is a nuclear protein expressed throughout the cell cycle in all active phases and absent in resting cells (Kiupel, 2017). It reflects the growth fraction and is independently related to survival, recurrence and metastasis, regardless of tumor grade (Blackwood et al., 2012; Gieger et al., 2005; Kiupel, 2017).

AgNORs, visualized by silver-based staining, represent how quickly cells progress through the cell cycle, the proliferation rate (Gieger et al., 2005; Welle et al., 2008). A higher AgNOR count is associated with higher tumor grade and shorter survival (Gieger et al., 2005). However, AgNORs are not considered independent prognostic indicators since their value is strongly related to tumor grade (Dobson & Scase, 2007).

When combined, the AgNOR \times Ki-67 score has demonstrated a higher predictive power. A score higher than 54 is strongly linked with an increased risk of MCT-related mortality and metastasis (Kiupel, 2017; Sledge et al., 2016). This score incorporates both the proliferation rate and growth fraction, resulting in a more complete assessment of tumor aggressiveness (Sledge et al., 2016).

PCNA is implicated in DNA replication and repair. It is thought to be less accurate for prognostic evaluation than Ki-67 and AgNOR (Bostock, 1986; M.Vail et al., 2020).

Although proliferation markers are useful, consistency between laboratories remains a challenge, particularly for Ki-67 cut-off, which can differ due to differences in staining and

counting methods (De Nardi et al., 2022; Webster et al., 2007). According to a study by Vascellari et al. (2013), the probability of mortality for a patient affected by a cMCT with a Ki-67 index > 10.6 was 8 times higher than one with a lower value. On the contrary, Scase et al. (2006) proposed a cut-off value for Ki-67 $> 1.8\%$ which was later confirmed by Maglennon et al. (2008) as a worse prognosis indicator for grade II MCTs. Additionally, Berlato et al. (2015) demonstrated that patients with grade II cMCTs with MI > 5 and Ki-67 $> 1.8\%$ had a significantly higher risk of MCT related death.

2.5.10. c-KIT mutation and KIT expression pattern

Mutations in the c-kit gene, which encodes the receptor tyrosine kinase KIT, play an important role in the pathogenesis and prognosis of MCTs (Bhanpattanakul et al., 2025). KIT promotes mast cell proliferation, differentiation, survival and migration (Kiupel & Camus, 2019; Webster et al., 2007). Under normal physiological settings, KIT activation is dependent on ligand binding, however, internal tandem duplications (ITDs) and other mutations in c-kit lead to consecutive activation of KIT, resulting in uncontrolled mast cell growth and neoplastic transformation (Kiupel, 2017).

The most prevalent c-kit mutations are ITDs located in exons 11 and 8 (Bhanpattanakul et al., 2025; Kiupel & Camus, 2019). Exon 11 mutations, accounting for 60-74% of all mutations, are linked to high-grade tumors and are considered a significant negative prognostic factor due to their correlation with higher local recurrence, metastasis and decreased ST (De Nardi et al., 2022; Kiupel, 2017). On the other hand, exon 8 mutations occur less frequently ($<5\%$) and have not been consistently associated with aggressive behavior (De Nardi et al., 2022; Willmann et al., 2021).

The incidence of c-kit mutations in canine MCTs ranges from 8-45%, with an increased prevalence in intermediate to high-grade tumors (Dobson & Scase, 2007; Gieger et al., 2005). Tumors with these mutations are considerably more likely to recur and metastasize than those without mutations (Kiupel, 2017). Molecular screening for c-kit mutations is indicated when TKI treatment is considered (Willmann et al., 2021).

KIT expression patterns assessed by immunohistochemistry (IHC) offer additional prognostic value (Kiupel & Camus, 2019), with abnormal localization associated with aggressive MCTs (Kiupel et al., 2004). Three KIT staining patterns have been described: pattern I (perimembranous), pattern II (focal cytoplasmic) and pattern III (diffuse cytoplasmic) (De Nardi et al., 2022; Kiupel & Camus, 2019). Patterns II and III, considered aberrant, correlate with higher proliferation indices, local recurrence, c-kit mutations and decreased ST (Webster et al., 2007; Welle et al., 2008). Aberrant cytoplasmic expression of KIT is frequently, but not always, associated with c-kit mutations (Sledge et al., 2016).

Given their prognostic significance, both c-kit mutation evaluation and KIT IHC staining patterns should be included in the diagnostic workup and staging (Bellamy & Berlato, 2022; Webster et al., 2007). Their combination with histologic grade and proliferation rates improves accuracy in predicting clinical behavior and directing treatment options (Bellamy & Berlato, 2022; Webster et al., 2007).

2.6. Treatment

Treatment options for mast cell tumors include local and systemic therapy or a combination of the different modalities (Macy, 1985; Oliveira et al., 2020). In most cases, the standard treatment is surgical excision. Even so, the treatment plan should be adapted to the individual characteristics of the MCT (Bhanpattanakul et al., 2025; De Nardi et al., 2022), considering factors such as histopathological grade, clinical stage, location (Bhanpattanakul et al., 2025; Martins et al., 2022) and overall clinical status of the patient (Rogers, 1996). Modalities such as radiotherapy or chemotherapy are considered for advanced cases or instances where surgical intervention alone proves inadequate (Bhanpattanakul et al., 2025) and can be used either as isolated or as adjuvant treatment (Martins et al., 2022). Treatment decisions are guided based on the presence or absence of negative prognostic factors (M.Vail et al., 2020; Welle et al., 2008).

Mast cell tumors manifest as a heterogenous disease (Rogers, 1996) and the complexity of cellular interactions between the tumour and immune system lead to a variety of treatment responses among patients (Bhanpattanakul et al., 2025). Treating both the primary disease and the paraneoplastic disorders commonly associated with MCT is imperative for optimal patient care (Rogers, 1996).

The following key questions should always be asked: (1) Is surgical excision possible?; (2) Is local therapy sufficient or is systemic therapy also required? (Blackwood et al., 2012).

2.6.1. Local treatment

2.6.1.1. Surgery

Surgery is the primary treatment option for MCTs and is curative in most cases, especially for localized, nonmetastatic tumors (Bellamy & Berlato, 2022; Blackwood et al., 2012; Webster et al., 2008). Traditionally, surgical excision with a 3 cm margin was suggested, although more recent studies propose a less aggressive approach with lateral margins of 1-2 cm and a deep margin of one fascial plane for low to intermediate-grade MCTs with a diameter of less than 5 cm (Blackwood et al., 2012; M.Vail et al., 2020; Welle et al., 2008). For high-grade tumors, larger margins of at least 3 cm laterally and one fascial plane deep are indicated (Blackwood et al., 2012; Donnelly et al., 2015; M.Vail et al., 2020; Sledge et al., 2016).

Preoperative diagnosis is critical for surgical planning, since it indicates the need for aggressive resection or further adjuvant treatment (Oliveira et al., 2020; Rogers, 1996). Planning the initial surgery to be definitive is crucial (Rogers, 1996), especially since scar tissue from past interventions might complicate later procedures (Rogers, 1996; Sledge et al., 2016).

New evidence indicates that certain low to intermediate-grade MCTs may not recur even without adjuvant therapy after excision with close margins (M.Vail et al., 2020; Vincenti & Findji, 2017). Nonetheless, complete surgical excision is critical for long-term management and cure of most MCTs, with the initial surgery giving the highest chance for a permanent cure (Donnelly et al., 2015; Sledge et al., 2016).

Surgical complications are most related to large, poorly differentiated MCTs and can include intraoperative hypotension, severe hemorrhage and tissue (De Nardi et al., 2022; Oliveira et al., 2020) as well as suture dehiscence, hemorrhage and delayed wound healing, linked mediators released by mast cells (Oliveira et al., 2020; Rogers, 1996).

Lymph node management, such as sentinel lymph node (SLN) mapping and lymphadenectomy, is becoming more significant in the surgical treatment of MCTs. Regional lymphadenectomy has shown therapeutic potential, considerably lowering the risk of distant metastasis and local recurrence while increasing survival outcomes (Bellamy & Berlato, 2022; De Nardi et al., 2022; Marconato et al., 2020).

However, identifying the correct lymph node can be difficult due to anatomical variances, small node size and overlaying adipose tissue, resulting in increased interest in SLN mapping techniques (Stefanello et al., 2024). From the SLN mapping techniques, lymphoscintigraphy is considered the gold standard (Chiti et al., 2025). When perioperative mapping is not available, intraoperative methylene blue staining alone provides a cost-effective option for successfully detecting SLNs in a high percentage of cases (Zanardi et al., 2025).

2.6.1.2. Radiotherapy

Radiotherapy (RT) is frequently used postoperatively when complete surgical resection is not possible (Blackwood et al., 2012; Dobson & Scase, 2007; Webster et al., 2008; Welle et al., 2008) or clean margins have not been achieved, particularly in grade II MCTs or tumors located on distal extremities (Dobson & Scase, 2007; Oliveira et al., 2020), giving long-term tumor control in the majority of cases (Oliveira et al., 2020). It is mostly effective against microscopic tumors, but tumor control has been documented in the presence of gross disease. Since it's a form of local treatment, it does not prevent LN metastasis or the formation of distant tumors (Oliveira et al., 2020). Combining surgery with adjuvant radiation has proven to be effective with 94-97% of cases remaining disease-free after 12 months (Dobson & Scase, 2007). Radiotherapy can also be the primary treatment when surgery is not an option due to tumor location or size (De Nardi et al., 2022).

Acute side effects of RT include erythema, moist desquamation and mucositis, which usually resolve with supportive care (Oliveira et al., 2020). Complications such as fibrosis, bone necrosis and secondary tumor formation might appear one year or longer after the treatment (De Nardi et al., 2022; Oliveira et al., 2020). Skin reactions are more common when irradiating areas as the face or perineum (Oliveira et al., 2020).

Access to radiation therapy is limited, either due to equipment availability, the high cost associated with the procedure or the requirement for several anesthetic sessions (Oliveira et al., 2020). Additionally, the necessity for specialized facilities and trained personnel makes this modality less accessible in general practice settings.

2.6.1.3. Eletrochemotherapy

Electrochemotherapy (ECT) combines the administration of chemotherapeutic drugs, usually bleomycin or cisplatin, with the application of electric pulses directly to the tumor. These pulses generate reversible momentary permeabilization of the cell membrane, increasing the local absorption and cytotoxic effects of chemotherapy drugs (De Nardi et al., 2022; Spugnini & Baldi, 2019).

ECT is considered a successful local treatment, producing complete and partial responses with minimal toxicity (Oliveira et al., 2020). It can be used as an isolated treatment, in conjunction with surgery or intraoperatively when complete surgical margins are not possible (De Nardi et al., 2022; Oliveira et al., 2020; Spugnini & Baldi, 2019). In cases involving large tumor masses (>3cm), the combination of surgery and intraoperative ECT is advised to avoid complications as delayed wound healing and extensive tissue necrosis (Oliveira et al., 2020).

2.6.2. Systemic treatment

2.6.2.1. Chemotherapy

Chemotherapy is usually indicated for dogs with high-grade, poorly differentiated or metastatic MCTs lesions (Oliveira et al., 2020). The primary objectives are to delay or prevent metastatic spread, give loco-regional control in microscopic disease, downstage tumors before surgery or radiation (neoadjuvant usage) and act as a palliative alternative in nonresectable or disseminated disease (Blackwood et al., 2012; Oliveira et al., 2020). Chemotherapy can also be used to minimize peritumoral inflammation and clinical signs, such as pruritus or ulceration, particularly in large or unresectable tumors (Rogers, 1996).

Several chemotherapeutic agents have been used in the treatment of canine MCTs, including vinblastine, lomustine, cyclophosphamide, vincristine, doxorubicin, methotrexate, chlorambucil and L-asparaginase (Rogers, 1996). Vinblastine and lomustine are the most used, typically in combination with corticosteroids (Blackwood et al., 2012; De Nardi et al.,

2022; Dobson & Scase, 2007). Vinblastine is considered a first-line agent, frequently administered at a dose of 2.0 mg/m² either weekly or every two weeks, combined with oral prednisolone (Blackwood et al., 2012; De Nardi et al., 2022). Lomustine, typically dosed at 60-90 mg/m² every 21 days, is widely used as a second-line agent or alternated with vinblastine in sequential protocols (De Nardi et al., 2022; Dobson & Scase, 2007). Combination therapy is generally more effective than monotherapy, with response rates reaching 64% (M.Vail et al., 2020; Welle et al., 2008).

Prednisolone exerts its effect by inhibiting growth factors and cytokines, suppressing granule formation in mast cells and decreasing peritumoral inflammation (De Nardi et al., 2022; Rogers, 1996). Although it has a limited cytotoxic effect, it contributes by reducing inflammation, edema and tumor volume, making it effective in both neoadjuvant and palliative contexts (Rogers, 1996). However, poorly differentiated tumors may be less sensitive due to reduced glucocorticoid receptor expression (Rogers, 1996).

Regarding chemotherapy toxicity, vinblastine is a recognized perivascular irritant that can induce myelosuppression, requiring hematological monitoring before each dosage (Blackwood et al., 2012; De Nardi et al., 2022). Lomustine is linked to both hepatotoxicity and myelosuppression, therefore, it is necessary to regularly check liver enzymes levels (Blackwood et al., 2012). Although 26% of dogs experience some side effects, the overall toxicity linked to combination procedures is usually manageable (Welle et al., 2008).

2.6.2.1.1. Neoadjuvant use of Vinblastine

In recent years, the use of vinblastine as a neoadjuvant therapy in canine cMCTs has increased, especially in cases where tumors are located in surgically challenging areas or too large for initial excision (Ossowska et al., 2023). The primary goal of this treatment is to enable complete excision without the necessity for radical surgery and potentially improving local control (Anderson et al., 2024; Ossowska et al., 2023).

One of the earliest studies to assess the efficacy of vinblastine in measurable MCTs was conducted by Rassnick et al. (2008), who reported response rates of 12% and 27%, at doses of 2.0 mg/m² and 3.5 mg/m², respectively, with only vinblastine. These results confirmed VBL cytoreductive properties, providing a foundation for its use in the neoadjuvant setting. More recently, (Ossowska et al., 2023) showed that combining both vinblastine and prednisolone produced a higher overall response rate of 40.9%, with a median tumor size reduction of 27%. Although this facilitated surgery in many patients, local recurrence still occurred in 20.8%, suggesting limited long-term impact (Ossowska et al., 2023).

To evaluate the outcomes of dogs receiving this treatment, Anderson et al., 2024 developed a study which demonstrated that neoadjuvant VBL prevented short-term tumor progression with an increased risk of local recurrence. Furthermore, there was no significant

difference in overall survival and prognosis appeared to be more related with tumor grade and mitotic index. Additionally, Olsen et al., (2018) reported a 90% response rate with VBL combined with prednisolone and toceranib, supporting its efficacy in more complex cases.

Vinblastine can effectively reduce tumor size, however, its impact on survival and recurrence remains uncertain and its use should be reserved for cases where surgery alone is insufficient or not an immediate option (Anderson et al., 2024; Ossowska et al., 2023; Rassnick et al., 2008).

2.6.2.2. Tigilanol tiglate

Another treatment modality for MCTs is intratumoral therapy with tigilanol tiglate (Stelfonta®, Virbac), an intratumoral antitumor agent that induces localized tumor destruction through selective protein kinase C activation (De Ridder, Campbell, et al., 2021). This mechanism triggers rapid vascular disruption, hemorrhagic necrosis and progressive elimination of the tumor mass, leading to local tumor elimination without the systemic effects associated with chemotherapy (Reddell et al., 2021). A recent study by Musser et al., (2024) demonstrated that 75% of tumors achieve complete response after a single intratumoral injection, with the possibility of further response after a second dose when indicated.

Tigilanol tiglate is indicated for non-metastatic cutaneous and subcutaneous MCTs that are not amenable to surgery or where adequate surgical margins would be difficult to achieve (Reddell et al., 2021). However, the usage of this agent is limited to localized disease, as it does not address nodal or distant metastasis and shows reduced effectiveness in high-grade or large-volume tumors (De Ridder, Reddell, et al., 2021)

Adverse effects are predominantly local wound formation, edema, pain and inflammatory exudate at the injection site, which typically resolves with appropriate wound management over several weeks (Musser et al., 2024).

2.6.2.3. Tyrosine Kinase Inhibitors

The introduction of tyrosine kinase inhibitors (TKIs), such as toceranib (Palladia®, Zoetis) and masitinib (Masivet®, AB Science), has increased therapy options for dogs with nonresectable, recurrent or metastatic grade II and III MCTs. These medications, by blocking ATP binding to the KIT receptor, inhibit the downstream signaling that is necessary for mast cell proliferation and angiogenesis (Blackwood et al., 2012; De Nardi et al., 2022; Kiupel, 2017). Toceranib has been associated to improved outcomes in dogs without lymph node metastasis (De Nardi et al., 2022). Masitinib is especially advised for tumors with confirmed c-Kit mutations since it has been found to extend time to progression and survival in these cases (Blackwood et al., 2012). Although KIT mutation status was before considered a key predictor of TKI

response, several studies have indicated that dogs without c-kit mutations, known as wild-type c-Kit, can also benefit from treatment (De Nardi et al., 2022; Sledge et al., 2016).

2.6.2.4. Complementary treatment

Complementary treatment is often required to manage the systemic effects of MC degranulation (De Nardi et al., 2022; M.Vail et al., 2020). Histamine release plays a major role in these effects and therapy typically includes H1 and H2 receptor blockers (Blackwood et al., 2012; De Nardi et al., 2022). Omeprazole, a proton pump inhibitor, may be beneficial in extensive disease or when antihistamines are insufficient (De Nardi et al., 2022).

These medications are advised in dogs with macroscopic disease, systemic signs or before surgery, radiotherapy or chemotherapy, where mast cell degranulation is expected (Oliveira et al., 2020; Welle et al., 2008). Antihistamines should be administered 30 to 60 minutes prior to manipulation or treatment to reduce complications (De Nardi et al., 2022).

Other MCs substances that may contribute to systemic effects is Heparin, released during surgical manipulation, resulting in bleeding complications. Protamine sulfate (heparin antagonist) can be used to control prolonged hemorrhage (De Nardi et al., 2022).

3. OBJECTIVES

This retrospective study aimed to: (1) characterize the population sample of dogs presented for consultation at the Oncology Referral Center - Anicura Atlântico Veterinary Hospital between January 2023 and December 2024, either referred by other veterinary centers for follow-up of a previously diagnosed mast cell tumor or as second-opinion cases; (2) evaluate the impact of epidemiological, clinical and histologic factors on overall survival; and (3) in dogs that received neoadjuvant vinblastine and prednisolone therapy, assess whether tumor reduction occurred, determine if the reduction was statistically significant and compare survival and disease-free interval outcomes with those in dogs that did not receive this treatment.

4. MATERIALS AND METHODS

4.1. Data collection

The medical records from dogs presented for consultation due to mast cell tumors from January 2023 to December 2024 were reviewed. The study was conducted retrospectively, analyzing data from cases that occurred during this period.

Data collection was performed in two steps. First, in order to compile the mast cell tumor cases database, patients with suspected or confirmed diagnosis of mast cell tumor within this period were selected using the hospital management software program OranGest Vet®. In a

second step, the clinical history of the previously selected cases with diagnosed cMCTs were collected, organized and imported into a Microsoft® Excel spreadsheet.

4.2. Patients' selection

Dogs were selected with the following inclusion criteria: (1) Dogs with a definitive cytologic or histopathologic diagnosis of cMCT between January 2023 and December 2024; (2) Dogs presented for first assessment at Anicura Atlântico Veterinary hospital or referred to this hospital from other veterinary centers due to cMCT.

Exclusion criteria consisted of the following: (1) Absence of a definitive diagnosis of cMCT; (2) Incomplete data regarding the animal, such as age and breed; (3) Incomplete medical history; (4) Patients with missing information relevant for the study; (5) Absence of complementary exams' results; (6) Presence of other type of neoplasm undergoing concurrent treatment.

4.3. Medical records review

Based on the data available in OranGest Vet®, the following information was collected for each patient:

- Patient signalment (age, sex, weight, reproductive status and breed), comorbidities Ongoing therapies, Referral status (referral from another clinic or in-patient) and presence of concomitant neoplasms.
- Method of diagnosis and date. If histopathology was performed, histopathological grade and margins, cell proliferation indicators, KIT expression pattern and lymph node grade were also collected.
- Clinical presentation of the cutaneous MCT (number and location), Tumor size, (measured with a caliper) and the presence or absence of systemic and local signs.
- Complementary exams for staging and presence of metastasis.
- Treatment protocol followed. If systemic chemotherapy was administered, the number of sessions and cytotoxic drug used, administration route and doses were recorded.
- Recurrence and clinical outcome (alive or dead, with date of death).

4.4. Parameters under analysis

Epidemiological data (such as breed, sex, age, weight and reproductive status), clinical data (including comorbidities, referral status, tumor location, method of diagnosis, type of lesion, number of lesions, tumor size, cell proliferation indicators, KIT expression pattern, histological grade as Patnaik and Kiupel grades, clinical stage and treatment) were collected from the clinical records of each animal. Survival time was calculated using the date of diagnosis and the date of death, or, for patients still alive, the date July 27, 2025. Disease free

interval was calculated for patients that underwent surgery using the date of the surgery and the date of recurrence or appearance of new nodules, for those with none of these, the date used was July 27, 2025. In case the clinical stage was missing from the clinical records, it was retrospectively determined using the World Health Organization Clinical Staging System for Mast Cell Tumors (Table 3).

4.5. Statistical analysis

Statistical analysis was conducted using IBM SPSS, version 30.0. Sample characterization was carried out using frequency tables for categorical variables and mean (M) and standard deviation (SD) for numerical variables.

Pearson correlation coefficients were used to examine associations between survival time and prognostic factors, including relationships between numerical and binary variables. Associations between prognostic factors and histological grade were analyzed using a series of chi-square tests. Whenever the asymptotic conditions for the chi-square test were not met, the exact significance value of the test statistics was assessed. For 2x2 tables, the Fisher's exact test was used and odds-ratio was estimated.

Survival analysis was carried out using the Kaplan-Meier method, with survival time as the dependent variable and survival status (alive or dead) as the censoring indicator. Animals that remained alive at the end of the study were classified as censored. Kaplan-Meier survival curves were generated for the categorical prognostic variables. Differences in survival distributions between groups were analyzed using the Log-Rank test, with results considered statistically significant at $p < 0.05$. Additionally, a Cox proportional hazards regression model was employed to evaluate the impact of the prognostic factors on survival time. The model included survival time as the dependent variable and event status as the censoring indicator.

To assess whether tumor dimensions were reduced following neoadjuvant vinblastine administration, the Shapiro-Wilk normality test was first applied to determine the appropriate use of parametric or non-parametric statistical tests for paired samples. Effect size was also calculated using Cohen's d , with interpretation thresholds defined as follows: $d < 0.20$ indicating a weak effect, $0.20 < d < 0.50$ a moderate effect, and $d > 0.50$ a strong effect (Rassnick et al., 2008). Similarly, normality tests were performed to decide the appropriate independent test for analyzing whether neoadjuvant VBL treatment increased survival time or DFI. To investigate the association between neoadjuvant VBL treatment and tumor recurrence, crosstabulation analysis was conducted and odds ratios were calculated.

For all the analysis 5% and 1% levels of significance were used.

5. RESULTS

5.1. Sample characterization

The sample consisted of 65 dogs. Regarding epidemiological data, 60.0% (N = 39) were male and 61.5% (N = 40) neutered. The most common breed was mixed breed, representing 24.6% (N = 16) of the sample, followed by the French Bulldog at 12.3% (N = 8). The dogs' ages ranged from 2 to 17 years, with a mean age of 9.0 years. Body weights varied between 2.6kg and 47.0kg, with a mean weight of 20.68 ± 11.90 kg. (Table 4)

As for clinical data, all dogs had cutaneous MCT, 64.6% (N = 42) presented no relevant comorbidities (NSAD, no significant abnormalities detected), with most being referrals from other veterinary centers (66.1%, N = 43). The limbs presented as the most common location (33.8%, N = 22), followed by the trunk (29.2%, N = 19) and head and neck (18.5%, N = 12). Diagnosis was established by cytology in 95.4% of the cases (N = 62) with 80.0% (N = 52) presenting as solitary lesions. Surgery was the first line of therapy for most cases (75.4%, N = 49), in 15.4% (N = 10) neoadjuvant vinblastine was administrated, 1.5% (N = 1) received either Palladia or Prednisolone and 6.2% (N = 4) had no treatment. Among the dogs with multiple lesions, the number ranged from 1 to 5, with an average of 3.33 ± 0.82 lesions per animal. The tumor dimension varied from 0.5 to 10.0 cm, with mean of 2.49 cm. (Table 5)

Table 4: Epidemiological variables characterization (N = 65)

Variables	Categories	n	%
Sex	Male	39	60.0
	Female	26	40.0
Reproductive status	No	40	61.5
	Yes	25	38.5
Dog Breed	Mixed	16	24.6
	French Bulldog	8	12.3
	Golden Retriever	6	9.2
	Labrador Retriever	6	9.2
	Boxer	4	6.2
	Pug	4	6.2
	Other	21	32.3
Variables		M	SD
Age (Years)		9.00	3.00
Weight (Kg)		20.68	11.90

Table 5: Clinical parameters characterization (N = 65)

Variables	Categories	n	%
Comorbidities	NSAD	42	64.6
	Dermatopathies	10	15.4
	Osteoarticular disease	5	7.7
	Cardiopathies	3	4.6
	Other	5	7.7
Referral cases	Yes	43	66.2
	No	22	33.8
Tumor Location	Limbs	22	33.8
	Trunk	19	29.2
	Head and Neck	12	18.5
	Scrotal	5	7.7
	Preputial	3	4.6
	Perineal	2	3.1
	Inguinal	2	3.1
Diagnosis Method	Histology	4	6.2
	Cytology	61	93.8
Type of lesion	Solitary	59	90.8
	Multiple	6	9.2
Treatment	Surgery	49	75.4
	Palladia	1	1.5
	Prednisolone	1	1.5
	Vinblastine	10	15.4
	No treatment	4	6.2
Variables		M	SD
Number of tumors (for those who had multiple)		3.33	0.82
Disease free interval (DFI)	(days)	362.5	287.7
Tumor dimension (cm)		2.49	1.94

5.1.1. Characterization of histological grade, cell proliferation rate, Kit expression pattern and staging

Regarding histological grade, most patients had MCT classified as Patnaik Grade II (76.5%, N = 39) and Kiupel Low Grade tumors (78.2%, N = 43). (Table 6)

As for cell proliferation markers, the majority showed a mitotic index of less than 1 per 10 HPF (84.3%, N = 43). Only 8 animals (15.7%) had a mitotic index higher than 1 per 10 HPF. Among these 8 animals, 5 (9.8%) exhibited a MI >5 per 10 HPF and Ki-67 index below than 1 in 77.0% (N = 30) of the cases. The KIT expression pattern was predominantly Pattern 1 (71.8%, N = 28). (Table 7)

Regarding tumor staging, 33.8% (N = 22) were classified as stage I(a), while 32.3% (N = 21) were stage III(a). Systemic signs were absent in 92.3% (N = 60) of patients and present in 7.7% (N = 5). (Table 8)

Table 6: Histological grade Characterization

Variables	Categories	<i>n</i>	%
Patnaik Grade (<i>N</i> = 51)	Grade I	9	17.6
	Grade II	39	76.5
	Grade III	3	5.9
	Missing or N/A	14	---
Kiupel Grade (<i>N</i> = 55)	Low Grade	43	78.2
	High Grade	12	21.8
	Missing or N/A	10	---

Table 7: Cell proliferation rate and KIT expression pattern Characterization

Variables	Categories	<i>n</i>	%
Mitotic Index (<i>N</i> = 51)	< 1/10 HPF	43	84.3
	> 1/10 HPF	8	15.7
	Missing or N/A	14	---
KIT (<i>N</i> = 39)	Pattern 1	28	71.8
	Pattern 2	8	20.5
	Pattern 3	3	7.7
	Missing or N/A	26	---
Ki-67 (<i>N</i> = 39)	Rate < 1	30	77.0
	Rate = 2	2	5.1
	Rate >2	7	17.9
	Missing or NA	26	---

Table 8: Staging Characterization (N = 61)

Variables	Categories	<i>n</i>	%
Staging	0(a)	7	11.5
	I(a)	22	36.1
	I(b)	1	1.6
	II(a)	3	4.9
	II(b)	3	4.9
	III(a)	21	34.4
	III(b)	2	3.3
	IV	2	3.3
	Missing	4	---

5.1.2. Characterization of patients that underwent surgical treatment as first-line therapy

Among the 49 dogs treated with surgery as the initial therapeutic approach, most tumors were classified as low grade (83.7%, *N* = 41) and Patnaik grade II (73.5%, *N* = 36), with clean margins (57.1%, *N* = 28) and no metastasis at the time of diagnosis (85.7%, *N* =

42). Of this population group, tumor recurrence was observed in only 18.4% (N = 9), while 81.6% showed no evidence of disease following surgery (N = 40). (Table 9)

Table 9: Characterization of patients with surgery as initial treatment by Patnaik grade, Kiupel grade, Margins, Metastasis and recurrence (N = 49)

Variables	Categories	n	%
Patnaik grade	Grade I	9	18.4
	Grade II	36	73.5
	Grade III	1	2.0
	Missing or N/A	3	---
Kiupel grade	Low-grade	41	83.7
	High-grade	8	16.3
Margins	Clean	28	57.1
	Close	14	35.9
	Infiltrated	7	14.3
Metastasis	No	42	85.7
	Yes	6	12.3
	Missing or NA	1	---
Recurrence	No	40	81.6
	Yes	9	18.4

5.2. Statistical associations

5.2.1. Statistical Association between binary or numeric Prognostic Factors with Survival

The variables age, tumor dimension and survival time were numeric and sex, reproductive status, tumor recurrence and systemic signs were binary, so Pearson correlation coefficient was used to analyze the relation between those. (Table 10)

The only statistically significant associations identified between survival time and the analyzed variables were with tumor size ($p = 0.028$) and reproductive status ($p = 0.001$). Specifically, larger tumor size was associated with shorter survival time, while intact dogs had a greater chance of survival. (Table 10)

Table 10: Association between prognostic factors and survival

Variables	Survival Time
Age (N = 65)	.034
Tumor dimension (N = 65)	-.272*
Time to recurrence (N = 10)	.124
Sex (N = 65)	.090
Reproductive status (N = 65)	.398**
Tumor Recurrence (N = 54)	.063
Systemic Signs (N = 65)	-.089
Mitotic Index (N = 51)	-.130

5.2.2. Statistical Association of reproductive status, sex and tumor dimension with Kiupel grade

The association between reproductive status and histological grade (Kiupel system) was assessed using the Chi-square test of independence and Fisher's exact test was additionally applied due to one expected frequency < 5. No statistically significant association was identified ($\chi^2 = 0.59$, $p = 0.44$; Fisher's exact test, $p = 0.32$). In the intact group, 30.0% of tumors were classified as high grade, compared to 17.1% in the neutered group. The odds ratio indicated that intact dogs had 2.05 times higher odds of presenting with a high-grade tumor (OR = 2.05, 95% CI = 0.51-8.24) compared to neutered animals, but this association did not reach statistical significance. (Table 11)

Table 11: Association between reproductive status and Kiupel Grade

Reproductive status	Kiupel-Grade		p	OR (95% CI)
	Low-grade	High-Grade		
Neutered	82.9% (29)	17.1% (6)	0.32	2.05 (0.51-8.24)
Intact	70% (14)	30% (6)		

The association between sex and Kiupel grade was obtained using the Chi-square test of independence and Fisher's exact test, applied due to one expected frequency < 5. Although no statistically significant association was identified ($\chi^2 = 0.53$, $p = 0.47$; Fisher's exact test, $p = 0.34$), the odds ratio indicated that males had 2.16 times higher odds of presenting with a high-grade tumor (OR = 2.16, 95% CI = 0.51-9.18) compared to females. In the male group, 26.5% of tumors were classified as high grade, compared to 14.3% in the female group. (Table 12)

Table 12: Association between Sex and Kiupel Grade

Sex	Kiupel-Grade		p-value	OR (95% CI)
	Low-grade	High-Grade		
Female	85.7% (18)	14.3% (3)	0.336	2.16 (0.51-9.16)
Male	73.5% (25)	26.5% (9)		

The relationship between tumor size (continuous variable) and Kiupel grade (binary: low vs. high) was evaluated using the Mann-Whitney U test, since tumor size did not follow a normal distribution. High-grade tumors had a median size of 3.25 cm, whereas low-grade tumors had a median of 2.00 cm. The difference between groups was statistically significant (Mann-Whitney U = 364.5, $p = 0.029$), indicating that larger tumors were more frequently classified as high grade. (Table 13)

Table 13: Association between tumor size and Kiupel Grade

Kiupel-Grade	<i>M ± SD (cm)</i>	<i>Median (cm)</i>	p-value	<i>Mann-Whitney</i>
Low-grade (n=43)	1.81 ± 0.90	2.00	0.029	364.5*
High-grade (n=12)	3.50 ± 2.56	3.25		

5.3. Survival analysis

Survival time ranged between 18 to 850 days, with median 539 days and mean 507.79 ± 211.98. From the 65 dogs, 84.6% (N = 55) were alive at the end of the study and 15.4% dead (N = 10).

5.3.1. Kaplan-Meier Survival Study

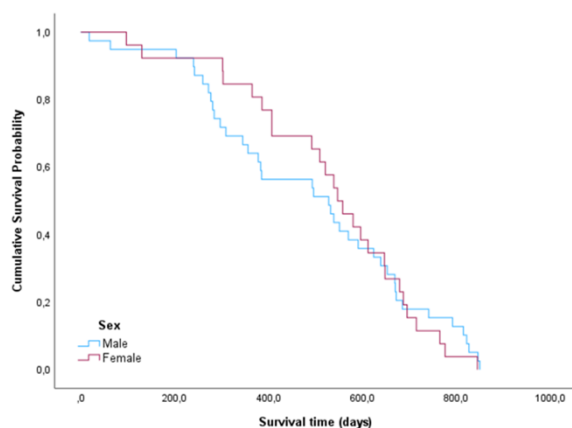
To facilitate the data visualization, the Kaplan-Meier curve is presented along with the median of the survival time.

In the survival analysis by sex, the median survival time (MST) was 547 days for females (95% confidence interval [CI]: 473.3 to 620.7; mean 531.1 ± 37.14) and 528 days for males (95% CI: 339.6 to 716.4; mean 492.3 ± 36.3). At 539 days (the overall median survival time), the probability of survival was 53.8% for females and 43.6% for males. The difference in survival time between sex was not statistically significant (p = 0.967) (Graphic 2).

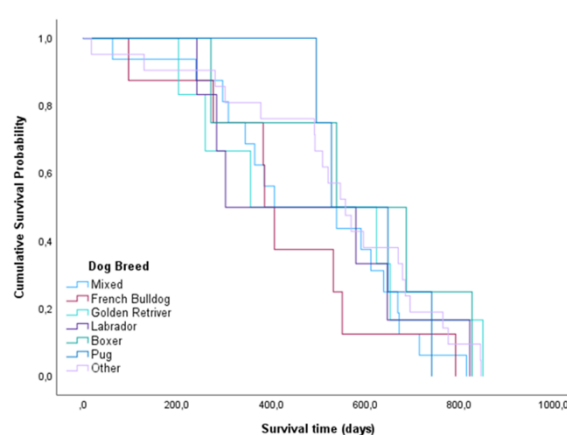
In the survival analysis by breed, Boxers had the highest MST at 539 days (95% CI: 132.3 to 945.7) with a mean of 581.3 ± 118.7. Pugs had a lower MST of 528 days (95% CI: 379.0 to 677.0) with a higher mean 603.3 ± 56.4. Mixed-breed dogs with 407 days (95% CI: 105.2 to 708.8) and mean 478.9 ± 51.6. Labradors had the lowest MST at 303 days and mean 479.9 ± 96.8.

At 539 days, the estimated survival probabilities were approximately 50.0% for Boxers and Pugs, 43.8% for mixed breeds, 38.4% for Golden Retrievers, 33.3% for Labradors and approximately 25.0% for French Bulldogs. However, no statistically significant differences in survival time were observed among breeds (p = 0.740) (Graphic 3).

Graphic 2: Survival Kaplan-Meier curve by Sex



Graphic 3: Survival Kaplan-Meier curve by Breed

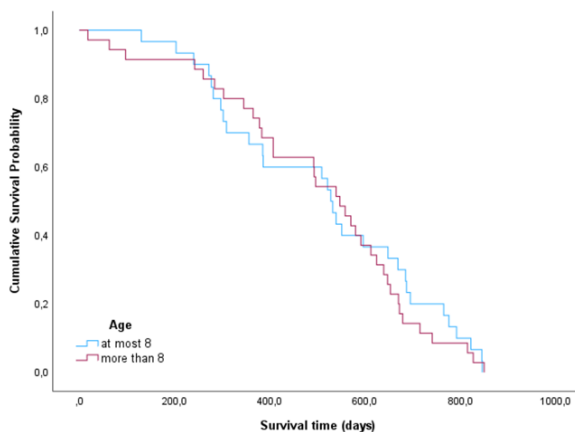


To assess the impact of age on survival, the numerical variable age was recoded into a binary variable, using a cut-off point of 8 years, allowing the comparison of survival outcomes between younger and older patients.

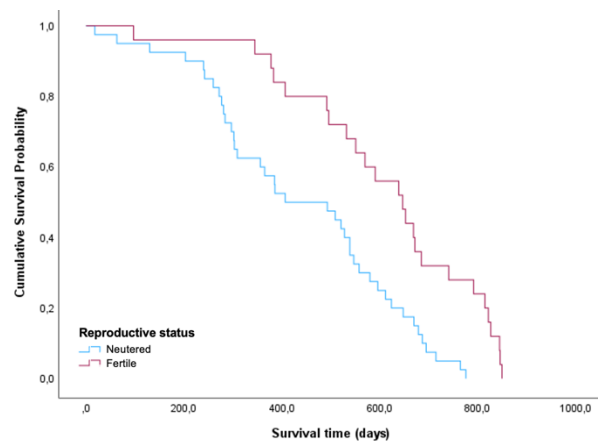
The analysis showed that patients aged eight years or younger had a MST of 528 days (95% confidence interval [CI]: 487.7 to 568.3) with a mean of 514.8 ± 39.4 days. Older patients (more than eight years) exhibited a MST of 547 days (95% CI: 457.8 to 636.2) and a mean of 501.8 ± 35.8 days. At the MST point, cumulative survival probabilities were 43.3% for the younger group and 51.4% for the older group. No statistically significant difference in survival was observed between the age groups ($p = 0.732$) (Graphic 4).

The analysis of reproductive status revealed that intact dogs had a median survival time of 647 days (95% [CI]: 545.8 to 748.2) and a mean survival of 613.8 ± 38.5 days. At the corresponding median point, the cumulative survival probability was approximately 40.0% for neutered dogs and 68.0% for intact dogs. The difference between groups was statistically significant ($p < 0.001$). (Graphic 5)

Graphic 4: Survival Kaplan-Meier curve by Age



Graphic 5: Survival Kaplan-Meier curve by Reproductive status

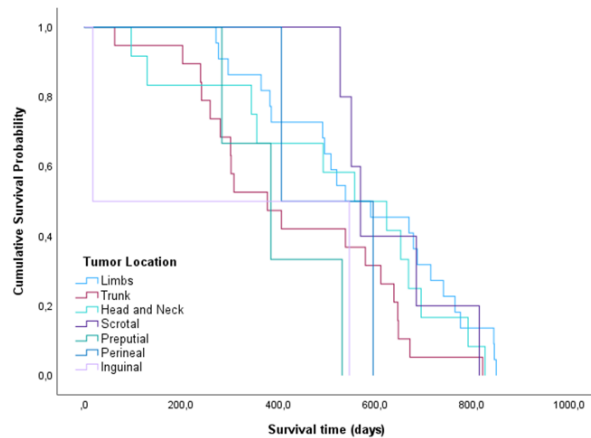


As for tumor location, the longest MST was observed when the tumor was located in the scrotum, with a median survival of 570 days (95% CI: 529.2 to 610.8) and a mean of 629.8 ± 53.6 days. The shortest ST was seen with tumors located in the inguinal region, with a survival time of 18 days, but only one case was observed. At 539 days, the estimated probability of survival was approximately 50.0% for tumors located on the limbs, 36.8% for the trunk, around 50.0% for head and neck tumors and 33.3% for preputial tumors. No significant difference in survival was found among tumor locations ($p = 0.084$) (Graphic 6).

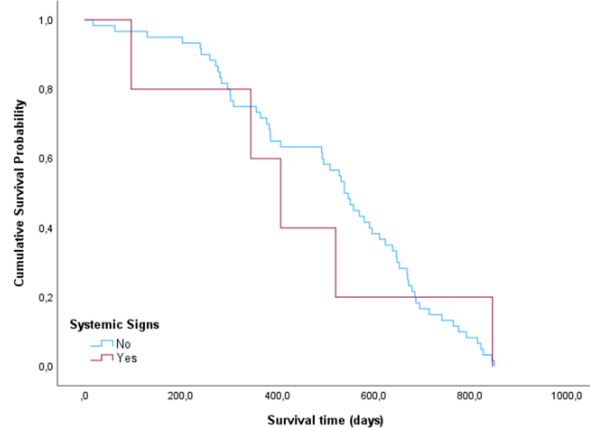
When systemic signs were absent, the MST was 539 days (95% confidence interval [CI]: 501.0 to 577.0) with a mean of 513.7 ± 26.9 days and the probability of survival was up to 50.0%. In contrast, when systemic signs were present, the MST decreased to 407 days (95% CI: 273.9 to 540.1), with an estimated survival probability of approximately 20.0%. However,

the difference in survival between the two groups was not statistically significant ($p = 0.925$) (Graphic 7).

Graphic 6: Survival Kaplan-Meier curve by Tumor location



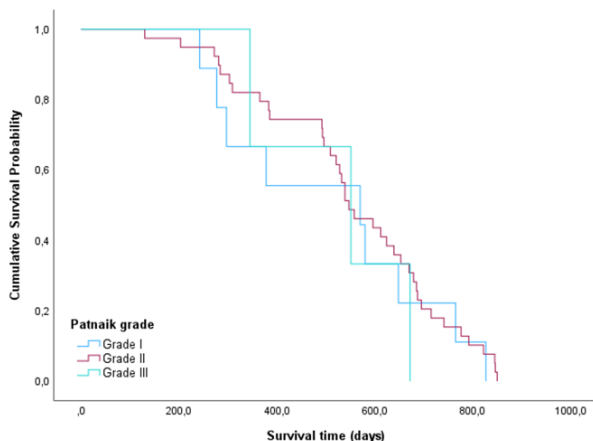
Graphic 7: Survival Kaplan-Meier curve by systemic signs



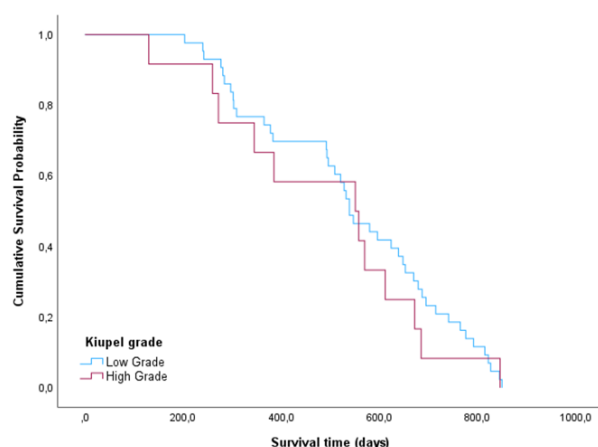
Regarding Patnaik grade, the MST was 570 days (95% confidence interval [CI]: 9.0 to 1131.0) with a mean of 509.3 ± 72.8 days for grade I; 547 days (95% CI: 468.7 to 625.3) with a mean of 553.7 ± 30.4 days for grade II; and 551 days (95% CI: 221.3 to 880.7) with a mean of 522.7 ± 95.5 days for grade III. The overall MST was 551 days. At this time point, survival probabilities were approximately 44.4% for grade I, 46.2% for grade II, and 33.3% for grade III tumors. No statistically significant differences in survival were observed among the grades ($p = 0.767$). (Graphic 8).

In the survival analysis by Kiupel grade, the overall MST was 547 days. For low-grade tumors, the median survival time was 539 days (95% CI: 472.2 to 605.8) with a mean of 546.0 ± 29.8 days. For high-grade tumors, the MST was 551 days (95% CI: 257.3 to 844.7) with a mean of 490.5 ± 60.9 days. At 547 days, the estimated probability of survival was 46.5% for low-grade and approximately 50.0% for high-grade tumors. No statistically significant difference in survival was observed between the grades ($p = 0.593$) (Graphic 9).

Graphic 8: Survival Kaplan-Meier curve by Patnaik grade



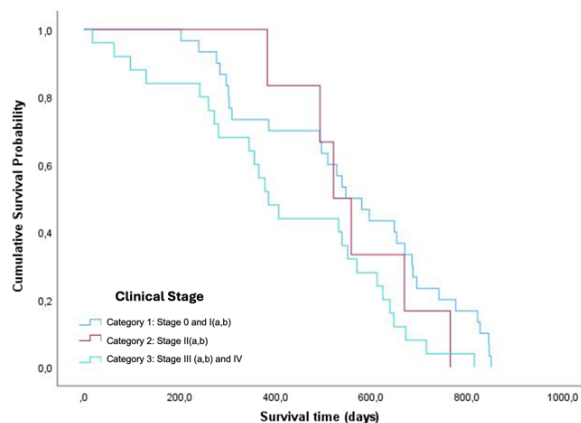
Graphic 9: Survival Kaplan-Meier curve by Kiupel grade



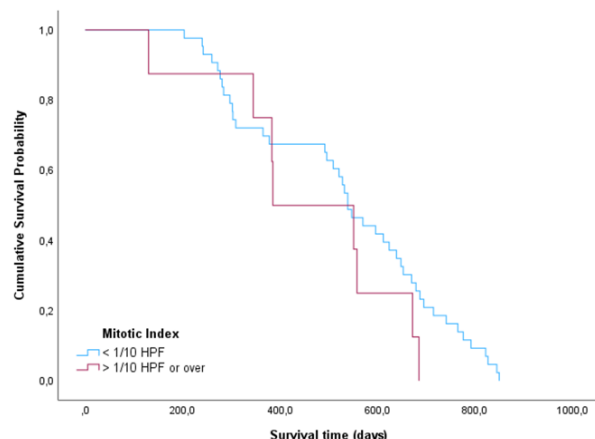
For the Clinical Stage, the analysis revealed that the highest MST was 547 days (95% confidence interval [CI]: 455.75 to 638.25) with a mean of 554.43 ± 37.75 days for Stages 0 and I and a mean of 564.83 ± 55.11 for Stage II. The lowest MST was observed for Stages III and IV tumors at 385 days (95% confidence interval [CI]: 316.46 to 453.55). The overall MST across the three stages was 532 days. At this median time point, the cumulative survival probabilities were approximately 53.3% for Stages 0 and I, 50.0% for Stage II and 40.0% for Stages III and IV tumors. Statistically significant differences in survival were observed among the stages ($p = 0.039$) (Graphic 10).

Regarding the mitotic index, the overall MST was 539 days. For tumors with a MI below 1 per 10 HPF, the MST was 539 days (95% confidence interval [CI]: 485.0 to 593.0) with a mean of 533.1 ± 30.3 days. For those with a MI above 1 per 10 HPF, the MST was 385 days (95% CI: 152.2 to 617.8) with a mean of 463.6 ± 66.4 days. At 539 days, the estimated probability of survival was 48.8% for patients with a MI below 1 per 10 HPF and 37.5% for those above 1 per 10 HPF. No statistically significant differences were observed between the groups ($p = 0.275$) (Graphic 11).

Graphic 10: Survival Kaplan-Meier curve by Clinical stage



Graphic 11: Survival Kaplan-Meier curve by Mitotic index

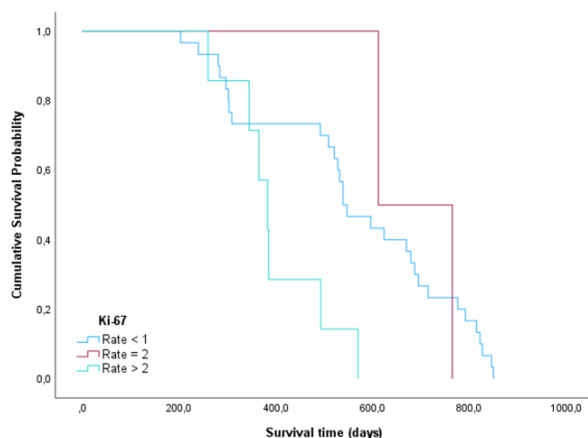


The MST based on the Ki-67 proliferation rate were as follows: 539 median days (95% confidence interval [CI]: 453.1 to 624.9) with a mean of 560.7 ± 37.5 days for a Ki-67 rates lower than 1; 612 median days with a mean of 688.5 ± 76.5 days for a rate of 2; and 383 days (95% CI: 336.8 to 429.2) with a mean of 400.1 ± 38.4 days for rates above 2. At 539 days, the survival probability was approximately 50.0% for patients with a Ki-67 index <1 and close to 0.0% for Ki-67 index >2. Statistically significant differences among these groups were observed ($p = 0.024$) (Graphic 12).

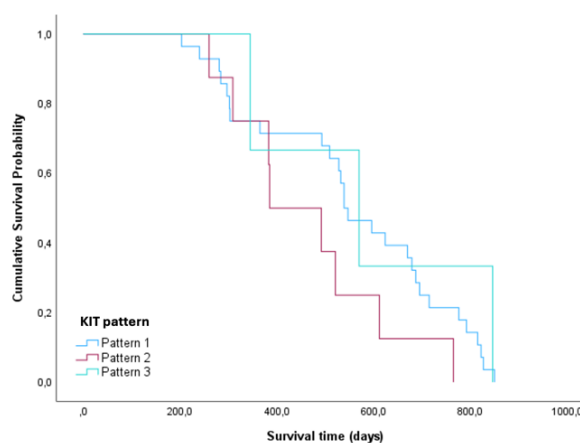
The survival analysis for the KIT expression pattern was 539 median days (95% confidence interval [CI]: 456.0 to 622.0) with a mean of 553.9 ± 38.4 days for pattern 1; 385 median days (95% CI: 234.0 to 536.1) with a mean of 465.9 ± 59.0 for pattern 2 and 570 median days (95% CI: 210.0 to 930.1) with a mean of 587.0 ± 144.9 days for pattern 3. At 539

days, the survival probability was approximately 50.0% for pattern 1, 25.0% for pattern 2 and 33.3% for pattern 3. No statistically significant differences among these groups were observed ($p = 0.225$). (Graphic 13)

Graphic 12: Survival Kaplan-Meier curve by Ki-67



Graphic 13: Survival Kaplan-Meier curve by KIT expression pattern



5.3.1.1. Impact of significant prognostic factors on survival

To understand which variables that showed differences in survival by Pearson correlation coefficient and Kaplan-Meier method (Ki-67 index, clinical stage, reproductive status and tumor size) had more impact on survival, a Cox regression was estimated considering only the 39 observations of Ki-67. The Omnibus test indicates a good model fit, $\chi^2(3) = 17.78$, $p < .001$.

The final model of cox regression showed reproductive status ($p=0.002$) and Ki-67, both as a continuous variable ($p=0.007$) and with a cut-off value of $Ki-67 > 2$ ($p=0.003$), as independent predictors of survival (Table 14). Clinical stage and tumor size were not significant factors when added to the model.

Table 14: Results of cox regression study evaluating the impact of significant Prognostic factors on Survival

Variables	Coefficient	df	Sig.	HR	95.0% CI HR	
					Lower	Upper
Reproductive status	-1.61	1	.002	.20	.07	.55
Ki-67 continuous		2	.007			
Ki-67 (Rate < 1)	-.71	1	.343	.49	.11	2.13
Ki-67 (Rate > 2)	1.47	1	.003	4.37	1.63	11.71

For the reproductive status, the cox regression showed a *Negative coefficient* and a Hazard Ratio (HR) of 0.20, $p = 0.002$. This indicates that intact dogs had an 80% lower risk of death compared to neutered dogs, which supports a strong protective of intact status, with retained gonads.

For Ki-67, using the baseline for Ki-67 as Rate = 2, the cox regression shows that Ki-67 (Rate < 1) had a *Negative coefficient* (-0.71), $HR = 0.49$, $p = 0.343$ and although the coefficient suggests a lower risk, this association is not statistically significant ($p > 0.05$). For Ki-67 (Rate > 2), the regression shows *Positive coefficient* (1.47), $HR = 4.37$, $p = 0.003$. This indicates that this level of Ki-67 is significantly associated with an increased risk of mortality compared to the reference group (Ki-67 Rate = 2), showing a strong adverse effect on survival.

5.4. Neoadjuvant vinblastine treatment

5.4.1. Characterization of patients receiving neoadjuvant vinblastine

To better understand the cases that underwent neoadjuvant vinblastine treatment (pre-sx VBL), a clinical characterization of these patients is summarized in Table 15. All patients in this treatment group received prednisolone concurrently and VBL was administered intravenously at a dose of 2mg/m².

Among the 10 cases, 80.0% (N = 8) exhibited local signs, such as ulceration and edema, while 20.0% (N = 2) presented systemic signs as anemia and vomiting. Metastasis was observed in 40% (N = 4) of the patients, all with lymph node involvement and in one case (10%, N = 1) metastasis extended to the spleen and intestines. Tumor sizes ranged from 1.0cm to 10.0cm, with a mean of 4.65 ± 3.53 cm.

Regarding tumor location, 40% (N = 4) were located on the Head and Neck, followed by 30% (N = 3) on the trunk. Most of the cases had stage III(a) MCT, representing 60% (N = 6) of the sample. The patients presenting with the 1.0cm tumors had lesions located in the head and neck. The number of vinblastine sessions ranged from 1 to 4, with a mean of 1.90 ± 1.00 sessions.

Table 15: Clinical Characterization of neoadjuvant vinblastine treatment cases (N = 10)

Variables	Categories	N	%
Metastasis	No	6	60.0
	Yes	4	40.0
Tumor location	Head and Neck	4	40.0
	Trunk	3	30.0
	Limbs	1	10.0
	Preputial	1	10.0
	Inguinal	1	10.0
Local Signs	No	2	20.0
	Yes	8	80.0
Systemic Signs	No	8	80.0
	Yes	2	20.0
Clinical Stage	I(a)	2	20.0
	III(a)	6	60.0
	III(b)	1	10.0
	IV	1	10.0
Variables		M	SD
Tumor dimension (cm)		4.65	3.53
Number of neoadjuvant VBL sessions		1.90	1.00

5.4.2. Effect of Neoadjuvant Vinblastine treatment in tumor size

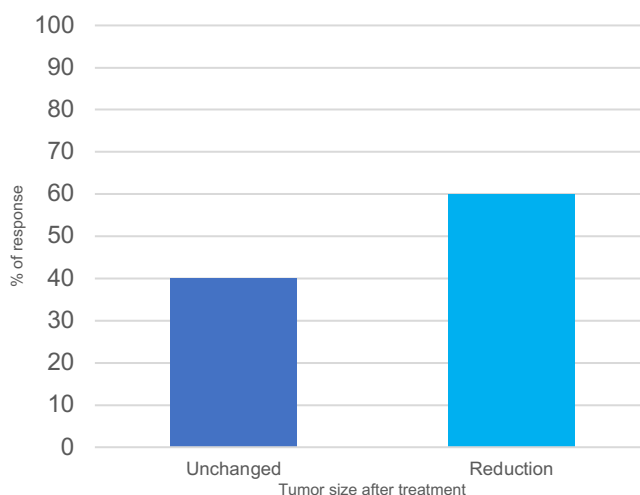
To evaluate whether neoadjuvant VBL administration effectively reduced tumor size, the 10 dogs that received the treatment were included in this analysis. The tumor's major axis (largest dimension) prior to VBL administration and after VBL, but before surgery, was considered. To compare these two measurements, the Shapiro-Wilk test was first applied to assess data normality. Since normality was not rejected in both cases ($p > 0.05$), a paired-samples Student's t-test was performed, using a one-tailed (right-sided) alternative hypothesis that tumor size was greater before VBL treatment.

A strong positive correlation was found between tumor dimensions before and after VBL administration ($r = 0.99$), indicating high consistency in measurement. The paired-samples t-test results ($p = 0.011$, one-tailed test) confirmed a statistically significant reduction in tumor size following treatment. Additionally, Cohen's d was calculated to assess the magnitude of this effect. Given the small sample size, the effect is particularly meaningful and the Cohen's result indicated a strong effect of neoadjuvant VBL on tumor dimension. (Table 16)

Table 16: Results of a paired sample t-Student test to compare tumor dimension before (pre-VBL) and after treatment the neoadjuvant vinblastine (Post-VBL) (N = 10)

Tumor dimension	<i>M ± SD</i>	<i>t</i>	<i>Cohen's d</i>
Pre-VBL	4.65 ± 3.53	2.75*	.87
Post-VBL	4.20 ± 3.48		

Graphic 14: Distribution of cases (%) per tumor size after treatment



By calculating the difference between tumor dimensions pre- and post-VBL treatment, it was found that in 40% of the cases the tumor size remained unchanged, while in 60% a reduction was observed. Among the six patients who experienced tumor size reduction, the smallest decrease was 0.2 cm and the largest 1.5 cm, with a mean reduction of 0.75 ± 0.46 cm. The percentage of decrease in tumor size ranged from 11.8% to 50.0%, with a mean reduction of $26.3\% \pm 16.51\%$. (Table 16, Graphic 14)

Of the 10 dogs treated with neoadjuvant VBL, 50.0% (N = 5) did not have surgery, while the remaining 50.0% (N = 5) underwent surgical excision.

5.4.3. Association of neoadjuvant VBL treatment with survival

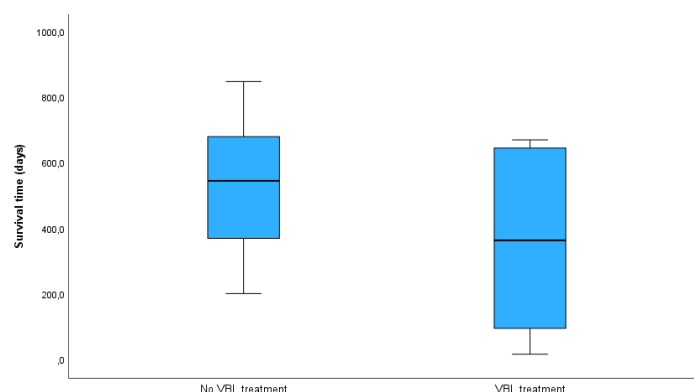
To compare survival times between individuals who received neoadjuvant VBL treatment and those who did not, tests of normality were first applied. The Kolmogorov-Smirnov test indicated that survival time in the group without VBL treatment was normally distributed ($p = 0.200$). Similarly, the Shapiro-Wilk test confirmed normality in the VBL group ($p = 0.114$). Therefore, to compare the two groups, the independent sample Student's t-test was used.

There was a significant difference in survival between the no treatment and neoadjuvant VBL treatment groups ($p = 0.012$). Examination of the means suggests that survival was longer in the group that did not receive neoadjuvant VBL treatment. Furthermore, the Cohen's d test result shows how the effect of treatment on survival was large (0.89). (Table 17, Graphic 15)

Table 17: Results of independent sample t-Student test to compare Survival between neoadjuvant vinblastine treatment and the no vinblastine treatment group

Neoadjuvant VBL	$M \pm SD$	t	Cohen's d
No ($n = 55$)	535.69 ± 191.21	2.60*	.89
Yes ($n = 10$)	354.30 ± 263.09		

Graphic 15: Bloxplot of survival with the neoadjuvant VBL treatment and the no vinblastine treatment group



5.4.4. Association of neoadjuvant VBL treatment with DFI

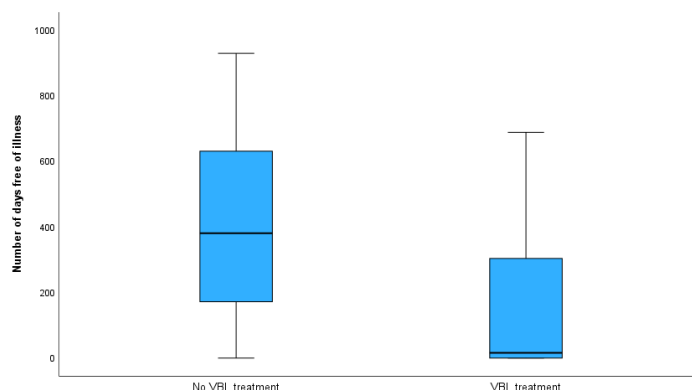
To compare DFI between patients who received neoadjuvant VBL treatment and those who did not, tests of normality were first applied. The Kolmogorov-Smirnov test indicated that DFI in the group without VBL treatment was normally distributed ($p = 0.091$). In contrast, the Shapiro-Wilk test in the VBL group ($p = 0.001$) indicated non-normality for DFI. Therefore, the nonparametric Mann-Whitney test was applied to compare the two groups.

The results revealed a statistically significant difference in DFI between the two groups ($p = 0.009$), with patients receiving neoadjuvant VBL exhibiting a shorter disease-free interval compared to the no VBL treatment group. (Table 18, Graphic 16)

Table 18: Results of Mann-Whitney test to compare DFI between neoadjuvant vinblastine treatment group and the no vinblastine group

DFI	$M \pm SD$	p-value	Mann-Whitney	Cohen's d
No ($n = 55$)	397.82 ± 280.09	0.009	2.61**	.32
Yes ($n = 10$)	168.40 ± 261.92			

Graphic 16: Bloxplot of survival with the neoadjuvant VBL treatment group and the no VBL treatment group



5.4.5. Association of neoadjuvant VBL treatment with recurrence

To analyze the association between neoadjuvant VBL treatment and recurrence, a Fisher’s exact test was performed. Only dogs who underwent surgical treatment were included, 49 cases for the no neoadjuvant VBL group and 5 cases for the neoadjuvant VBL.

There was no statistically significant association between recurrence and neoadjuvant VBL treatment ($p = 0.571$). However, an odds ratio of 1.519 comparing the two groups means that among patients who did not undergo neoadjuvant VBL treatment, the odds of remaining recurrence-free are about 52% higher than in those who did receive neoadjuvant VBL treatment. In other words, having the neoadjuvant VBL treatment is associated with a higher chance of recurrence compared to not having the treatment, based on the odds. (Table 19)

Table 19: Association of neoadjuvant vinblastine treatment and no vinblastine treatment groups with recurrence

Neoadjuvant VBL	Recurrence		p-value	OR (95% CI)
	Yes	No		
No (N = 49)	18.4% (9)	81.6% (40)	0.571	1.519
Yes (N = 5)	40.0% (2)	60.0% (3)		

6. DISCUSSION

In the present study, 65 animals with diagnosed cMCTs were included. All cases were retrieved from the Anicura Atlântico Veterinary Hospital database (Orangest Vet®).

Mast Cell Tumors distribution per breed was wide, with a major frequency in mixed breed dogs (24.8%), as described in previous studies (De Nardi et al., 2022; Kiupel et al., 2011; Ribeiro et al., 2022; Villamil et al., 2011). Regarding pure-breed dogs, the French Bulldog (12.8%), Golden Retriever (9.2%), Labrador retriever (9.2%), Boxer (6.2%) and Pug (6.2%) were the most common breeds in the study, which is in agreement with Mochizuki et al. (2017), O'Connell & Thomson 2013; Ribeiro et al., 2022) who described these breeds as some of the ones with an increased risk of MCT development.

The age of the included animals ranged from 2 to 17 years, with a mean age of 9 years, corroborating with the European Consensus document on MCTs in dogs and cats (Blackwood et al., 2012). Similarly, the studies carried by Donnelly et al. (2015), Escoda Llorens et al. (2025) and Mullins et al. (2006) also reported the mean age of 9 years, with Donnelly et al. (2015) being 8.85 years.

As for sex distribution, 60% were male and 40% female, suggesting no clear sex predisposition. Similar findings have been reported in several studies, where no statistically significant association between sex and the occurrence of MCT was detected (Mochizuki et al., 2017; O'Connell & Thomson 2013; Villamil et al., 2011). Although Ribeiro et al. (2022) have documented a slight predominance of males, as in the present study, these differences are often attributed to sample composition or local population demographics rather than true biological predisposition (Ribeiro et al., 2022).

In the literature, the trunk, perineal and inguino-genital regions are the most common sites of cutaneous MCTs ($\pm 50\%$), followed by the limbs ($\pm 40\%$) and the head and neck ($\pm 10\%$) (Bellamy & Berlato, 2021; De Nardi et al., 2022; M.Vail et al., 2020; Welle et al., 2008). In the concurrent study, the proportions were similar, with the trunk, perineum and inguino-genital region representing 47.7% of the sample, the limbs 33.8% and the head and neck 18.8%. When separating the trunk from the other regions usually taken together (perineal region), the present study is even closer to the one carried by Catarino et al. (2025), with 43.2% located on the limbs, 36.1% on the trunk, 15.3% on the head and neck and 5.4% on the perineal region.

Of the animals included in the study, 80% presented with solitary tumors, consistent with the literature identifying solitary nodules as the most common form of manifestation (Escoda Llorens et al., 2025; Kiupel, 2017; M.Vail et al., 2020). The remaining 20% had multiple tumors, ranging from 3 to 5 nodules, a proportion notably higher than the approximately 10% reported by Gieger et al. (2005) at the time of diagnosis. This difference

may be explained by the fact that HVA serves as a referral center for oncology, where more complex and advanced cases might be overrepresented.

As for treatment, surgery was the first-line therapy in 75.4% cases, reflecting its status as the gold-standard for local control (Bellamy & Berlato, 2022; Kiupel, 2017; M.Vail et al., 2020; Webster et al., 2008). This approach is supported by the European consensus document on MCTs in dogs and cats, which emphasizes that surgery remains the treatment of choice for localized disease and offers the highest likelihood of long-term control when appropriate margins are achieved (Blackwood et al., 2012). A small proportion of cases (6.2%) did not receive any treatment, which might be explained by an advanced stage of the disease leading to owner's personal decisions not to proceed or owner's financial constraints.

Of the cases submitted to surgery as first-line therapy, most were of low or intermediate grade, with a high proportion of clean margins and absence of metastasis at diagnosis. As a result, surgery was curative in the majority of cases (81.6%), with less than 20% exhibiting recurrence. These findings agree with previous studies showing surgery as curative in a high percentage of cases (Bellamy & Berlato, 2022; Welle et al., 2008). The recurrence rate observed was lower than those reported in other studies, which generally range from 20-50% (Ong et al., 2025), probably related to the high frequency of low-grade tumors and large number of cases with clean surgical margins.

In the present study, 76.5 of cases fell under the intermediate grade (Patnaik System), which is comparable with previous studies by Kiupel & Camus (2019) and Stefanello et al. (2015) reporting similar findings. Regarding the 2-tier grading system, although Kiupel et al. (2011) reported that 90% of MCT are classified as low grade, a lower percentage of cases were classified as so in this study (78.2%). Sabattini et al. (2015) and Stefanello et al. (2015) also reported the same findings, 75.4% and 65%, respectively. Similarly to the number of tumors, this percentage may be explained by the complexity of the cases presented for consult in a referral center.

The main objective of this study was to evaluate how epidemiological, clinical and histological factors would affect survival time in the whole sample (65 patients included). As previously described in the literature review, no single factor can fully predict the outcome (M.Vail et al., 2020; Ribeiro et al., 2022; Webster et al., 2008; Welle et al., 2008), **it** is the combination of different factors that offers the most reliable approach to prognosis (M.Vail et al., 2020; Ong et al., 2025; Ribeiro et al., 2022; Webster et al., 2007).

To determine the effect of the different prognostic indicators on survival time, both association tests and a survival analysis were conducted. In the survival analysis, survival time in days, as previously described, was used as the temporal measure.

The survival analysis and associations identified clinical stage, Ki-67 index, reproductive status and tumor size as significant prognostic factors, while other variables such

as sex, age, breed, tumor location, histological grade (Patnaik and Kiupel), mitotic index, KIT expression pattern and systemic clinical signs did not show a statistically significant association with survival.

As expected, patients with lower clinical stages (0/I and II) showed longer mean survival times (554 and 564 days, respectively) compared with those in advanced stages (III/IV), which had a shorter median survival of 385 days. These results are consistent with the findings of Welle et al. (2008), who reported that MCT with lower stages have longer survival times than higher stages. This association reinforces the importance of clinical stage in outcome and corroborates previous reports that staging is a key prognostic indicator in canine MCT (Giantin et al., 2025; M.Vail et al., 2020; Welle et al., 2008).

The Ki-67 proliferation rate also proved to be a significant prognostic factor in this study. Tumors with higher proliferative activity (Ki-67 >2) were associated with decreased survival time (median survival time of 383 days), compared to Ki-67 <1 with a median survival time of 560 days. Similar results have been described by Berlato et al. (2021), Scase et al. (2006) and Webster et al. (2007), who showed that increased Ki-67 expression correlates with poor outcome. Specifically, the study by Scase et al. (2006) reported the similar median survival times, such as 395 days for Ki-67>1.8.

Interestingly, reproductive status emerged as a significant prognostic factor in this study. Neutered dogs presented shorter survival times compared to intact dogs and the Cox regression revealed reproductive intact status as a strong protective effect on survival, with an 80% lower risk of death in intact dogs. No reports associating reproductive status alone and survival were found. When assessing the relationship between reproductive status and histological grade, no statistically significant association was identified, although intact animals had twice the odds of presenting with high-grade tumors compared to neutered ones. This trend is consistent with the findings of Mochizuki et al. (2017), who reported that neutered dogs were less likely to present high-grade tumors.

The apparent contradiction between these results (survival analysis vs association with histological grade) may be explained by several factors, starting with the relatively small number of intact animals in this study and that only two deaths occurred among them, which may have increased the protective effect observed in the Cox regression. Second, despite higher odds for intact dogs to present with high-grade tumors, the majority (70%) of intact animals in this study were classified as low-grade MCT, which could bias survival outcomes towards longer survival. Moreover, histological grade itself did not show a significant statistical association with survival in this study, which could further explain why reproductive status appeared as an independent prognostic factor. Finally, the possibility of a biological effect cannot be excluded, as previous studies have suggested that sexual hormones may influence mast cell tumor behavior (Mochizuki et al., 2017).

Tumor size, analyzed as a continuous variable, also showed a statistically significant association with survival time. Larger tumors were correlated with reduced survival times, consistent with the findings of Rogers (1996) and Welle et al. (2008). In this study, as in the study by Moore et al. (2020) tumors classified as high-grade had significantly larger dimensions compared to low grade tumors, further supporting tumor size as a significant prognostic factor.

The absence of significance for sex and age with survival time contrasts with several reports. Age has often been reported as a prognostic factor, with older animals tending to have shorter survival times (Berlato et al., 2021; Ribeiro et al., 2022). In the present study, age was recoded from a continuous variable into a binary variable using a cut-off point of 8 years, allowing the comparison of survival outcomes between younger and older dogs. However, even after this categorization, no statistically significant association between age and survival was detected. In agreement with these results, other retrospective studies also failed to confirm sex or age as independent prognostic factors (Catarino et al. 2025; Shoop et al. 2015), suggesting that these variables may be influenced by tumor biology or differences in population distribution. Although the association between sex and Kiupel grade was not statistically significant in the present study, the same trend described by Mochizuki et al. (2017) was observed, with male dogs being approximately twice as likely to present with high-grade tumors compared to females.

Breed was also not significantly statistically associated with survival in the present study. While it is widely recognized that mixed-breeds and certain pure-breeds are predisposed to developing mast cell tumors (De Nardi et al., 2022; Mochizuki et al., 2017; M.Vail et al., 2020; Oliveira et al., 2020; Patnaik et al., 1984), the literature indicates that breed itself is not consistently predictive of prognosis (Kiupel, 2017; M.Vail et al., 2020). These findings suggest that while genetic predisposition might affect tumor incidence, it does not necessarily determine biological behavior, such as outcome.

Tumor location did not influence survival in this study. These results contrast with some reports that associate different locations (as perianal and inguinal regions) with more aggressive behavior and shorten survival time (J. M. Dobson & Scase, 2007; Gieger et al., 2005; M.Vail et al., 2020; Ribeiro et al., 2022; Welle et al., 2008). On the other hand, other studies have failed to confirm a consistent correlation between location and survival (Ong et al., 2025). Although the difference between these locations and survival was not significant, it was equally found better survival probabilities for tumors located on the limbs, trunk and head and neck compared to tumors on the preputial region, since at 539 days, the estimated survival probability was 50% for tumors on the limbs and head and neck, 36.8% for tumors on the trunk and the lowest of 33.3% for tumors on the preputial region.

Both histological grading systems (Patnaik and Kiupel) failed to reach statistical significance with survival in the present study, which diverges from most of the published

literature that considers histological grade as the most reliable prognostic tool (Berlato et al., 2021; Gieger et al., 2005; M.Vail et al., 2020; Ong et al., 2025; Sabattini et al., 2015). A possible explanation is that the sample size and retrospective nature of the study may have limited statistical power. Moreover, not all dogs were initially evaluated at HVA and histologic assessments were performed by different pathologists and laboratories, increasing the risk of variability in grading. The Patnaik grading system, in particular, is known to have poor inter-observer reproducibility, with agreement rates below 63% for grades II (Kiupel, 2017; Kiupel et al., 2011; Kiupel & Camus, 2019; M.Vail et al., 2020; Northrup et al., 2005a, 2005b), which represents 76.5% of the sample.

Similarly, the mitotic index, previously reported as relevant prognostic indicators (Berlato et al., 2015, 2021; Romansik et al., 2007; Vascellari et al., 2013), was not significant in this study. Different cut-off values have been proposed in the literature, such as $MI > 5$ (Romansik et al., 2007) and $MI > 7$ (Sledge et al., 2016). In the present study, MI was also a binary variable, but with a cut-off value of 1 ($MI < 1$ and $MI > 1$), since only 5 animals (9.8% of the 51 animals with a mitotic index description) had MI above 5 and was too small to be used as a threshold. This difference in distribution might explain the absence of significance when compared to other studies. Nevertheless, the survival probability at 539 days was 48.8% for patients with $MI < 1$ and 37.5% for patients with $MI > 1$, which, although not statistically significant, indicates a trend towards the shorter survival time in cMCTs with $MI > 1$.

Regarding KIT expression pattern, MCTs with patterns 2 and 3 have been associated with decreased survival times compared to tumors with expression pattern 1 (Kiupel et al., 2004; Webster et al., 2007; Welle et al., 2008). Although the differences were not statistically significant, the present study also found better survival probabilities in mast cell tumors with expression pattern 1 than in those with patterns 2 and 3. At 539 days, the survival probability was 50% for patients with pattern 1, compared to 25% for pattern 2 and 33.3% for pattern 3.

Systemic signs are usually associated with degranulation, indicating extensive disease and frequently linked with a reserved prognosis (Ledur et al., 2023; M.Vail et al., 2020), particularly in cases which gastrointestinal signs are present, recognized as negative prognostic factors for survival (Mullins et al., 2006). In this study, however not statistically significant, the same results were found, with an estimated survival probability of only 20% at 407 days when systemic signs were present.

An additional objective of this study was to evaluate whether the administration of neoadjuvant vinblastine resulted in measurable tumor size reduction, including the 10 patients who underwent this treatment approach.

VBL is typically administered as a neoadjuvant approach in patients for whom surgery presents a challenge, as in large tumors not suitable for initial excision (Ossowska et al., 2023) and advanced clinical stages (Rassnick et al., 2008). The sample analyzed regarding the

neoadjuvant VBL treatment is comparable with previous reports (Ossowska et al., 2023; Rassnick et al., 2008), since among the 10 dogs included, 40% had tumors located on the head and neck, where achieving adequate margins without radical surgery is often difficult, and a mean size of 4.65 cm, considered as large according to the >3cm reported in the literature (De Nardi et al., 2022). Furthermore, the majority were in advanced clinical stages (70% with Stage III), 80% exhibiting clinical signs (either local, systemic or both) and 40% with lymph node involvement at the time of diagnosis.

In the present study, the use of vinblastine in association with prednisolone as neoadjuvant chemotherapy resulted in a statistically significant difference between tumor sizes before and after treatment, confirming the cytoreductive potential of this drug. Among dogs treated, most achieved partial remission or stable disease, without any case of local progression. This mirrors the findings of Anderson et al., (2024); Ossowska et al. (2023) and Thamm et al. (1999), supporting the role of neoadjuvant vinblastine-based protocols in local disease control.

The proportion of dogs achieving tumor size reduction in the concurrent study (60%) was higher than the overall response rate reported by Ossowska et al. (2023) (40.9%) and Thamm et al., (1999) (47%), both of which, like the present study, used vinblastine combined with prednisolone. In contrast, vinblastine as a monotherapy in the trial by Rassnick et al. (2008) produced lower response rates of 12% and 27%, at doses of 2.0 mg/m² and 3.5 mg/m², respectively. When compared to protocols with additional drugs, as the study carried by Olsen et al (2018), who reported a 90% overall response rate with vinblastine, prednisolone and toceranib phosphate, the present study suggest that multi-agent therapy can substantially higher response rates on cytoreduction. Nevertheless, the increased complexity and cost of such protocols, along with potential additive toxicities must be considered with the benefits for each individual case.

As in the study by Ossowska et al. (2023), no complete responses were documented, likely reflecting case selection and differences in tumor chemotherapy sensitivity. The mean tumor size reduction in the present study (26.3%) is comparable to the reduction of 27% reported by Ossowska et al., (2023). This degree of reduction remains clinically meaningful, as partial cytoreduction can facilitate surgical excision and potentially reduce the morbidity associated with more radical procedures.

Surgical excision was performed in 50% of cases after neoadjuvant VBL treatment. This proportion is lower than the 68.2% reported by Ossowska et al. (2023) under a similar protocol and below the 80% described in studies of neoadjuvant prednisolone (Stancliff & Gilson, 2008). This discrepancy may reflect differences in case selection, with this study including a higher proportion of dogs with possibly unresectable MCTs at presentation. Specifically, among the dogs that did not proceed to surgery, one had stage IV MCT in the

head and neck region, three belonged to the subgroup showing no tumor size reduction and one had a tumor on the limb where achieving adequate surgical margins is challenging.

When outcomes were compared between patients that received neoadjuvant VBL and those managed with other first-line treatments (predominantly surgery), a statistically significant difference was shown both in Survival and DFI, presenting as shorter in the neoadjuvant group, with 535.69 days vs 354.30 days for Survival and 397.82 vs 168.40 days for DFI. These findings contrast with those of Anderson et al. (2024), who reported no significant differences in survival when comparing vinblastine-based protocols to other treatment strategies. However, the patients included in the current study for the neoadjuvant treatment typically presented with large tumors and advanced disease stages, factors known to adversely affect both survival and DFI (O'Connell & Thomson, 2013; Rogers, 1996; Welle et al., 2008). Under such conditions, shorter survival and DFI in the neoadjuvant group are an expected. The true role of neoadjuvant VBL in Survival and DFI remains to clarify.

Regarding recurrence, no statistically significant association was identified between neoadjuvant VBL treatment and recurrence rates. However, the odds ratio (OR = 1.52) suggests a trend toward higher recurrence risk in the neoadjuvant group. In the study by Ossowska et al. (2023), local recurrence was documented in 20.8% of dogs treated with neoadjuvant vinblastine-prednisolone, but, as in the present study, it did not represent a significant difference compared to the control group. In contrast, Anderson et al. (2024) found that neoadjuvant vinblastine was significantly associated with a higher risk of local recurrence, suggesting that while short-term cytoreduction may facilitate surgery, it does not necessarily prevent subsequent local tumor regrowth. This higher risk of recurrence may happen due to the bigger sizes of MCT before neoadjuvant VBL, that may have microsatellite metastasis that even with adequate surgical margins are not enough to fully resect the tumor.

As for limitations, one of the main one's found in this study is its retrospective nature, which limits the quality and consistency of available data.

Clinical records were often incomplete or inconsistent, patients were managed with different diagnostic tools and therapeutic protocols and follow-up times varied between patients. In addition, histopathological evaluation was not always performed by the same pathologist and there is no standardization regarding where to perform the analysis, so even if the same pathologist saw the same slide twice, different grades could be obtained, since a lower mitotic count or fewer atypical cells in the analyzed section may result in a lower histological grade. Similarly, the assessment of the Ki-67 index lacks a universally standardized method and cut-off, which introduces additional variability and may affect comparability across cases and studies.

Another important limitation was the relatively small sample size, particularly for the neoadjuvant vinblastine group, which only included 10 animals. Although meaningful, it potentially reduced statistical power and limited the ability to perform further analysis.

The study was also limited by its reliance on medical records from a single hospital (HVA), which may restrict the applicability of conclusions to other clinical settings. Initially, the objective was to collect cases from multiple veterinary centers to conduct a multicenter study that could provide a more representative overview of the Portuguese canine population with cutaneous mast cell tumors. However, this was not possible to achieve due to difficulties in establishing collaborations and the short timeframe available.

Limitations were also present in the statistical methods applied. Survival analysis was performed using Kaplan-Meier curves and log-rank tests, which are appropriate for initial univariate comparisons, but do not adjust for other influencing factors. Cox regression was used to identify independent predictors of survival, but the small number of events in some subgroups, such as Ki-67 >2, limited the strength of the model. Several continuous variables, such as tumor size and mitotic index, had to be divided into categories due to the distribution of the data, which reduces statistical power and may underestimate differences.

It should also be noted that the number of animals across the groups of each variable was different, a factor that may have negatively influenced statistical associations and survival analysis.

7. FUTURE PROSPECTS

This study contributes to the understanding of canine cutaneous mast cell tumors by identifying relevant prognostic factors and exploring the role of neoadjuvant vinblastine treatment as a cytoreductive.

For future research, prospective studies with larger and more diverse groups are needed to clarify the influence of maintaining the intact status, on survival times and determine whether it genuinely influences tumor biology or whether these results reflect sample size and retrospective design limitations. Additional research should explore efficacy of neoadjuvant vinblastine, and which patients may benefit most from this approach.

8. CONCLUSION

Canine cutaneous mast cell tumors are among the most frequent skin neoplasms in dogs, with highly variable clinical behavior, making prognosis and treatment planning particularly challenging.

In this study, reproductive status, clinical stage, tumor size and Ki-67 index were identified as significant prognostic factors. Among these, reproductive status was especially important as intact dogs presented with longer survival times, making this the first study to

suggest reproductive status intact as a prognostic factor in canine mast cell tumors. This present study also demonstrates how vinblastine can effectively reduce MCTs size in a neoadjuvant therapy in the majority of cases, showing its potential despite the small number of cases treated.

Overall, these findings highlight the importance of a multifactorial evaluation that combines epidemiological, clinical and histological variables, while also supporting the role of neoadjuvant vinblastine in the management of canine mast cell tumors.

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ANNEXES

Annex i: Poster 1 accepted for presentation at the XX Montenegro International Veterinary Congress (10 - 11 October 2025)

IMPACT OF REPRODUCTIVE STATUS ON SURVIVAL IN CANINE CUTANEOUS MAST CELL TUMORS

A RETROSPECTIVE STUDY OF 65 CASES

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1 INTRODUCTION

Mast cell tumors (MCT) are among the most common neoplasms in dogs, representing up to 21% of skin tumors (Catarino et al., 2025). Prognosis evaluation of canine MCTs is highly variable, depending on multiple factors. No single factor can fully predict biological behavior or treatment outcomes, it is the combination of several factors that offers the most reliable approach to prognosis, guiding therapeutic decision-making and avoiding under or over treatment.

Some epidemiological factors, such as age, breed and sex correlate with prognosis and tumor grade. On the other hand, no studies evaluating the effect of reproductive status alone (fertile or neutered) with survival have been described.

2 OBJECTIVES

Evaluate the impact of reproductive status on overall survival of dogs affected by cutaneous mast cell tumours

3 MATERIALS AND METHODS

The retrospective study was performed using the medical records from 65 dogs diagnosed with cutaneous mast cell tumors and presented for consultation at Anicura Atlântico Veterinary Hospital between January 2023 and December 2024;

Only dogs with a complete identification data (age, sex and reproductive status) and full medical history were included. Animals were excluded in case of presenting with other types of neoplasia undergoing concurrent treatment.

Overall survival, in days, was considered from the date of tumor diagnosis to date of death of any cause or, for patients still alive, the date July 27, 2025;

Pearson correlation coefficients (r) were used to examine associations between survival time and epidemiological factors. Additionally, a Cox proportional hazards regression model was employed to evaluate the impact of the significant factors on survival time. The model included survival time as the dependent variable and event status as the censoring indicator.

For all the analysis 5% and 1% levels of significance were used.

4 RESULTS

The only statistically significant associations identified between survival time and the analyzed variables were reproductive status (p = 0.001), with fertile dogs showing a greater chance of survival. In contrast, both age (r = 0.034) and sex (r = 0.090) showed only weak correlations with survival time, which were not statistically significant. (Table 1)

Table 1 - Association between epidemiological factors and survival

Variables	Survival
Age	0.034
Sex	0.090
Reproductive status	0.398**

*p < 0.05, **p < 0.01

To further explore the effect of reproductive status on survival, a Cox regression analysis was performed, since this was the only variable significantly correlated with survival.

The model confirmed reproductive status as an independent predictor of survival (p = 0.002). Specifically, the regression showed a negative coefficient (-1.67) and a hazard ratio (HR) of 0.20, indicating that fertile dogs had an 80% lower risk of death compared to neutered dogs, which supports a strong protective effect of fertility. (Table 2)

Table 2 - Results of cox regression study evaluating the impact of significant epidemiological factors on Survival

Variable	Coefficient	p-value	HR
Reproductive status	-1.61	0.002	0.20

*p < 0.05, **p < 0.01

5 CONCLUSION

The present study is the first to suggest reproductive status alone as a significant prognostic factor in canine cutaneous Mast Cell Tumors. Neutered dogs presented shorter survival times compared to fertile dogs and the Cox regression revealed fertility as a strong protective effect on survival.

Although sex and age did not show a significant association with survival time in this study, other authors have also failed to confirm these variables as independent prognostic factors (Catarino et al., 2025; Shoop et al., 2015). This suggests that their prognostic value may be influenced by tumor biology or by differences in the studied populations.

For future research, prospective multicentric studies with larger groups are needed to clarify the influence of fertility on survival time and determine whether it genuinely influences tumor biology or if these results reflect sample size and retrospective design limitations.

Catarino, J., Pinello, K., Niza-Ribeiro, J., Santos, J., Payan-Carreira, R., Reis, J., & Faisca, P. (2025). Exploring canine mast cell tumors: An investigation into demographic characteristics, and grading system analysis from a pathology lab data (2019–2021). *Preventive Veterinary Medicine*, 236. <https://doi.org/10.1016/j.prevetmed.2025.106416>

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RESPONSE TO NEOADJUVANT VINBLASTINE IN CANINE CUTANEOUS MAST CELL TUMORS

A RETROSPECTIVE STUDY OF 10 CASES



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1 INTRODUCTION

Cutaneous Mast Cell Tumors (MCT) are among the most frequent skin neoplasms in dogs, with highly variable clinical behavior. Although surgery remains the gold-standard for low-grade MCT, in large or advanced-stage tumors, achieving complete excision with adequate margins can be particularly challenging. In such cases, neoadjuvant chemotherapy with vinblastine represents a treatment option with the objective of achieving cytoreduction, facilitating complete excision without the need for radical surgery, and potentially improving local control.

Previous studies reported overall response rates of 40 - 47%, with median tumor size reductions of approximately 27%.

2 OBJECTIVES

Evaluate whether neoadjuvant vinblastine administration effectively reduced tumor size

3 MATERIALS AND METHODS

The retrospective study was performed using the medical records from 10 dogs diagnosed with cutaneous mast cell tumors and presented for consultation at Anicura Atlântico Veterinary Hospital between January 2023 and December 2024;

Only dogs with measured MCTs and treated with a combination of vinblastine (2mg/m²) and prednisolone were included; Tumor size pre and post neoadjuvant treatment were collected;

To compare these two measurements, the Shapiro-Wilk test was first applied to assess data normality. Since normality was not rejected in both cases ($p > 0.05$), a paired-samples Student's t-test was performed (t-value), using a one-tailed (right-sided) alternative hypothesis that tumor size was greater before VBL treatment. For both measurements, a mean size (M) and a standard deviation (SD) was calculated. Effect size was also calculated using Cohen's d, with interpretation thresholds defined as follows: $d < 0.20$ indicating a weak effect, $0.20 < d < 0.50$ a moderate effect, and $d > 0.50$ a strong effect;

4 RESULTS

Table 1 - Results of a paired sample t-Student test to compare tumor size before (Pre-VBL) and after treatment the neoadjuvant vinblastine (Post-VBL) (N = 10)

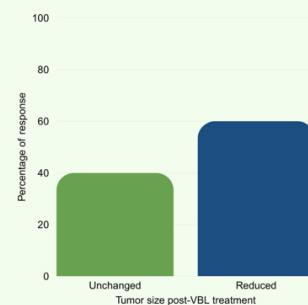
Tumor dimension	M ± SD	t-value	Cohen's d
Pre - VBL	4.65 ± 3.53 cm	2.75*	0.87
Post - VBL	4.20 ± 3.48 cm		

* $p < 0.05$, ** $p < 0.01$

A statistically significant difference in tumor size was observed between groups ($p = 0.011$). The Cohen's d test result indicated a strong effect of neoadjuvant VBL on tumor dimension. Given the small sample size, the effect is particularly meaningful (Table 1).

By calculating the difference between pre and post-VBL treatment groups, it was found that in 40% of the cases the tumor size remained unchanged, while in 60% a reduction was observed (Figure 1). No cases of tumor progression were documented. The mean tumor size reduction was 26.3%.

Figure 1: Response rates of tumor size post neoadjuvant VBL treatment



5 CONCLUSION

The use of vinblastine in combination with prednisolone as neoadjuvant chemotherapy resulted in a statistically significant difference between tumor sizes before and after treatment, confirming the cytoreductive potential of this drug. Most dogs achieved partial remission or stable disease, without any case of local progression, supporting the role of neoadjuvant vinblastine-based protocols in local disease control.

The proportion of dogs achieving tumor size reduction (60%) was higher than the 40.9% and 47% reported by Ossowska et al., (2023) and Thamm et al., (1999), respectively, no complete responses were documented, likely reflecting case selection and differences in tumor chemotherapy sensitivity. The mean tumor size reduction of 26.3% remains clinically meaningful, as even partial cytoreduction can facilitate surgical excision and potentially reduce the morbidity associated with tumour burden and more radical procedures.

Further studies with a higher number of cases are needed to compare response rates and which cases may benefit from this therapy.

Ossowska, M., Picornell, J. A., Finotello, R., Amores-Fuster, I., & Tanis, J. B. (2023). Pre-operative neoadjuvant vinblastine-prednisolone in canine mast cell tumours: A single-centre retrospective cohort study. *Veterinary and Comparative Oncology*, 21(3), 447-459. <https://doi.org/10.1111/vco.12904>

Thamm, D. H., Mauldin, E. A., & Vail, D. M. (1999). Prednisone and Vinblastine Chemotherapy for Canine Mast Cell Tumor-41 Cases (1992-1997). *In J Vet Intern Med* (Vol. 13).