UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
CÂMPUS DE JABOTICABAL

AVALIAÇÃO DA TERAPIA COM TALIDOMIDA EM NEOPLASIAS MALIGNAS DA GLÂNDULA MAMÁRIA CANINA

Cecília Bonolo de Campos
Médica Veterinária

2016

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
CÂMPUS DE JABOTICABAL

AVALIAÇÃO DA TERAPIA COM TALIDOMIDA EM NEOPLASIAS MALIGNAS DA GLÂNDULA MAMÁRIA CANINA

Cecília Bonolo de Campos
Orientador: Prof. Dr. Geovanni Dantas Cassali
Coorientadoras: Dra. Gleidice Eunice Lavalle
Profa. Dra. Renee Laufer Amorim

Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias - UNESP, Câmpus de Jaboticabal, como parte das exigências para obtenção do título de Doutor em Medicina Veterinária: Clinica Médica Veterinária.

2016

Campos, Cecília Bonolo de
C198a Avaliação da terapia com talidomida em neoplasias malignas da glândula mamária / Cecília Bonolo de Campos. - - Jaboticabal, 2016
xiii, 83 p. : il. ; 29 cm

Tese (doutorado) - Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, 2016
Orientador: Geovanni Dantas Cassali
Coorientadora: Gleidice Eunice Lavalle, Renee Laufer Amorim
Banca examinadora: Débora Balabram, Erika Maria Terra, Mirela Tinucci Costa, Rosemeri de Oliveira Vasconcelos
Bibliografia

1. Cão. 2. Mama. 3. Metástase. 4. Quimioterapia metronômica. 5. Oncologia. 6. Sobrevida global. I. Título. II. Jaboticabal-Faculdade de Ciências Agrárias e Veterinárias.

CDU 619:616-006:636.7

Ficha catalográfica elaborada pela Seção Técnica de Aquisição e Tratamento da Informação – Serviço Técnico de Biblioteca e Documentação - UNESP, Câmpus de Jaboticabal.
e-mail: arnold@cnpso.embrapa.br

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: AVALIAÇÃO DA TERAPIA COM TALIDOMIDA EM NEOPLASIAS MALIGNAS DA GLÂNDULA MAMÁRIA CANINA

AUTORA: CECÍLIA BONOLO DE CAMPOS
ORIENTADOR: GEOVANNI DANTAS CASSALI
COORIENTADORA: RENEE LAUFER AMORIM
COORIENTADORA: GLEIDICE EUNICE LAVALLE

Aprovada como parte das exigências para obtenção do Título de Doutora em MEDICINA VETERINÁRIA, área: CLÍNICA MÉDICA VETERINÁRIA pela Comissão Examinadora:

Professor Doutor GEOVANNI DANTAS CASSALI
Departamento de Patologia Geral / Universidade Federal de Minas Gerais - Belo Horizonte/MG

Pesquisadora Dra. DÉBORA BALABRAM
UFMG - Belo Horizonte/MG

Profa. Dra. ERIKA MARIA TERRA
Departamento de Medicina Veteinária / UNICEP - São Carlos/SP

Profa. Dra. MIRELA TINUCCI COSTA
Departamento de Clínica e Cirurgia Veterinária / FCAV / UNESP - Jaboticabal

Profa. Dra. ROSEMERI DE OLIVEIRA VASCONCELOS
Departamento de Patologia Veterinária / FCAV / UNESP - Jaboticabal

Jaboticabal, 02 de setembro de 2016

DADOS CURRICULARES DO AUTOR

Cecília Bonolo de Campos nasceu em Belo Horizonte, no dia 02 de março de 1986, filha de Francisco Eduardo de Campos e Palmira de Fátima Bonolo. Graduada no curso de Medicina Veterinária na Universidade Federal de Minas Gerais (UFMG), no ano de 2010. Desenvolveu projeto de Iniciação Científica sob orientação do Prof. Dr. Geovanni Dantas Cassali, auxiliando no desenvolvimento da tese "Avaliação terapêutica do uso de inibidor de Cox-2 no câncer de mama avançado em cadelas" da Dra. Gleidice Eunice Lavalle. Também participou de cursos e realizou estágios principalmente nas áreas de Clínica e Cirurgia de Pequenos Animais e de Oncologia Veterinária. Ingressou no curso de Mestrado do Programa de Pós-graduação em Patologia da Faculdade de Medicina da UFMG, em 2011, orientada pelo Prof. Dr. Geovanni Dantas Cassali e coorientada pela Dra. Gleidice Eunice Lavalle, obtendo o título de Mestre em Patologia Investigativa com a dissertação intitulada "Avaliação de fatores prognósticos e tratamento quimioterápico adjuvante em neoplasias mamárias malignas felinas", no ano de 2013. Realizou Pós-graduação *Lato Sensu* em Clínica Médica e Cirúrgica em Pequenos Animais no Instituto Qualittas entre 2013 e 2015. Iniciou o Doutorado em 2014 no Programa de Pós-graduação em Medicina Veterinária na Universidade Estadual Paulista "Júlio de Mesquita Filho" – Câmpus de Jaboticabal, sob orientação do Prof. Dr. Geovanni Dantas Cassali e coorientada pela Dra. Gleidice Eunice Lavalle e Profa. Dra. Renee Laufer Amorim. Ministrou aulas e palestras, participou de congressos nacionais e internacionais com apresentação de trabalhos, auxiliou na organização de eventos, é revisora de periódicos científicos e publicou artigos na área de Oncologia Veterinária em periódicos nacionais e internacionais.

Aos obstáculos que impulsionam.

AGRADECIMENTOS

A Fifi, Hanna, Lily, Luna, Luck, Belinha, Melissa, Jade, Kira, Willa, Angel, Bebela, Friska, Mel, Pituca, Vick, Diana, Laika, Nika, Pretinha, Julie, Deby, Ianka, Luma, Megg, Kika, Ju e tantos outros que nos inspiram a conseguir melhores tratamentos para nossos pacientes.

À minha família pelos nossos laços nada convencionais, mas cheios de apoio. Sei que posso ir atrás das minhas ambições porque tenho vocês na base de suporte. À minha mãe por ensinar amor, respeito e compreensão de uma forma muito sábia. Ao meu pai, de onde herdei meu lado obsessivo tão benéfico na vida acadêmica, pela ajuda inicial para acreditarmos na viabilidade do projeto. Aos meus irmãos, Luís e Gustavo, por saber que sempre irão ajudar prontamente, e por todas nossas semelhanças.

Ao Be, por caminhar comigo, por ser minha maior fonte de conforto, carinho, risadas, compreensão, cumplicidade e (grande monte) amor. Por entender que as minhas batalhas são suas, e suas são minhas, e que juntos conseguiremos conquistar tudo que planejamos.

Ao Professor Geovanni, meu orientador há tanto tempo, responsável por incentivar meus principais méritos acadêmicos. Agradeço pela dedicação, por estar sempre disponível, por embarcar nos sucessos e nas furadas junto com os alunos, querendo sempre nosso sucesso.

À Gleidice, minha coorientadora, por muito mais do que isso. Por um curso cheio de fascínio pela oncologia em 2007 que direcionou toda minha vida acadêmica, por todos os ensinamentos, conselhos, amizade, pela família maravilhosa e por sempre lembrar o verdadeiro motivo de todas essas pesquisas.

À Professora Renee, pela disponibilidade e vontade de ajudar, pelo apoio em todos os assuntos paulistas, sempre com muito bom humor e paciência.

Ao Professor Rubens, pela colaboração fundamental no trabalho, por sempre ser acessível e por um empurrão no HV para vencer a timidez e começar a trabalhar com oncologia.

À Silvia Fialho e todos da Divisão de Desenvolvimento Tecnológico Farmacêutico da Fundação Ezequiel Dias pela colaboração indispensável, por acreditarem no nosso projeto e no potencial terapêutico da talidomida.

À Professora Rose, todos os programas de pós-graduação deveriam ter professores tão dedicados como você, que se importam e que doam seu tempo para tentar ajudar.

À Fernanda, Stefane, Rúbia, Artur, Miriã, Carla e Antônio por toda a ajuda com os pacientes desse estudo e por fazerem uma rotina pesada ganhar leveza e se tornar prazerosa. Um agradecimento especial à Fernanda por toda a parceria (e pela memória muito melhor do que a minha) e à Miriã pela dedicação.

A todos do Laboratório de Patologia Comparada, ao Ênio; aos mais antigos, Karine, Conrado, Lili e Marina; aos atuais, Diego, Fernanda, Lidi, Stefane, Miriã, Michelle, Breno, Emerson, Taty e Ivy; e aos ICs, Gabriela, Izabel, Fernanda, Aline, Ana Carolina, Bárbara, Ana Silva, Maria, Jennifer e Juliane. Trabalhar e crescer com todos vocês, foi um prazer. À Lidianne e Gabriela, fundamentais para o término desse trabalho em um prazo tão corrido, desculpa pela pressa e agradeço toda a disposição e cuidado com a tese.

A todas as pessoas que conheci em Jaboticabal. À Erika que já me chamou atenção no primeiro momento que encontramos, já me deixando à vontade, me acolhendo e apresentando para todo mundo. Não sabia que iria encontrar tantas pessoas entusiasmadas pela oncologia e foi um prazer conviver com todos vocês. Também à Erika, Talita, Rosana, Marília, Cadu e Letícia pelas infinitas ajudas com os relatórios e prestações de contas.

À Ro, que, sem nenhum bom senso, insistiu para eu sempre ficar na casa dela e que, de repente, era como se a gente se conhecesse desde sempre, aparecendo uma afinidade inexplicável. À Marília, por abrir as portas da sua casa, pela capacidade de resolver tudo instantaneamente e pela cumplicidade.

A todos meus amigos. À Rabelo e Marcos pela ajuda fundamental em um momento de desespero, à Vanessa por estarmos juntas nessa trajetória e pelos auxílios diversos, à Mariana pelo companheirismo de sempre e pela revisão do artigo, à Júlia pela disponibilidade e pelas palavras de incentivo, à Adriana pela pulseira azul

e Rebeca pelo apoio, e aos agregados. Aos amigos do kdolimite por estarem sempre presentes e por todas as ótimas memórias.

À todos das famílias Bonolo e Campos pela torcida.

À Lavínia por me acolher tão bem na sua família, Evandro pelas bobagens, Renato, Snarf e todos os Dolabellas pela convivência prazerosa. Ao Marcelo (pelo favorzinho nada usual) e Ro pelo descanso necessário em Cachoeira.

Ao Rhino e Jota, meus meninos, por melhorarem minha qualidade de vida diariamente.

À Fundação de Apoio a Pesquisa do Estado de São Paulo pelo apoio financeiro através da bolsa de doutorado referente à essa pesquisa (Processo número: 2014/01329-9).

SUMÁRIO

Páginas

RESUMO ..... **xii**

ABSTRACT ..... **xiii**

CAPÍTULO 1 – Considerações gerais **14**

INTRODUÇÃO 14

OBJETIVOS 15

Objetivo Geral 15

Objetivos Específicos 15

REVISÃO DE LITERATURA 16

Neoplasias da Glândula Mamária 16

Angiogênese e Quimioterapia Metronômica como Terapia Antiangiogênica 20

Talidomida 21

REFERENCIAS 25

CAPÍTULO 2 – Absence of significant adverse events following thalidomide administration in bitches diagnosed with mammary gland carcinomas **32**

ABSTRACT 32

INTRODUCTION 32

MATERIALS AND METHODS 34

RESULTS 36

DISCUSSION 40

CONCLUSION 44

ACKNOWLEDGEMENTS 44

REFERENCES 45

CAPÍTULO 3 – Thalidomide treatment in an advanced stage canine mammary gland carcinosarcoma 49

ABSTRACT 49

INTRODUCTION 49

CASE REPORT 50

DISCUSSION 55

ACKNOWLEDGEMENTS 57

REFERENCES 57

CAPÍTULO 4 – Thalidomide and metronomic chemotherapy in addition to surgery and chemotherapy for the treatment of canine malignant mammary gland neoplasms 61

ABSTRACT 61

INTRODUCTION 61

MATERIAL AND METHODS 63

RESULTS 66

DISCUSSION 71

CONCLUSION 77

ACKNOWLEDGEMENTS 77

REFERENCES 77

CAPÍTULO 5 – CONSIDERAÇÕES FINAIS 83

unesp UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

C E R T I F I C A D O

Certificamos que o Protocolo nº 021846/14 do trabalho de pesquisa intitulado **"Avaliação da terapia com talidomida e quimioterapia metronômica em neoplasias mamárias malignas em estadiamento avançado em cadelas",** sob a responsabilidade do Prof. Dr. Geovanni Dantas Cassali, está de acordo com os Princípios Éticos na Experimentação Animal adotado pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), em reunião ordinária de 07 de novembro de 2014.

Jaboticabal, 07 de novembro de 2014.

Profª Drª Paola Castro Moraes
Coordenadora – CEUA

Faculdade de Ciências Agrárias e Veterinárias
Via de Acesso Prof. Paulo Donato Castellane, s/n CEP 14884-900 - Jaboticabal - SP - Brasil
Tel. 16 3209 2600 - fax 16 3202 4275 www.fcav.unesp.br

AVALIAÇÃO DA TERAPIA COM TALIDOMIDA EM NEOPLASIAS MALIGNAS DA GLÂNDULA MAMÁRIA CANINA

RESUMO - As neoplasias da glândula mamária canina são as neoplasias mais comuns em cadelas não castradas, sendo pelo menos 50% malignas. A maioria dos carcinomas invasores é relacionado a uma sobrevida inferior a dois anos quando tratados apenas com cirurgia, sendo a principal forma de tratamento da doença. A talidomida tem demonstrado benefício clínico em diversas doenças neoplásicas e não-neoplásicas, principalmente devido aos seus efeitos antiangiogênicos e imunomodulatórios. Assim, o objetivo do presente trabalho foi avaliar os efeitos terapêuticos da talidomida em cadelas diagnosticadas com neoplasias malignas da glândula mamária. Inicialmente, os eventos adversos do tratamento com talidomida foram avaliados em 29 cadelas tratadas com 20 e 10 mg/kg/dia, durante 6 meses. O fármaco foi bem tolerado, não interferindo na qualidade de vida dos animais. Em casos de sonolência excessiva a dose de 10 mg/kg deve ser preconizada. Em seguida, 58 cadelas foram divididas entre quatro diferentes tratamentos propostos: tratamento cirúrgico; cirurgia seguido de quimioterapia em dose máxima tolerada (QDMT); cirurgia, QDMT e talidomida; e cirurgia, QDMT e quimioterapia metronômica (QM). Não houve diferença estatística significativa na sobrevida global (SG) entre os quatro tratamentos propostos quando animais de todos os estadiamento clínicos foram avaliados (p=0,3177). Porém, quando avaliamos a sobrevida de animais diagnosticados com metástase à distância, os tratamentos com cirurgia, QDMT e talidomida ou QM apresentaram maiores SG (463 e 376,5 dias, respectivamente) quando comparados aos tratamentos consistindo em cirurgia e cirurgia e QDMT (150 e 148 dias, respectivamente). Além disso, relatamos o caso de uma cadela diagnosticada com um carcinossarcoma que, após diagnóstico de metástase pulmonar, sobreviveu 20 meses sendo submetida apenas ao tratamento com talidomida. O tratamento com cirurgia, QDMT e talidomida foi considerado eficaz e seguro em pacientes com estadiamento avançado, aumentando a SG de pacientes com metástase à distância. A QM pode ser associada ao tratamento cirúrgico e quimioterápico quando a talidomida não estiver disponível.

Palavras-chave: Cão, Mama, Metástase, Quimioterapia Metronômica, Oncologia, Sobrevida Global

EVALUATION OF THALIDOMIDE IN THE TREATMENT OF CANINE MALIGNANT MAMMARY GLAND NEOPLASMS

ABSTRACT - The neoplasms of the canine mammary gland are the most common neoplasm in intact female dogs, and at least 50% are malignant. Most invasive carcinomas present a survival lower than two years when treated solely with surgery, which consists in the main treatment approach for the disease. Thalidomide has demonstrated clinical benefit in several neoplastic and non-neoplastic diseases, mainly due to its antiangiogenic and immunomodulatory properties. Therefore, the aim of the present study was to evaluate the therapeutic effects of thalidomide in female dogs diagnosed with malignant neoplasms of the mammary gland. Initially, the adverse events of the thalidomide treatment were evaluated in 29 female dogs treated with 20 and 10 mg/kg/day during 6 months. The drug was well tolerated, not interfering with the activities of daily living of most of the studied population. In cases where excessive somnolence was present the 10 mg/kg/day should be indicated. Afterwards, 58 female dogs were divided into four different treatment proposals: surgical treatment, surgery followed by maximum tolerated dose chemotherapy (MTDC), surgery, MTDC, and thalidomide, and surgery, MTDC, and metronomic chemotherapy (MC). No statistically significant difference in overall survival (OS) was observed between the four proposed treatments when animals presenting all clinical stages were evaluated ($p=0.3177$). However, the analysis of OS of animals presenting distant metastasis, the surgery, MTDC associated to thalidomide and MC presented a longer OS, 463 and 376.5 days, respectively, when compared to the surgical and surgery plus MTDC protocols, 150 and 148 days, respectively. Furthermore, we report the case of a female dog diagnosed with a carcinosarcoma that presented a 20-month OS following the diagnosis of pulmonary metastasis solely treated with thalidomide. The treatment with surgery, MTDC and thalidomide was considered effective and safe in canine patients with advanced stage mammary gland neoplasms, increasing the OS of patients diagnosed with distant metastasis. MC may be associated to surgery and chemotherapy when thalidomide is not available.

Key-words: Dog, Mammary, Metastasis, Metronomic Chemotherapy, Oncology, Overall Survival

CAPÍTULO 1 – CONSIDERAÇÕES GERAIS

INTRODUÇÃO

A prevalência do câncer em animais de companhia continua aumentando (WITHROW; VAIL; PAGE, 2013), sendo estimado que um em cada quatro cães e gatos irão vir a óbito devido à doença. O aumento da prevalência pode ser justificado pela melhora da saúde e bem-estar, além dos avanços na medicina veterinária, que geram não somente um aumento na expectativa de vida, mas também no diagnóstico de neoplasias (DOBSON, 2011).

Médicos veterinários apresentam papel fundamental no avanço do diagnóstico, tratamento e prevenção do câncer em espécies animais, por meio de investigações contínuas sobre biologia tumoral e tratamento (WITHROW; VAIL; PAGE, 2013). Muitas neoplasias espontâneas em cães e gatos compartilham de características e comportamentos biológicos semelhantes aos humanos (DOBSON, 2011). Investigações em oncologia comparada são importantes na busca da cura ou na transformação do câncer de qualquer espécie em uma doença crônica manejável (WITHROW; VAIL; PAGE, 2013).

O câncer de mama é o tumor de maior incidência e mortalidade na população feminina em todo o mundo (INSTITUTO NACIONAL DO CÂNCER JOSÉ ALENCAR GOMES DA SILVA. CORDENAÇÃO DE PREVENÇÃO E VIGILÂNCIA, 2015). A glândula mamária canina também é um sítio comum para o desenvolvimento tumoral (DOBSON, 2011), representando cerca de 50% dos diagnósticos em fêmeas caninas (VON EULER, 2011). Idade, exposição hormonal, raça, dieta e obesidade influenciam o risco do aparecimento de neoplasias da glândula mamária (NGM) em cães. Tumores espontâneos em cães e gatos são excelentes modelos para tumores humanos, sendo interessantes para avaliar a eficácia de novas terapias (SORENMO, 2003) e a carência de padrões estabelecidos para o tratamento (SORENMO; WORLEY; GOLDSCHMIDT, 2013; SORENMO, 2003) torna relevante o estudo de novas abordagens terapêuticas eficazes para as NGM caninas.

OBJETIVOS

Objetivo Geral

- Avaliar os efeitos terapêuticos da talidomida em cadelas diagnosticadas com neoplasias malignas da glândula mamária.

Objetivos Específicos

- Avaliar os efeitos adversos associados à administração de talidomida em cadelas diagnosticadas com neoplasias malignas da glândula mamária;
- Avaliar o benefício clínico da administração da talidomida associado a exérese cirúrgica e quimioterapia com carboplatina, comparado com a exérese cirúrgica como único tratamento e com a exérese cirúrgica seguido de quimioterapia com carboplatina na terapia de cadelas diagnosticadas com neoplasias malignas da glândula mamária, por meio da avaliação da sobrevida global;
- Comparar o benefício clínico associado ao protocolo de tratamento consistindo em exérese cirúrgica, quimioterapia com carboplatina e talidomida com o protocolo consistindo em exérese cirúrgica, quimioterapia com carboplatina e quimioterapia metronômica com ciclofosfamida e firocoxibe, por meio da avaliação da sobrevida global;
- Avaliar o estadiamento clínico, diagnóstico histopatológico, graduação histopatológica e o índice de proliferação celular nas neoplasias primárias de cadelas submetidas à medicação com Talidomida, correlacionando esses fatores prognósticos à resposta terapêutica dos pacientes e à sobrevida global;
- Avaliar a densidade de microvasos e do infiltrado inflamatório nas neoplasias primárias de cadelas submetidas à medicação com Talidomida, correlacionando esses fatores prognósticos à resposta terapêutica dos pacientes e a sobrevida global;
- Avaliar os efeitos antiangiogênicos e imunomodulatórios esperados do tratamento com talidomida pela comparação da densidade de microvasos e do infiltrado inflamatório nas neoplasias primárias e metástases à distância de cadelas submetidas à medicação com Talidomida.

REVISÃO DE LITERATURA

Neoplasias da Glândula Mamária

As NGM são as neoplasias mais comuns em cadelas não castradas (BRODEY; GOLDSCHMIDT; ROSZEL, 1983). Cadelas castradas antes do primeiro cio apresentam 0,5% de risco para NGM malignas, cadelas castradas antes do segundo cio apresentam 8% de risco e cadelas castradas após o segundo cio apresentam 26% de risco (SCHNEIDER; DORN; TAYLOR, 1969).

A aparência macroscópica dos tumores é muito variável: nódulos podem ser únicos ou múltiplos, móveis ou aderidos à pele e ulcerados (BOSTOCK, 1986). O comportamento biológico dessas neoplasias também é consideravelmente variável. O prognóstico é influenciado por diversos fatores, tais como: idade do paciente, tipo histológico, estadiamento clínico, tamanho tumoral, índice mitótico, grau histológico, metástase regional ou à distância, densidade de microvasos e marcadores moleculares (CASSALI et al., 2014; SORENMO, 2003). Piores prognósticos são associados a tumores grandes (maiores que 3 cm), ulcerados, aderidos e indiferenciados; diagnóstico histológico de carcinoma sólido, anaplásico, inflamatório ou sarcomas; presença de metástases regionais ou à distância, invasão vascular ou linfática; ausência de expressão de receptor de estrógeno e progesterona e altos índices de proliferação celulares (VON EULER, 2011). Predizer o prognóstico de animais com neoplasias é importante para a determinação do tratamento adequado (QUEIROGA; LOPES, 2002). Entretanto, estabelecer precisamente o prognóstico de um paciente canino com NGM pode ser um desafio devido às grandes variações no comportamento biológico dessas neoplasias (CASSALI et al., 2014).

O carcinoma inflamatório é a apresentação clínica mais agressiva de NGM caninas (CLEMENTE et al., 2009; VON EULER, 2011), caracterizado por eritema cutâneo, elevada temperatura e por vezes ulceração e linfedema (VON EULER, 2011). O tratamento deve ser iniciado nos estágios iniciais da doença e terapias multimodais podem ser indicadas. Porém, a sobrevida global é menor quando comparado à sobrevida global de carcinomas não inflamatórios da glândula mamária (CLEMENTE et al., 2009).

A idade média de cadelas, no momento do diagnóstico das NGM, foi descrita como 10,58 ± 2,83 anos (SCHNEIDER; DORN; TAYLOR, 1969) e 10,04 ± 2,6 anos (NUNES, 2015). O estadiamento clínico, descrito por Owen (1980), foi relacionado com prognóstico em cadelas diagnosticadas com NGM malignas, onde os estadio IV e V apresentaram menor sobrevida global quando comparados aos estádios I, II e III (NUNES, 2015). Tumores múltiplos foram observados em mais de 60% dos casos de NGM (SCHNEIDER; DORN; TAYLOR, 1969), sendo a maioria das NGM localizadas nas glândulas mamárias abdominais e inguinais (NUNES et al., 2014). Aproximadamente 50% das NGM são malignas, dos quais metade metastatiza (VON EULER, 2011). Bostock (1986) também relatou incidência de 50% de tumores benignos na glândula mamária canina. Nunes (2015), por sua vez, relatou incidência de 84% de neoplasias malignas, sendo 92% composto por carcinomas.

As NGM são divididas entre lesões não-neoplásicas, benignas e malignas (SORENMO; WORLEY; GOLDSCHMIDT, 2013), geralmente progredindo de lesões benignas para lesões malignas invasivas (SORENMO; WORLEY; GOLDSCHMIDT, 2013). O tumor misto benigno é a neoplasia benigna mais comum (NUNES et al., 2014; QUEIROGA; LOPES, 2002) e o carcinoma em tumor misto é a neoplasia maligna mais prevalente na glândula mamária canina (NUNES, 2015; NUNES et al., 2014; QUEIROGA et al., 2005). No entanto, existe uma grande variedade de tipos histológicos malignos de NGM e o diagnóstico histopatológico adequado é importante para a definição do prognóstico e do tratamento (VON EULER, 2011). Os carcinomas micropapilares, sólidos, tubulares e carcinosarcomas apresentaram menor sobrevida global quando comparados aos carcinomas em tumores mistos e carcinomas papilares (NUNES, 2015; NUNES et al., 2014). Queiroga e Lopes (2002) também relataram maior sobrevida em pacientes com tumores mistos.

Uma sobrevida global inferior a dois anos é esperada para aproximadamente 80% dos animais diagnosticados com carcinomas invasores tratados apenas com cirurgia (BOSTOCK, 1986), sendo que a maioria dos óbitos relacionados à neoplasia ocorre durante o primeiro ano pós-cirúrgico (BOSTOCK, 1986; SCHNEIDER; DORN; TAYLOR, 1969). A mediana de sobrevida global de NGM malignas foi descrita como 7 meses por Schneider et al. (1969).

Metástases à distância são mais comumente encontradas no pulmão (SORENMO, 2003; VON EULER, 2011), seguido de fígado, rins, baço, ossos, sistema nervoso central e pleura (VON EULER, 2011), e são associadas a diminuição da sobrevida global do paciente (QUEIROGA; LOPES, 2002; YAMAGAMI et al., 1996), com taxa de sobrevida de 13,6%, um ano após a mastectomia (YAMAGAMI et al., 1996). Animais diagnosticados com metástases à distância no momento do diagnóstico apresentaram pior prognóstico, com mediana de sobrevida pós-cirúrgica de 5 meses, quando comparados aos 28 meses para animais sem evidência de metástase no momento do diagnóstico (PHILIBERT et al., 2003).

A cirurgia é a principal forma de tratamento para NGM canina e é a modalidade de terapia local mais eficaz para controle da neoplasia (NOVOSAD, 2003; SORENMO, 2003; VON EULER, 2011). Porém, a extensão ideal da excisão cirúrgica não está bem estabelecida (SORENMO; WORLEY; GOLDSCHMIDT, 2013). As possíveis abordagens cirúrgicas variam entre nodulectomia, mastectomia simples, em bloco e radical e não há evidência de benefício clínico associado a exéreses mais amplas em tumores bem definidos e móveis (BOSTOCK, 1986; VON EULER, 2011). Uma excisão cirúrgica ampla o suficiente para remover completamente um tumor de uma única glândula mamária é considerado adequado, enquanto múltiplas neoplasias mamárias podem necessitar de ressecções mais extensas (SORENMO; WORLEY; GOLDSCHMIDT, 2013).

Não existem padrões estabelecidos para o tratamento das NGM (SORENMO; WORLEY; GOLDSCHMIDT, 2013; SORENMO, 2003), entretanto, as recomendações geralmente intensificam com o avanço do estadiamento clínico e dos fatores de pior prognóstico (NOVOSAD, 2003; SORENMO, 2003). Quimioterapia é frequentemente administrada em cães diagnosticados com NGM com maior risco de metástase ou recorrência (tipos histológicos agressivos, tumores grandes e com metástase à distância) (SORENMO; WORLEY; GOLDSCHMIDT, 2013). Estudos prévios recomendam que as cadelas diagnosticadas com carcinomas sólidos, micropapilares, anaplásico e carcinosarcomas devem ser submetidos a quimioterapia (CASSALI et al., 2014; NUNES, 2015).

A quimioterapia adjuvante ao tratamento cirúrgico das NGM pode aumentar a eficácia do tratamento (KARAYANNOPOULOU et al., 2001). Entretanto, não existe

recomendação padrão para a definição dos protocolos quimioterápicos (CASSALI et al., 2014). Estudos clínicos de alta qualidade são necessários para avaliar a eficácia de protocolos quimioterápicos e estabelecer diretrizes para o tratamento de NGM de alto risco (SORENMO; WORLEY; GOLDSCHMIDT, 2013; SORENMO, 2003).

Lavalle et al. (2012) descreveram aumento da sobrevida global em cadelas tratadas com quimioterapia com carboplatina associado ou não a inibidores de Cox-2, comparado às cadelas tratadas apenas com excisão cirúrgica. Karayannopoulou et al. (2001) relataram aumento de sobrevida livre de doença e global em cadelas tratadas com cirurgia e quimioterapia com 5-fluoracil e ciclofosfamida quando comparado às cadelas tratadas apenas com cirurgia. A literatura também descreve os seguintes protocolos: doxorrubicina associado a ciclofosfamida, doxorrubicina associado a carboplatina, carboplatina associado a gencitabina e paclitaxel como único fármaco (CASSALI et al., 2014).

Quimioterapia causa dano e interfere na replicação do DNA em células em proliferação (HANAHAN; BERGERS; BERGSLAND, 2000), resultando em efeitos antiproliferativos e citotóxicos (EHRKE, 2003). Protocolos de quimioterapia em dose máxima tolerada (QDMT) tem como objetivo o máximo de citotoxicidade das células tumorais possível, necessitando de intervalo para a recuperação de tecidos normais. Esses protocolos frequentemente são inicialmente eficazes e resultam em regressão ou estabilização do tumor, aumento da sobrevida e até cura. Entretanto, algumas respostas podem apresentar curta duração, com recorrências resultando em neoplasias de pior comportamento biológico e resistência à fármacos citotóxicos (HANAHAN; BERGERS; BERGSLAND, 2000).

O principal objetivo do tratamento das NGM em cães deve ser não submeter pacientes a tratamentos agressivos desnecessários ou não tratar os pacientes que seriam beneficiados (CASSALI et al., 2014). A qualidade de vida do paciente deve sempre ser prioritária (CASSALI et al., 2014; PIERINI et al., 2012), assim, novas estratégias quimioterápicas devem objetivar minimizar efeitos colaterais (PIERINI et al., 2012).

Angiogênese e Quimioterapia Metronômica como Terapia Antiangiogênica

A angiogênese tem sido claramente associada a metástase e progressão tumoral em tumores sólidos (RAJE; ANDERSON, 2002). Células tumorais necessitam de perfusão induzida por novos capilares para exceder 2-3 mm de diâmetro. Com a inibição da angiogênese, o tumor pode permanecer em um estado não-vascularizado e dormente, dificultando a progressão metastática e aumentando a suscetibilidade das células tumorais ao sistema imunológico. Assim, a inibição da angiogênese também pode ser sinérgica com a imunoterapia (FOLKMAN, 1971).

A combinação de estratégias antiangiogênicas com protocolos de quimioterapia antineoplásica são provavelmente associados a benefícios clínicos no tratamento de tumores malignos (PIERINI et al., 2012). Tratamentos com agentes únicos são geralmente considerados menos eficazes quando comparado aos tratamentos multimodais envolvendo radioterapia e quimioterapia e possivelmente terapias que promovem as defesas antitumorais do hospedeiro (EHRKE, 2003).

Em 2000, regimes metronômicos de fármacos citotóxicas foram estabelecidas como terapias antiangiogênicas promissoras (HANAHAN; BERGERS; BERGSLAND, 2000). Apesar da ausência de citotoxicidade direta da quimioterapia metronômica (QM) contra células neoplásicas, o tratamento modifica o microambiente tumoral, principalmente dificultando a angiogênese estromal (PIERINI et al., 2012) e também tem sido associada ao aumento na imunidade antitumoral em animais (EHRKE, 2003). A QM tem como objetivo eliminar ou minimizar o intervalo entre as administrações de quimioterapia, limitando o reparo e a replicação celular (PIERINI et al., 2012). A abordagem da terapia é alterada, sem ênfase nas células neoplásicas, oferecendo potencial no tratamento de neoplasias refratárias a determinados fármacos ou usando fármacos considerados ineficazes para determinada neoplasia (HANAHAN; BERGERS; BERGSLAND, 2000). Ao contrário da QDMT, onde o objetivo é a redução do tumor, a inibição da progressão da doença assume uma grande importância na QM (COLLEONI et al., 2006).

Os protocolos contínuos da QM exigem significativa redução da dose do agente quimioterápico (MUTSAERS, 2013). A QM é atraente por geralmente ser bem tolerada, apresenta baixa toxicidade, fácil administração e baixo custo (MUTSAERS,

2013; PIERINI et al., 2012). Contudo, os estudos clínicos em medicina veterinária ainda estão em fases iniciais (MUTSAERS, 2013).

Diversos fármacos podem ser combinados com a QM, incluindo anti-inflamatórios não estereoidais e inibidores de tirosina quinase (PIERINI et al., 2012). Vários estudos clínicos envolvendo QM e diversas neoplasias malignas foram realizados em medicina veterinária. Lomustina foi administrada em neoplasias primárias não ressecáveis, sem margens cirúrgicas, refratárias a quimioterapia ou metastáticas, sendo bem tolerada e considerada uma opção terapêutica viável nesses casos (TRIPP et al., 2011). Clorambucil demonstrou atividade antitumoral no tratamento em cães com neoplasias malignas espontâneas (LEACH et al., 2012) e o fármaco foi administrado em carcinomas de células de transição da bexiga urinária, sendo considerada bem tolerada e uma opção de tratamento para essa neoplasia em cães (SCHREMPP et al., 2013). Ciclofosfamida foi associada a toceranib e administrado em várias neoplasias malignas (excluindo mastocitomas), sendo observados efeitos antitumorais (MITCHELL; THAMM; BILLER, 2012); foi associado a etoposide e piroxican no tratamento de hemangiossarcomas esplênicos estadio II, sendo o tratamento bem tolerado e considerado uma alternativa eficiente ao tratamento de QDMT (LANA et al., 2007); e associado ao piroxican em sarcomas de tecidos moles sem margens cirúrgicas, sendo muito eficaz na prevenção de recorrências tumorais (ELMSLIE; GLAWE; DOW, 2008).

Talidomida

A talidomida foi sintetizada na Alemanha em 1954 e comercializada mundialmente como um sedativo e hipnótico, sendo mais eficaz como antiemético indicado no início da gestação (MCBRIDE, 1977). O primeiro efeito colateral do fármaco, a neuropatia periférica, foi descrito por Florence (1960) em quatro pacientes sendo tratados com 100 mg de talidomida por períodos prolongados e, após a interrupção do medicamento, houve melhora dos sintomas. Após o relato de Florence, sintomas similares foram descritos por Burley (1961), Kuenssber, Simpson e Stanton (1961) e Shafar (1961). O efeito colateral de malformações nas extremidades foi descrito por McBride (1961). Assim, talidomida foi associada a deformidades congênitas e o fármaco foi retirado do mercado (LENZ, 1988; MCBRIDE, 1977).

A talidomida tem sido usada em investigações de tratamentos de inúmeras doenças (PARAVAR; LEE, 2008). A atividade terapêutica da talidomida em humanos foi descrita para lesões não neoplásicas, como o eritema nodoso hansênico e caquexia em pacientes com síndrome da imunodeficiência adquirida, e doenças neoplásicas, como mieloma múltiplo e sarcoma de Kaposi (DIMOPOULOS; ELEUTHERAKIS-PAPAIAKOVOU, 2004; SINGHAL; MEHTA, 2002). As propriedades antiangiogênicas e imunomodulatórias do fármaco e seus análogos estimulam a investigação desses agentes no tratamento de cânceres hematológicos e sólidos e de doenças não neoplásicas, havendo estudos clínicos em combinação com agentes quimioterápicos ou como agentes únicos (MELCHERT; LIST, 2007).

O exato mecanismo de ação da talidomida em neoplasias não é completamente conhecido (SINGHAL; MEHTA, 2002). As propriedades antiangiogênicas foram descritas como uma possível consequência da terapia com talidomida, não explicando o efeito clínico do fármaco (STEWART, 2014). Ito et al. (2010) e Chamberlain et al. (2014) demonstraram a importância da proteína Cereblon, que forma um complexo Cul4–Rbx1–DDB1–Cereblon E3 ubiquitina ligase, como alvo da talidomida e seus análogos. A ligação da talidomida ao complexo promove o recrutamento, ubiquitinação e degradação dos substratos Ikaros e Aiolos, levando aos efeitos terapêuticos e teratogénicos dos fármacos.

A descoberta das propriedades antiangiogênicas da talidomida coincidiu com a evidenciação da importância da angiogênese no crescimento e progressão tumoral. A terapia antiangiogênica consiste em uma estratégia atraente para doenças resistentes e há evidência de que a quimioterapia citotóxica e terapias antiangiogênicas possuem maiores efeitos antitumorais quando realizadas em combinação, quando comparadas a cada terapia isolada (RICHARDSON; HIDESHIMA; ANDERSON, 2002).

Talidomida e seus análogos inibem diversas citocinas, como interleucina-6 (IL-6), fator de necrose tumoral-α (TNF-α), fator de crescimento do endotélio vascular (VEGF) e fator de crescimento de fibroblastos básico (bFGF), resultando em inibição de crescimento, sobrevida, migração, resistência a fármacos e angiogênese de células tumorais; e podem estimular interleucina-2 (IL-2) e interferon-γ (IFN-γ), favorecendo a imunidade antitumoral (RAJE; ANDERSON, 2002). Os efeitos

imunomodulatórios da talidomida dependem da doença, do status imunológico, do tipo de célula imunológica ativada e do tipo de estímulo que a célula recebe (TEO, 2005).

O fármaco é geralmente administrado em doses únicas a noite, devido aos efeitos adversos de sonolência e hipotensão ortostática (ADLARD, 2000). Teratogenicidade, sedação, neuropatia periférica, sonolência, constipação, náusea, fraqueza, fadiga, letargia, formigamento e/ou dormência nas mãos e nos pés, tontura, tromboembolismo, dor de cabeça e erupções cutâneas são os principais efeitos adversos da talidomida em humanos (DIMOPOULOS; ELEUTHERAKIS-PAPAIAKOVOU, 2004; ESCUDIER et al., 2002; SINGHAL; MEHTA, 2002). Se os efeitos adversos são considerados toleráveis, os pacientes são incentivados a manter a dose (SINGHAL; MEHTA, 2002). A dose de 4-8 mg/kg/dia de talidomida é usada na manutenção de várias doenças neoplásicas em humanos (TEO, 2005). A combinação da atividade antiangiogênica, disponibilidade de apresentação oral bem tolerada, disponibilidade de métodos contraceptivos seguros e eficazes e a importância da angiogênese no desenvolvimento, crescimento e metástase de neoplasias malignas, induziram estudos clínicos com talidomida no tratamento do câncer (BAIDAS et al., 2000).

Estudos prévios, avaliando o tratamento do carcinoma mamário murino 4T1 com talidomida, demonstraram benefício claro no controle do desenvolvimento do tumor primário e da metástase (DE SOUZA et al., 2012, 2014; DOS REIS et al., 2014). Estudos com câncer de mama avançado em mulheres foram realizados apenas com a talidomida como agente único ou com duração limitada, sendo que maior benefício clínico poderia ter sido observado se a talidomida fosse combinada com a quimioterapia e depois continuada como terapia de manutenção por tempo prolongado (SINGHAL; MEHTA, 2002).

Em medicina veterinária, o perfil de segurança da talidomida foi avaliado em beagles saudáveis, em doses entre 43 e 1000 mg/kg, administradas diariamente durante 53 semanas. A dose de 200 mg/kg foi considerada a dose limite sem efeitos adversos observados. Os efeitos adversos observados nesse estudo foram urina esverdeada, partículas esbranquiçadas nas fezes, aumento do tecido mamário, duração prolongada do estro, descoloração óssea amarelada e acúmulo de pigmento biliar no fígado. Neuropatia periférica não foi descrita (TEO et al., 2001). Três estudos

piloto foram realizados: não foram observados efeitos adversos em cães com várias neoplasias malignas tratados com talidomida (JANKOWSKI et al., 1999); talidomida induziu respostas clínicas prolongadas como único fármaco em hemangiossarcomas caninos (WOODS; MATHEWS; BINNINGTON, 2004); e uma abordagem multimodal incluindo bleomicina, piroxican, talidomida, radioterapia e cirurgia teve resultados encorajadores em carcinomas de células escamosas não ressecáveis de cabeça e pescoço em felinos (MARCONATO et al., 2013). Apesar de não estar determinada a dose e protocolo de administração ideal em cães, a ausência de efeitos adversos importantes, incluindo mielosupressão, permite que a talidomida possa ser usada em combinação com protocolos quimioterápicos, possivelmente representando uma nova forma de tratamento para os tumores e microambientes tumorais (WOODS; MATHEWS; BINNINGTON, 2004). Entretanto, atualmente, a talidomida está disponível apenas para uso humano, por meio de prescrição médica, não estando disponível para prescrição médica veterinária (TEO et al., 2000).

Dois estudos prévios avaliaram a associação da QM com talidomida. Um cão diagnosticado com tumor de células de Leydig maligno metastático foi tratado com exérese cirúrgica e tratamento adjuvante com QM com ciclofosfamida e talidomida na dose de 4,46 mg/kg (TOGNI et al., 2015). O tratamento de hemangiossarcomas caninos de comportamento biológico agressivo com QDMT associada ou não à QM com ciclofosfamida e talidomida revelou que a associação foi bem tolerada e aumentou a sobrevida dos pacientes. Os autores descrevem que enquanto a QDMT diminuem o número das células neoplásicas, a terapia de manutenção da QM inibe a angiogênese, impedindo a recorrência e levando a benefícios terapêuticos (FINOTELLO et al., 2016). Colleoni et al. (2006) avaliaram a QM (com ciclofosfamida e metotrexato) com ou sem a adição de talidomida no tratamento de câncer de mama metastático em mulheres, demonstrando eficácia clínica da QM, e confirmando o importante papel dessa terapia. O tratamento foi bem tolerado pelas pacientes mas administração da talidomida não gerou benefício clínico adicional. Baidas et al. (2000), relatam que a talidomida apresentou pouca ou nenhuma atividade como único fármaco no tratamento de mulheres com câncer de mama metastático previamente tratados com quimioterapia. Entretanto, os autores não excluem a possibilidade de ação da talidomida em combinação com outras modadlidades terapêuticas ou em

câncer de mama com micrometástases ou outras neoplasias. Eisen et al. (2000) avaliaram talidomida no tratamento de melanoma, carcinoma de células renais e câncer ovariano e de mama em estadio avançado, com respostas encorajadoras nos carcinomas de células renais. Apesar da ausência de respostas objetivas nos outros tipos tumorais, houve melhora na qualidade do sono e no apetite dos pacientes.

Desde que a talidomida foi retirada do mercado, vários países estabeleceram regulamentações para a prescrição e descarte do medicamento para humanos, como o programa THALIDOMID REMS^{TM} dos Estados Unidos da América (CELGENE, 2015). No Brasil, a Agência Sanitária de Vigilância Sanitária (ANVISA) autoriza a fabricação do medicamento somente em laboratórios oficiais da programação do Ministério da Saúde, sendo proibida sua remanipulação. O tratamento com talidomida é indicado na hanseníase, na reação hansênica tipo eritema nodoso ou tipo II; em DST/AIDS, em úlceras aftoides idiopáticas; doenças crônico-degenerativas: em lúpus eritematoso sistêmico e discoide, lúpus eritematoso cutâneo subagudo e doença do enxerto versus hospedeiro; mieloma múltiplo; e síndrome mielodisplásica em pacientes refratários a eritropoietina: anemia refratária sem sideroblastos em anel, com sideroblastos em anel e não especificada. Os pacientes recebem um termo de esclarecimento e termo de responsabilidade, e mulheres em idade fértil necessitam comprovar a utilização de, no mínimo, dois métodos de contracepção. A prescrição não pode ser superior a 30 dias de tratamento e poderá ser realizada somente por médicos devidamente cadastrados (AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA: RESOLUÇÃO - RDC N^{o} 11, 2011). Caso ocorra contato com cápsulas de talidomida que não estejam intactas ou se os prestadores de cuidados de saúde são expostos aos fluidos corporais de pacientes recebendo talidomida, a área exposta deve ser lavada com água e sabão. Precauções apropriadas, como o uso de luvas, devem ser utilizadas com o objetivo de prevenir de potenciais exposições cutâneas ao fármaco (CELGENE, 2015).

REFERENCIAS

ADLARD, J. W. Thalidomide in the treatment of cancer. **Anti-Cancer Drug**, v. 11, p. 787–791, 2000.

AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA: RESOLUÇÃO - RDC Nº 11, D. 22 D. M. D. 2011. **Regulamento Técnico sobre as atividades que envolvam a substância Talidomida (DCB 08266 e CAS nº 50-35-1) e o medicamento que a contenha**. No 57 ed. Brasília: Diário Oficial da União, 2011.

BAIDAS, B. S. M.; WINER, E. P.; FLEMING, G. F.; HARRIS, L.; PLUDA, J. M.; CRAWFORD, J. G.; YAMAUCHI, H.; ISAACS, C.; HANFELT, J.; TEFFT, M.; FLOCKHART, D.; JOHNSON, M. D.; HAWKINS, M. J.; LIPPMAN, M. E.; HAYES, D. F. Phase II Evaluation of Thalidomide in Patients With Metastatic Breast Cancer. **Journal of Clinical Oncology**, v. 18, n. 14, p. 2710–2717, 2000.

BOSTOCK, D. E. Canine and feline mammary neoplasms. **British Veterinary Journal**, v. 142, p. 506–515, 1986.

BRODEY, R. S.; GOLDSCHMIDT, M. H.; ROSZEL, J. R. Canine mammary gland neoplasms. **Journal of the American Animal Hospital Association**, v. 19, p. 61–90, 1983.

BURLEY, D. Is thalidomide to blame? **BMJ: British Medical Journal**, v. 1, n. 5219, p. 130, 1961.

CASSALI, G.; LAVALLE, G.; FERREIRA, E.; ESTRELA-LIMA, A.; NARDI, A.; GHEVER, C.; SOBRAL, R.; AMORIM, R.; OLIVEIRA, L.; SUEIRO, F.; BESERRA, H.; BERTAGNOLLI, A.; GAMBA, C.; DAMASCENO, K.; CAMPOS, C.; ARAUJO, M.; CAMPOS, L.; MONTEIRO, L.; NUNES, F.; HORTA, R.; REIS, D.; LUVIZOTTO, M.; MAGALHAES, G.; RAPOSO, J.; FERREIRA, A.; TANAKA, N.; GRANDI, F.; UBUKATA. R.; BATSCHINSKI, K.; TERRA, E.; SALVADOR, R.; JARK, P.; DELECRODI, J.; NASCIMENTO, N.; SILVA, D.; SILVA, L.; FERREIRA, K.; FREHSE, M.; SANTIS, G.; SILVA, E.; GUIM, T.; KERR, B.; CINTRA, P.; SILVA, F.; LEITE, J.; MELLO, M.; FERREIRA, M.; FUKUMASU, H.; SALGADO, B,. TORRES, R. Consensus for the diagnosis, prognosis and treatment of canine mammary tumors - 2013. **Brazilian Journal of Veterinary Pathology**, v. 7, n. 2, p. 38–69, 2014.

CELGENE. **Thalidomid REMS**. Disponível em: <http://www.thalomidrems.com/>. Acesso em: 5 out. 2016.

CHAMBERLAIN, P..; LOPEZ-GIRONA, A.; MILLER, K.; CARMEL, G.; PAGARIGAN, B.; CHIE-LEON, B.; RYCHAK, E.; CORRAL, L.; REN, Y.; WANG, M.; RILEY, M.; DELKER, S.; ITO, T.; ANDO, H.; MORI, T.; HIRANO, Y.; HANDA, H.; HAKOSHIMA, T.; DANIEL, T.; CATHERS, B. Structure of the human Cereblon-DDB1-lenalidomide complex reveals basis for responsiveness to thalidomide analogs. **Nature Structural & Molecular Biology**, v. 21, n. 9, p. 803–810, 2014.

CLEMENTE, M. DE ANDRÉS, P.; PEÑA, L.; PEREZ-ALENZA, M. Survival time of dogs with inflammatory mammary cancer treated with palliative therapy alone or palliative therapy plus chemotherapy. **Veterinary Record**, v. 165, p. 78–81, 2009.

COLLEONI, M. ORLANDO, L.; SANNA, G.; ROCCA, A.; MAISONNEUVE, P.; PERUZZOTTI, G.; GHISINI, R.; SANDRI, M.; ZORZINO, L.; NOLÈ, F.; VIALE, G.; GOLDHIRSCH, A. Metronomic low-dose oral cyclophosphamide and methotrexate plus or minus thalidomide in metastatic breast cancer: Antitumor activity and biological effects. **Annals of Oncology**, v. 17, n. 2, p. 232–238, 2006.

DE SOUZA, C.; DE CARVALHO, L.; VIEIRA, T.; ARAÚJO E SILVA, A.; LOPES, M.; FERREIRA, M.; ANDRADE, S.; CASSALI, G. Thalidomide attenuates mammary cancer associated-inflammation, angiogenesis and tumor growth in mice. **Biomedicine and Pharmacotherapy**, v. 66, n. 7, p. 491–498, 2012.

DE SOUZA, C.; ARAÚJO E SILVA, A.; FERRACIOLLI, C.; MOREIRA, G.; CAMPOS, L.; DOS REIS, D.; LOPES, M.; FERREIRA, M.; ANDRADE, S.; CASSALI, G. Combination therapy with carboplatin and thalidomide suppresses tumor growth and metastasis in 4T1 murine breast cancer model. **Biomedicine and Pharmacotherapy**, v. 68, p. 51–57, 2014.

DIMOPOULOS, M. A.; ELEUTHERAKIS-PAPAIAKOVOU, V. Adverse effects of thalidomide administration in patients with neoplastic diseases. **American Journal of Medicine**, v. 117, n. 7, p. 508–515, 2004.

DOBSON, J. M. Introduction: Cancer in cats and dogs. In: DOBSON, J. M.; LASCELLES, B. D. X. (Eds.). **BSAVA Manual of Canine and Feline Oncology**. 3. ed. Gloucester: British Small Animal Veterinary Association, 2011. p. 1–5.

DOS REIS, D.; SOUZA, C.; CAMPOS, L.; SILVA, A.; LOPES, M.; CASSALI, G.; FERREIRA, E. Thalidomide promotes leukocytosis in mice inoculated with 4T1 mammary carcinoma. **Journal Brasileiro de Patologia e Medicina Laboratorial**, v. 50, n. 1, p. 64–66, 2014.

EHRKE, M. J. Immunomodulation in Cancer Therapeutics. **International Immunopharmacology**, v. 3, p. 1105–1119, 2003.

EISEN, T.; BOSHOFF, C.; MAK, I.; SAPUNAR, F.; VAUGHAN, M.; PYLE, L.; JOHNSTON, S.; AHERN, R.; SMITH, I.; GORE, M. Continuous low dose Thalidomide: a phase II study in advanced melanoma , renal cell , ovarian and breast cancer. **Brithish Journal of Cancer**, v. 82, n. 4, p. 812–817, 2000.

ELMSLIE, R. E.; GLAWE, P.; DOW, S. W. Metronomic therapy with cyclophosphamide and piroxicam effectively delays tumor recurrence in dogs with incompletely resected soft tissue sarcomas. **Journal of Veterinary Internal Medicine**, v. 22, n. 6, p. 1373–1379, 2008.

ESCUDIER, B.; LASSAU, N.; COUANET, D.; ANGEVIN, E.; MESRATI, F.; LEBORGNE, S.; GAROFANO, A.; LEBOULAIRE, C.; DUPOUY, N.; LAPLANCHE, A. Phase II trial of thalidomide in renal-cell carcinoma. **Annals of Oncology**, v. 13, n. 7, p. 1029–1035, 2002.

FINOTELLO, R.; HENRIQUES, J.; SABATTINI, S.; STEFANELLO, D.; FELISBERTO, R.; PIZZONI, S.; FERRARI, R.; MARCONATO, L. A retrospective analysis of chemotherapy switch suggests improved outcome in surgically removed, biologically aggressive canine haemangiosarcoma. **Veterinary and Comparative Oncology**, p. 9–11, 2016.

FLORENCE, A. L. Is Thalidomide to Blame? **BMJ: British Medical Journal**, v. 2, n. 5217, p. 1954, 1960.

FOLKMAN, J. Tumor angiogenesis: therapeutic implications. **The New England Journal of Medicine**, v. 285, n. 21, p. 1182–1186, 1971.

HANAHAN, D.; BERGERS, G.; BERGSLAND, E. Less is more, regularly: Metronomic dosing of cytotoxic drugs can target tumor angiogenesis in mice. **The Journal of Clinical Investigation**, v. 105, n. 8, p. 1045–1047, 2000.

INSTITUTO NACIONAL DO CÂNCER JOSÉ ALENCAR GOMES DA SILVA. CORDENAÇÃO DE PREVENÇÃO E VIGILÂNCIA. **Estimativa 2016: Incidência de câncer no Brasil**. Rio de Janeiro: INCA, 2015.

ITO, T.; ANDO, H.; SUZUKI, T.; OGURA, T.; HOTTA, K.; IMAMURA, Y.; YAMAGUCHI, Y.; HANDA, H. Identification of a primary target of thalidomide teratogenicity. **Science**, v. 327, n. 5971, p. 1345–1350, 2010.

JANKOWSKI, M.; FULTON, L.; SHEAFOR, S.; PRESCOTT, D.; KHANNA, C. Ongoing evaluation of single agent thalidomide in dogs with measurable cancer. **Annals of the Veterinary Cancer Society 19th Annual Meeting**. Wood's Hole, MA: 1999.

KARAYANNOPOULOU, M.; KALDRYMIDOU, E.; CONSTANTINIDIS, T.; DESSIRIS, A. Adjuvant Post-operative Chemotherapy in Bitches with Mammary Cancer. **Journal of Veterinary Medicine Series A**, v. 48, n. 2, p. 85–96, 2001.

KUENSSBERG, E. V; SIMPSON, J. A.; STANTON, J. B. Is thalidomide to blame? **BMJ: British Medical Journal**, v. 1, n. 5221, p. 291, 1961.

LANA, S.; LANCE, U.; PLAZA, S.; ELMSLIE, R.; GUSTAFSON, D.; MORLEY, P.; DOW, S. Continuous Low-Dose Oral Chemotherapy for Adjuvant Therapy of Splenic Hemangiosarcoma in Dogs. **Journal of Veterinary Internal Medicine**, v. 21, p. 764–769, 2007.

LAVALLE, G.; CAMPOS, C.; BERTAGNOLLI, A.; CASSALI, G. Canine malignant mammary gland neoplasms with advanced clinical staging treated with carboplatin and cyclooxygenase inhibitors. **In Vivo**, v. 26, p. 375–379, 2012.

LEACH, T.; CHILDRESS, M.; GREENE, S.; MOHAMED, A.; MOORE, G.; SCHREMPP, D.; LAHMAN, S.; KNAPP, D. Prospective trial of metronomic chlorambucil chemotherapy in dogs with naturally occurring cancer. **Veterinary and Comparative Oncology**, v. 10, n. 2, p. 102–112, 2012.

LENZ, W. A short history of thalidomide embryopathy. **Teratology**, v. 38, n. 3, p. 203–215, 1988.

MARCONATO, L.; BUCHHOLZ, J.; KELLER, M.; BETTINI, G.; VALENTI, P.; KASER-HOTZ, B. Multimodal therapeutic approach and interdisciplinary challenge for the treatment of unresectable head and neck squamous cell carcinoma in six cats: A pilot study. **Veterinary and Comparative Oncology**, v. 11, n. 2, p. 101–112, 2013.

MCBRIDE, W. G. Thalidomide and congenital abnormalities. **Public Health**, v. 278, n. 7216, p. 1358, 1961.

MCBRIDE, W. G. Thalidomide embryopathy. **Teratology**, v. 16, n. 1, p. 79–82, 1977.

MELCHERT, M.; LIST, A. The thalidomide saga. **International Journal of Biochemistry and Cell Biology**, v. 39, n. 7-8, p. 1489–1499, 2007.

MITCHELL, L.; THAMM, D. H.; BILLER, B. J. Clinical and Immunomodulatory Effects of Toceranib Combined with Low-Dose Cyclophosphamide in Dogs with Cancer. **Journal of Veterinary Internal Medicine**, v. 26, n. 2, p. 355–362, 2012.

MUTSAERS, A. J. Antiangiogenic and Metronomic Therapy. In: WITHROW, S. J.; VAIL, D. M. (Eds.). . **Withrow & MacEwen's Small Animal Clinical Oncology**. 5. ed. Philadelphia: W. B. Saunders Company, 2013. p. 229–237.

NOVOSAD, C. A. Principles of treatment for mammary gland tumors. **Clinical Techniques in Small Animal Practice**, v. 18, n. 2, p. 107–109, 2003.

NUNES, F.; GAMBA, C.; DAMASCENO, K.; CAMPOS, C.; HORTA, R.; ARAUJO, M.; MONTEIRO, L.; LAVALLE, G.; FERREIRA, E.; CASSALI, G. Analisis of clinico-pathological data, therapeutical conduct and overall survival of canine mammary lesions attended at the veterinary hospital of the Federal University of Minas Gerais (UFMG). **Brazilian Journal of Veterinary Pathology**, v. 7, n. 2, p. 122–126, 2014.

NUNES, F. C. **Diagnóstico, prognóstico e tratamento dos carcinomas de glândulas mamárias de cadelas atendidas no hospital veterinário da UFMG – Estudo retrospectivo**. Federal University of Minas Gerais, 2015. p.1-75.

OWEN, L. N. **TNM Classification of tumors in domestic animals**. 1. ed. Geneva: World Health Organization, 1980. p. 1-53.

PARAVAR, T.; LEE, D. J. Thalidomide: mechanisms of action. **International Reviews of Immunology**, v. 27, n. 3, p. 111–135, 2008.

PHILIBERT, J.; SNYDER, P.; GLICKMAN, N.; GLICKMAN, L.; KNAPP, D.; WATERS, D. Influence of Host Factors on Survival in Dogs with Malignant Mammary Gland Tumors. **Journal of Veterinary Internal Medicine**, v. 17, n. 1, p. 102–106, 2003.

PIERINI, A.; BOCCI, G.; GIORGI, M.; OWEN, H.; MARCHETTI, V. From humans to dogs and back: The translational lesson of metronomic chemotherapy. **American Journal of Animal and Veterinary Sciences**, v. 7, n. 4, p. 198–212, 2012.

QUEIROGA, F.; PEREZ-ALENZA, M.; SILVAN, G.; PENÃ, L.; LOPES, C.; ILLERA, J. Cox-2 Levels in Canine Mammary Tumors , Including Inflammatory Mammary Carcinoma: Clinicopathological Features and Prognostic Significance. **Anticancer Research**, v. 4276, p. 4269–4275, 2005.

QUEIROGA, F.; LOPES, C. Tumores mamários caninos, pesquisa de novos factores de prognóstico Canine mammary tumours, research on new prognostic factors. **Revista Portuguesa de Ciências Veterinárias**, v. 97, n. 543, p. 119–127, 2002.

RAJE, N.; ANDERSON, K. C. Thalidomide and immunomodulatory drugs as cancer therapy. **Current Opinion in Oncology**, v. 14, p. 635–640, 2002.

RICHARDSON, P.; HIDESHIMA, T.; ANDERSON, K. THALIDOMIDE: Emerging Role in Cancer Medicine. **Annual Review of Medicine**, v. 53, p. 629–657, 2002.

SCHNEIDER, R.; DORN, C. R.; TAYLOR, D. O. N. Factors Influencing Canine Mammary Cancer Development and Postsurgical Survival. **Journal of the National Cancer Institute**, v. 43, n. 6, p. 1249–1261, 1969.

SCHREMPP, D., CHILDRESS, M.; STEWART, J.; LEACH, T.; TAN, K.; ABBO, A.; DE GORTARI, A.; BONNEY, P.; KNAPP, D. Metronomic administration of chlorambucil for treatment of dogs with urinary bladder transitional cell carcinoma. **Journal of the American Veterinary Medical Association**, v. 242, n. 11, p. 1534–1538, 2013.

SHAFAR, J. Is Thalidomide to Blame? **BMJ: British Medical Journal**, v. 1, n. 5228, p. 829, 1961.

SINGHAL, S.; MEHTA, J. Thalidomide in cancer. **Biomedicine & Pharmacotherapy**, v. 56, p. 4–12, 2002.

SORENMO, K. U. Canine mammary gland tumors. **The Veterinary clinics of North America. Small animal practice**, v. 33, p. 573–596, 2003.

SORENMO, K. U.; WORLEY, D. R.; GOLDSCHMIDT, M. H. Tumors of the Mammary Gland. In: WITHROW, S. J.; VAIL, D. M.; PAGE, R. L. (Eds.). . **Withrow & MacEwen's Small Animal Clinical Oncology**. 5. ed. Saint Louis: Elsevier Saunders, 2013. p. 538–556.

STEWART, A. K. How thalidomide works against cancer. **Science**, v. 343, n. 6168, p. 256–7, 2014.

TEO, S.; EVANS, M.; EHRHART, J.; BROCKMAN, M.; ALLEN, D.; MORGAN, M.; STIRLING, D.; THOMAS, S. Lack of peripheral neuropathy in Beagle dogs after 53 weeks oral administration of thalidomide capsules. **Human & Experimental Toxicology**, v. 19, p. 615–622, 2000.

TEO, S. K.; EVANS, M.; BROCKMAN, M.; HERHART, J.; MORGAN, J.; STIRLING, D.; THOMAS, S. Safety Profile of Thalidomide after 53 Weeks of Oral Administration in Beagle Dogs. **Toxicological Sciences**, v. 59, p. 160–168, 2001.

TEO, S. K. Properties of thalidomide and its analogues: implications for anticancer therapy. **The AAPS journal**, v. 7, n. 1, p. 14–19, 2005.

TOGNI, A.; RÜTTEN, M.; BLEY, C.; HURTER, K. Metastasized Leydig cell tumor in a dog. **Schweizer Archiv für Tierheilkunde**, v. 157, n. 2, p. 111–5, 2015.

TRIPP, C.; FIDEL, J.; ANDERSON, C.; PATRICK, M.; PRATT, C.; SELLON, R.; BRYAN, J. Tolerability of Metronomic Administration of Lomustine in Dogs with Cancer. **Journal of Veterinary Internal Medicine**, v. 25, n. 2, p. 278–284, 2011.

VON EULER, H. Tumors of the mammary gland. In: DOBSON, J. M.; LASCELLES, B. (Eds.). . **BSAVA Manual of Canine and Feline Oncology**. 3. ed. Gloucester: British Small Animal Veterinary Association, 2011. p. 237–247.

WITHROW, S. J.; VAIL, D. M.; PAGE, R. L. Why worry about cancer in companion animals? In: WITHROW, S. J.; VAIL, D. M.; PAGE, R. L. (Eds.). **Withrow & MacEwen's Small Animal Clinical Oncology**. 5. ed. Saint Louis: Elsevier Saunders, 2013. p. xv–xvi.

WOODS, J. P.; MATHEWS, K. A.; BINNINGTON, A. G. Thalidomide for the treatment of hemangiosarcoma in dogs. **Veterinary and Comparative Oncology**, v. 2, n. 2, p. 108–109, 2004.

YAMAGAMI, T.; KOBAYASHI, T.; TAKAHASHI, K.; SUGIYAMA, M. Prognosis for canine malignant mammary tumors based on TNM and histologic classification. **Journal of Veterinary Medical Science**, v. 58, n. 11, p. 1079–1083, 1996.

CAPÍTULO 2 – ABSENCE OF SIGNIFICANT ADVERSE EVENTS FOLLOWING THALIDOMIDE ADMINISTRATION IN BITCHES DIAGNOSED WITH MAMMARY GLAND CARCINOMAS

ABSTRACT - The aim of the study was to evaluate the incidence of adverse events (AE) in female dogs diagnosed with advanced clinical stage mammary gland neoplasms following treatment with thalidomide. A prospective analysis of 29 female dogs treated with a high dose of 20 mg/kg/day of thalidomide (HD) for 3 months followed by a low dose of 10 mg/kg/day of thalidomide (LD) for 3 months was performed. All patients underwent physical examination, complete blood count, serum biochemistry profile, thoracic radiographs, and abdominal ultrasound analysis before the treatment and after the HD and LD. Clinical AE were absent in 16/29 (55.17%) patients following HD. An initial 3-5 days period of somnolence was described in 4/29 (13.79%), prolonged somnolence in 5/29 (17.24%), a short period of somnolence lasting only a few hours in 3/29 (10.34%), and difficulty to rouse was described in 5/29 (17.24%) cases. Two patients (6.89%) presented prolonged somnolence that interfered with activities of daily living, resulting in anticipation of the dose reduction to the proposed LD after 15 days of the HD treatment. Following dose reduction, AE improvement was observed in all patients. Albeit remaining within the reference ranges, erythrocytes, haematocrit, total leucocyte count, neutrophils, lymphocytes, monocytes, and GGT showed significant alteration associated to thalidomide treatment.

Key-Words: Adverse Events, Canine, Neoplasia, Thalidomide

INTRODUCTION

The images of devastating birth defects caused by thalidomide remains firmly embedded in the public consciousness (Stewart 2014). Thalidomide was synthesized in Germany in 1954 and marketed in various parts of the world as a sedative and a hypnotic, considered most effective as an anti-emetic in early pregnancy (McBride

Artigo apresentado nas normas do periódico *Veterinary Record* e publicado em 24 de Agosto de 2016 (doi: 10.1136/vr.103764).

1977). The first toxic effect of thalidomide was recorded by Florence (1960), who reported the early signs of peripheral neuropathy, followed by reports on malformations of the extremities (McBride 1961). Thalidomide was associated with congenital deformities and the drug was withdrawn from the market (McBride 1977; Lenz 1988).

Angiogenesis inhibition, immunomodulation and cytokine modulation have been considered, but the exact mechanism of action of thalidomide for neoplastic diseases remains unknown (Singhal and Mehta 2002). Antiangiogenic properties were described as a possible consequence of thalidomide therapy, but not the mechanism of action that explained its clinical effect (Stewart 2014). Chamberlain and others (2014) and Ito and others (2010) demonstrated the importance of thalidomide and related IMiD immunomodulatory agents binding to the Cul4–Rbx1–DDB1–Cereblon E3 ubiquitin ligase complex, leading to the therapeutic and teratogenic effects of these drugs. Thalidomide has shown activity in the treatment of erythema nodosum leprosum, oral ulcers in Behçet disease, wasting syndrome in patients with acquired human immunodeficiency disease, and is under investigation for rheumatoid arthritis, inflammatory bowel diseases, and other autoimmune disorders (Dimopoulos and Eleutherakis-Papaiakovou 2004). Thalidomide has also been studied in several types of human neoplastic diseases, such as multiple myeloma, Kaposi's sarcoma, breast cancer, gliomas, colon cancer, prostate cancer, renal cell carcinoma, and others (Singhal and Mehta 2002; Dimopoulos and Eleutherakis-Papaiakovou 2004). Although Teo and others (2001) evaluated the safety profile of thalidomide in healthy dogs, few studies have attempted to evaluate the efficacy of thalidomide in canine clinical trials.

Mammary gland tumours are the most common neoplasms in intact female dogs (Brodey and others 1983). Surgery is the mainstay of treatment for canine mammary gland tumours and is the single most effective modality for local tumour control. More advanced tumours may require adjuvant therapy (Novosad 2003; Sorenmo 2003), and chemotherapy is often recommended for dogs with mammary tumours considered to be at risk for metastasis or recurrence (Sorenmo and others 2013). However, there is no standard recommendation for the use of chemotherapy (Cassali and others 2014). High-quality trials are needed to evaluate the efficacy of chemotherapy protocols as well as to provide guidance for treating high-risk mammary gland tumours (Sorenmo 2003; Sorenmo and others 2013). Previous studies

evaluating thalidomide in the 4T1 mice mammary tumour model have demonstrated a clear benefit in controlling primary tumour development, as well as metastatic progression (De Souza and others 2012; De Souza and others 2014; Dos Reis and others 2014). Two pilot studies involving thalidomide were performed in veterinary medicine: thalidomide induced prolonged responses as a single drug in canine hemangiosarcomas (Woods and others 2004), and a multimodal approach including bleomycin, piroxican, thalidomide, radiotherapy, and surgery was considered to have encouraging results in feline unresectable head and neck squamous cell carcinoma (Marconato and others 2013).

An adverse event (AE) is defined as any unfavourable and unintended sign, clinical sign, or disease temporally associated with the use of a medical treatment that may or may not be considered related to the medical treatment (Veterinary Co-operative Oncology Group 2004). The aim of the present study was to evaluate AE in female dogs diagnosed with advanced clinical stage mammary gland neoplasms following two different doses of thalidomide.

MATERIALS AND METHODS

A prospective analysis of female dogs admitted to the Veterinary Hospital of the Federal University of Minas Gerais (UFMG), Brazil, diagnosed with mammary gland neoplasms was performed. The neoplasms were diagnosed at the Laboratory of Comparative Pathology of the Institute of Biological Sciences, UFMG, Brazil.

Clinical staging according to the TNM system established by the World Health Organization for canine mammary tumours was performed. This system evaluates: tumour size (T_1: 0-3 cm; T_2: 3-5 cm; T_3: >5 cm); neoplastic involvement of regional lymph nodes (N_0: non metastatic; N_1: metastatic); and presence of distant metastasis (M_0: non metastatic; M_1: metastatic). Afterwards, cases were divided into five stages: I ($T_1N_0M_0$), II ($T_2N_0M_0$), III ($T_3N_0M_0$), IV ($T_{1-3}N_1M_0$), and V ($T_{1-3}N_{0-1}M_1$) (Owen 1980; Sorenmo and others 2013). Patients presenting with advanced clinical staging (stages IV and V) were included in the study.

Thalidomide was administered in single daily oral doses for 6 months. In order to avoid possible undesirable effects of the drug due to its sedative properties, pet

owners were instructed to administer the medication at night. During the first three months, patients were subjected to a high dose (HD) (20 mg/kg) of thalidomide, followed by a low dose (LD) (10 mg/kg) for 3 months. The doses used in the present study were based on two previous canine studies that used daily doses of 100-400 mg to dogs diagnosed with hemangiosarcomas (Woods and others 2004) and proposed 3.3-6.5, 6.6-13, 13.3-26 mg/kg to dogs with varied malignant neoplasms (Jankowski and others 1999). Neither study found any important AE. Treatment with thalidomide was initiated following a minimum period of two weeks after the previous treatment (mainly chemotherapy) in order to allow patient recovery. Informed client consent was obtained in all cases.

Thalidomide tablets were manufactured at the Ezequiel Dias Foundation (EDF), Brazil, which meets the Brazilian Health Surveillance Agency's (ANVISA) good manufacturing practices for this production. The EDF is the only producer of thalidomide in Brazil.

Pet owners were instructed to administer the medication using disposable gloves. Individual prescriptions were given for a 28-day period and pet owners signed an informed-consent form acknowledging that the intact bitches were not allowed to reproduce, that the administration of thalidomide was exclusively for the canine enrolled in the study, and that any unused medication should be returned for adequate disposal at the EDF. Thalidomide was given to pet owners in tablets weighting 370 mg (100 mg of thalidomide + excipients). Doses were rounded to the nearest whole tablet size since reformulation was not possible. Therefore, the HD and LD the dose range was approximately 18-22 mg/kg and 8-12 mg/kg, respectively. The effect of food intake on thalidomide absorption in humans is considered minimal (Teo and others 2004). Pet owners were instructed to be careful while collecting and disposing of faeces, urine and other bodily fluids of the animals.

All patients underwent complete history evaluation, physical examination, serum exams consisting of a complete blood count (CBC), serum biochemistry profile (SBP), and thoracic radiographs and abdominal ultrasound at the baseline. Patients were clinically evaluated monthly and serum and imaging exams were repeated every three months. Additional exams or more frequent serum and imaging exams were requested when necessary, e.g. respiratory clinical signs leading to additional thoracic

radiographs. Pet owners were interviewed every month to assess any clinical observations of the patients that could be associated to the thalidomide treatment.

Clinical history, physical examination, serum and imaging exams performed during the HD and LD thalidomide treatment were compared to the baseline evaluation. In addition, VCOG-CTCAE criteria was applied to describe AE. AE were classified as: mild (grade 1), moderate (grade 2), severe (grade 3), life-threatening (grade 4), and death related to the AE (grade 5) (Veterinary Co-operative Oncology Group 2004).

Descriptive statistics are presented for AE findings. Statistical analyses of CBC and SBP were performed with Student's t-test, analysis of variance (ANOVA), Wilcoxon signed-rank, Friedman, and Kruskal-Wallis tests, depending on normality, the number of observations, and whether the observations were paired. Results were considered significant when $p \leq 0.05$.

All procedures were performed under the appropriate guidelines and with the approval of the Ethics Committee for Animal Experimentation of the Federal University of Minas Gerais (CETEA/UFMG) (protocol number 132/2011) and the Ethics Committee for Animal Experimentation of the São Paulo State University (Jaboticabal Campus) (protocol number 021846/14).

RESULTS

Twenty-nine patients were studied. Mean age was 12.6 ± 2.1 years. Six patients (20.69%) presented distant metastasis, classified as clinical stage V. All patients were submitted to surgical excision of the primary neoplasm and adjuvant chemotherapy was performed in 28/29 (95.55%) cases. Chemotherapy consisted of carboplatin, one dose every 21 days for a total of four doses given intravenously at 300 mg/m^2. Seven patients were not subjected to the low dose treatment: 6/29 (20.69%) patients due to death during the first three months of the HD treatment, and 1/29 (3.45%) due to treatment abandonment by the pet owner.

No significant alterations were found when evaluating the thoracic radiographs following the HD and LD of thalidomide. Abdominal ultrasounds demonstrated spleen

enlargement in 4/18 (22.22%) cases, increased renal cortical echogenicity in 3/18 (16.66%) cases, and diffuse hepatomegaly in 2/18 (11.11%) cases.

Pet owner interviews demonstrated some AE related to the thalidomide treatment. HD was not associated with AE in 16/29 (55.17%) patients. An initial 3-5 days period of somnolence was described in 4/29 (13.79%) cases, a short somnolence period lasting only a few hours following thalidomide administration in 3/29 (10.34%), while prolonged somnolence during the entire HD treatment period was found in 5/29 (17.24%), and 5/29 (17.24%) animals were found to be difficult to rouse in the morning. The somnolence AE described were considered grade 2. In addition, loss of appetite with no significant association to weight loss (grade 1) was found in 3/29 (10.34%). One (3.44%) animal presented transient mild ataxia (grade 2) during the first days of thalidomide administration and symptoms disappeared without additional treatment or thalidomide interruption. Following dose reduction, all patients that reported AE presented a significant improvement of all clinical findings. Two patients (6.89%) presented prolonged somnolence that was found to interfere with activities of daily living, resulting in anticipation of the dose reduction to the proposed LD after 15 days of the HD treatment. Following dose reduction, AE improvement was observed in all patients. However, 1/29 (3.44%) animal still presented a short period somnolence lasting only a few hours following thalidomide administration and 1/29 (3.44%) animal was found to be difficult to rouse in the morning.

Patients did not present significant body weight alteration, gastrointestinal, cardiac, dermatologic, endocrine, respiratory, genitourinary or neurological signs associated to the studied treatment. No definitive signs of peripheral neuropathy (PN) were observed.

CBC and SBP results at the baseline, following three months of the HD, and three months of the LD thalidomide administration are presented in table 1. Basophils and band neutrophils were only found in four and six CBC, respectively, not allowing statistical analysis. Baseline evaluations were performed after chemotherapy in the majority of patients, which explains the relatively low CBC values.

Table 1. Complete blood count (CBC) and serum biochemistry profile (SBP) results before thalidomide treatment (BT), following three months of the high dose (20 mg/kg/day) treatment (HD) and following three months of the low dose (10 mg/kg/day) treatment (LD) of female dogs with advanced stage mammary neoplasms receiving thalidomide therapy.

CBC / SBP	BT + HD[c]		BT + HD + LD[d]	
Erythrocytes[b] (10^6/mm^3)	BT 5.6 ± 0.9 HD 5.8 ± 1.1	p=0.2903	BT 5.5 ± 1 HD 6.1 ± 1 LD 6.4 ± 1.2	p=0.0201*
Haemoglobin[b] (%)	BT 13.1 ± 2.3 HD 13.3 ± 2.4	p=0.6184	BT 13.3 ± 2.5 HD 14.1 ± 1.8 LD 14.7 ± 2.4	p=0.1010
Haematocrit[a] (%)	BT 41 (24-51) HD 41 (27-50)	p=0.2124	BT 40.5 (26-44) HD 43.5 (34-48) LD 43 (29-50)	p=0.0121*
MCV[a] (fl)	BT 70.9 (61.4-80.7) HD 70.2 (61-95.7)	p=0.8124	BT 70 (61.4-76.1) HD 70.2 (61-77.6) LD 64.8 (58.5-79)	p=0.2359
MCH[b] (pg)	BT 23.5 ± 1.9 HD 23.3 ± 2.5	p=0.6818	BT 23.9 ± 2.5 HD 23.2 ± 1.8 LD 22.7 ± 1.7	p=0.3910
MCHC[b] (%)	BT 32.9 ± 3.8 HD 32.8 ± 2.6	p=0.8392	BT 34.1 ± 4.4 HD 33.4 ± 1.2 LD 34.7 ± 1.4	p=0.4595
RDW[a] (%)	BT 14.1 (0-19.3) HD 14.2 (0-19.5)	p=0.6944	BT 14.4 (13.4-19.3) HD 14.8 (12.1-19.5) LD 13.1 (0-16.2)	p=0.3519
Total Leucocyte Count[a] (/mm^3)	BT 8540 (1970-22300) HD 10900 (5850-44000)	p=0.0073*	BT 8540 (2010-13500) HD 9150 (5850-23200) LD 10435 (8260-30200)	p=0.1870
Segmented Neutrophils[a] (/mm^3)	BT 6448 (281.4-20516) HD 8719 (4471-41360)	p=0.0064*	BT 6536 (281.4-11745) HD 7247 (4856-21576) LD 9877 (4862-27180)	p=0.3046
Lymphocytes[a] (/mm^3)	BT 1364 (476-2291) HD 751.4 (240-4128)	p=0.0451*	BT 1467 (956.2-1806) HD 758.7 (351-1926) LD 1416 (712-1830)	p=0.0854
Eosinophils[a] (/mm^3)	BT 272 (0-3060) HD 332.5 (0-2091)	p=0.9999	BT 258 (0-956.2) HD 321 (105-520.1) LD 302 (0-1791)	p=0.9563
Monocytes[a] (/mm^3)	BT 165.8 (0-2314) HD 494 (0-2976)	p=0.0064*	BT 40.2 (0-751.3) HD 420 (148.6-929) LD 472 (341.2-1661)	p=0.0272*
Platelet Count[a] (/mm^3)	BT 180500 (27000-715000) HD 277500 (64400-834000)	p=0.3683	BT 265500 (106000-715000) HD 267000 (99000-399000)	p=0.9674

			LD 208000 (160000-532000)	
BUN[a] **(mg/dL)**	BT 41.2 (20.8-100.8) HD 49.9 (15.4-165.5)	p=0.1688	BT 28.93 (20.8-57) HD 43.18 (28.9-61.1) LD 42.5 (20.3-69)	p=0.6197
Creatinine[a] **(mg/dL)**	BT 1 (0.6-2.6) HD 1 (0.2-4.9)	p=0.9880	BT 1.1 (0.6-1.6) HD 0.9 (0.6-1.6) LD 1.5 (0.5-1.8)	p=0.9563
AST[a] **(U/L)**	BT 54.4 (0-117.8) HD 60.2 (29-216.9)	p=0.1726	BT 50.7 (0-117.8) HD 64.5 (40.2-77) LD 37.3 (33.8-103.2)	p=0.9537
ALT[a] **(U/L)**	BT 81.5 (34.2-423.2) HD 93.9 (31.5-271)	p=0.7609	BT 138.5 (60.2-313) HD 95.3 (75.8-271) LD 67.9 (38-136.1)	p=0.5705
GGT[a] **(U/L)**	BT 1.8 (0-17) HD 2.3 (0-38.5)	p=0.3101	BT 4.5 (0-17) HD 8.9 (0-38.5) LD 0.6 (0-1.2)	p=0.0206[*]
Alkaline Phosphatase[a] **(U/L)**	BT 59 (6-889.2) HD 82 (13.5-500)	p=0.2334	BT 75.4 (10-348) HD 108.8 (15-500) LD 61.4 (25-266.5)	p=0.6914
Amylase[a] **(U/L)**	BT 650 (484-2115) HD 818.5 (5.8-1230)	p=0.9453	BT 694 (627-761) HD 784.6 (547-1022) LD 905.2 (671-1139)	p=0.5000
Total Protein[a] **(g/dL)**	BT 6.6 (5.5-12.8) HD 6.7 (5.6-9.2)	p=0.9460	BT 6.1 (5.5-12.8) HD 7.4 (5.6-9.2) LD 6.6 (6.1-7.9)	p=0.7682
Albumin (g/dL)[b]	BT 2.8 ± 0.3 HD 2.7 ± 0.5	p=0.6642	BT 3 ± 0.5 HD 3.1 ± 0.1 LD 2.8 ± 0.5	p=0.4940
Globulin[a] **(g/dL)**	BT 4 (2.6-9.3) HD 4.1 (2.7-6.1)	p=0.7493	BT 3.8 (2.9-9.3) HD 4.625 (3-6.1) LD 4 (2.9-5.8)	p=0.6528

[a] Non-parametric values expressed as median (range)
[b] Parametric values expressed as mean ± standard deviation
Differences in BT and HD values in [c] and [d] are due to different sample sizes (seven more animals in [c])
MCH: Mean corpuscular haemoglobin; *MCHC*: Mean corpuscular haemoglobin concentration; *MCV*: Mean corpuscular volume; *RDW*: Red cell distribution width; *ALT*: Alanine aminotransferase; *AST*: Aspartate aminotransferase; *BUN*: Blood urea nitrogen; *GGT*: γ-glutamyltranspeptidase

Mean and median values of the CBC and SBP components were also compared with reference laboratory values for each component. CBC evaluation demonstrated a statistically significant increase in erythrocytes, haematocrit, total leucocyte count, segmented neutrophils, and monocytes, and a statistically significant decrease in lymphocytes after the thalidomide treatment. However, erythrocyte, haematocrit, total

leucocyte count, segmented neutrophil, and monocyte median and mean values remained within reference ranges. Lymphocyte median values were slightly lower than the 1000/mm^3 minimum reference value. Thalidomide administration did not statistically influence haemoglobin, MCV, MCH, MCHC, RDW, eosinophil, and platelet median and mean values.

SBP evaluation demonstrated HD thalidomide associated with a statistically significant decrease in GGT values following the LD thalidomide treatment. However, GGT values remained within reference ranges. Thalidomide administration did not statistically influence BUN, creatinine, AST, ALT, alkaline phosphatase, amylase, total protein, albumin, and globulin median and mean values.

CBC and SBP evaluation through VCOG-CTCAE criteria demonstrated an absence of grade 5 AE and only one grade 4 AE. The grade 4 finding referred to serum creatinine levels of a patient presenting bilateral renal metastasis, and therefore was not considered an AE related to therapy. No statistical difference was found when evaluating the frequency of grade 1, 2, and 3 findings at the baseline and after three months of the HD and LD of thalidomide.

DISCUSSION

Standard treatment for dogs with mammary gland tumours consists of surgery and there are no established guidelines for adjunctive therapy. However, systemic therapy is routinely recommended in high-risk cases characterized by regional lymph node metastasis, large tumours, and aggressive histology (lymphatic and vascular invasion or high histological grade) (Sorenmo 2003; Sorenmo and others 2013). Lavalle and others (2012) described a benefit in canine malignant mammary gland neoplasms with advanced clinical staging from complementary therapy with chemotherapy with or without Cox-2 inhibitors. Clinical benefit of adjuvant chemotherapy in disease free interval and overall survival was also described by Karayannopoulou and others (2001). More studies involving different effective adjuvant therapeutic protocols are warranted.

Woods and others (2004) suggested that, while the optimal dose and schedule of thalidomide administration in dogs remains to be determined, the absence of

myelosuppressive and other important AE enables thalidomide to be used in combination with chemotherapy, possibly representing a novel treatment approach that targets tumours and their microenvironment.

Severe teratogenicity, drowsiness, constipation, nausea, weakness, fatigue, lethargy, tingling and/or numbness in the hands and the feet, dizziness, thromboembolism, headache, and skin rash are predominant AE of thalidomide in humans. Sedation is a major AE. Furthermore, PN has been described in 30-70% of patients (Escudier and others 2002; Singhal and Mehta 2002; Dimopoulos and Eleutherakis-Papaiakovou 2004). PN is defined as any form of damage, inflammation, or degeneration of peripheral nerves, and is typically symmetrical and characterized by painful paraesthesia of the hands and feet, often accompanied by sensory loss in the feet. Dose and treatment duration of thalidomide are the two most crucial risk factors for development of PN (Delforge and others 2010). Teo and others (2001) suggest a difference in thalidomide toxicity among species. In contrast to humans, Teo and others (2000) did not describe thalidomide-induced PN in Beagles. The lack of PN in dogs might be due to the absence of a particular neurotoxic product, possibly resulting from the production of different metabolites or hydrolysis products by canines and humans that may be involved in thalidomide-induced PN.

In addition, the incidence of canine PN may be underestimated due to limitations in the perception of symptoms by humans and insufficient thorough routine neurological examination and exclusion of PN is not possible without more invasive tests. In the present study, the short thalidomide treatment length may also be associated to lower risk of PN. Nonetheless, PN should not be considered a limiting factor for canine thalidomide treatment (Teo and others 2000).

In humans, if the AE are tolerable, patients should be encouraged to continue the drug at the same dose. Occasionally, the drug may need to be discontinued for a period of 2–3 weeks and then restarted at a lower dose. Most studies have utilized thalidomide in a single bedtime dose. Patients with excessive drowsiness in the morning may occasionally divide the dose. Since the half-life of the drug in humans is 6–7 hours, this may be a better way to administer the drug (Singhal and Mehta 2002). In general, management of AE is based on four principles: dose adjustment or temporary interruption; avoidance of agents that enhance thalidomide-induced AE;

administration of agents or measures that prevent or relieve side effects; and clinical and laboratory monitoring for detection or prevention of toxicity (Dimopoulos and Eleutherakis-Papaiakovou 2004). The effort in altering the dosing strategy in order to maintain dose intensity is expected to be beneficial in tumour control.

Teo and others (2001) evaluated the safety profile of thalidomide in beagles in higher dosages (43-1000 mg/kg) than the present study, and the daily administration of thalidomide for 53 weeks was generally well tolerated. The 200 mg/kg dose was conservatively regarded as the NOAEL (no-observed-adverse effect levels). Green-coloured urine, white particulate matter in the faeces, enlarged female mammary tissue with dilatation of ducts and hyperplasia of the glandular epithelium, prolonged duration of oestrus, osseous yellow discoloration, and accumulation of bile pigment in the liver were attributed to different doses of thalidomide administration.

Sedation in healthy dogs following up to 1000 mg/kg of thalidomide administration was not observed (Teo and others 2001). In contrast, somnolence was present in approximately 50% of the patients submitted to the HD, and less than 10% presented a significant impact in the quality of life and activities of daily living. The difference in sedation observations may be due to an increased perception of somnolence by client-owned dogs compared to study dogs, the subjective evaluation of pet owners in the present study, and differences in studied populations: healthy and young beagles (Teo and others 2001) and older patients presenting advanced clinical stage mammary gland neoplasms and other comorbidities.

The low incidence of discrete ultrasound alterations found in the present study were considered incidental and possibly unrelated to thalidomide administration. No thalidomide-related differences in the absolute and relative organ weights or microscopic changes were observed in healthy beagles (Teo and others 2001). Patients were re-staged every three months, which may not be enough to precisely diagnose the moment of the disease progression in advanced clinical stage mammary gland neoplasms. Disease progression may not be associated to clinical signs and the chosen interval may be inadequate to evaluate the clinical benefit of the thalidomide treatment.

Blood work in most dogs with mammary gland tumours is normal, unless they have other concurrent medical problems or nonspecific age-related changes (Sorenmo

2003). Most of the significant hematologic and serum biochemistry changes following thalidomide administration described in healthy beagle dogs were small in magnitude, with no morphological and histopathological correlates, and all parameters returned to normal in the recovery dogs (Teo and others 2001). The significant CBC and SBP alterations of the present study were also considered small in magnitude, and were not related to treatment interruption or dose reduction. The majority of patients enrolled in the present study had been recently submitted to chemotherapy before thalidomide treatment was initiated. Therefore, the increases described in CBC following thalidomide administration may be partially due to a reversal of the myelosuppresive effects of chemotherapy. The bone marrow is sensitive to the toxic effects of chemotherapy, and neutropenia, thrombocytopenia, and, rarely, anaemia are described (Lana and Dobson 2011).

CBC analysis in mice treated with carboplatin followed by thalidomide demonstrated an increase in haematocrit, erythrocytes, haemoglobin, MCV, total leucocyte count, lymphocytes and platelets, and a decrease in eosinophils, neutrophils, and monocytes, when compared to the control group. No alterations were found in the biochemical evaluation of AST, GGT, urea and creatinine (De Souza and others 2014). Leucocytosis may be an important event in the antineoplastic immunomodulatory response of thalidomide and should be considered a relevant clinical parameter (Dos Reis and others 2014). Hematologic improvement of the erythroid and, to a lesser extent, the megakaryocytic lineages, and rare neutrophil responses, were also described in acute myeloid leukaemia in humans (Steins and others 2003). The limited CBC and SBP alterations described in HD and LD thalidomide were desirable, particularly for elderly patients with advanced neoplasms. The frequency of CBC and SBP monitoring every three months was considered adequate due to the limited alterations caused by thalidomide administration. However, specific patients may need more careful monitoring, specially in cases with pre-existing abnormalities or progressive disease.

The THALIDOMID REMS^{TM} program, a restricted program under a Risk Evaluation and Mitigation Strategy (REMS) of the United States of America, recommends that if there is contact with non-intact thalidomide capsules or powder contents or if healthcare providers or other care givers are exposed to bodily fluids

from patients receiving thalidomide, the exposed area should be washed with soap and water. Appropriate precautions should be utilized, such as wearing gloves to prevent the potential cutaneous exposure to the drug (Celgene 2015). Teo and others (2001) periodically observed a white particulate residue in the faeces of Beagles treated with thalidomide that appeared to be unabsorbed thalidomide, although chemical analysis was not performed. For safety reasons, the pet owners of the present study were requested to avoid cutaneous exposure to the drug by wearing disposable gloves and to avoid contact with bodily fluids of the animals. In the case of an accidental exposure, the recommendation was to wash the exposed area with soap and water.

CONCLUSION

Thalidomide administration was well tolerated in canine patients presenting advanced clinical staging mammary gland neoplasms. Both studied doses did not impair the activities of daily living of most of the studied population; however, the proposed low dose should be considered when excessive somnolence is found. Further studies are warranted to investigate the therapeutic benefit of thalidomide in canine mammary gland neoplasms and other neoplasms. The lack of a washout period between the HD and LD is a limitation of the present study and the toxicity evaluation of the LD individually were therefore hindered. Future thalidomide kinetic studies should be performed in canines in order to better evaluate the absorption and metabolization of the drug in several different moments in order to choose optimal doses and administration periods for specific diseases.

ACKNOWLEDGEMENTS

This research was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP Grant 2014/01329-9) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq Grant 302449/2013-2).

REFERENCES

BRODEY, R.S., GOLDSCHMIDT, M.H. and ROSZEL, J.R. (1983) Canine mammary gland neoplasms. *Journal of the American Animal Hospital Association* **19**, 61–90.

CASSALI, G.D., LAVALLE, G.E., FERREIRA, E., ESTRELA-LIMA, A., NARDI, A.B. De, GHEVER, C., and others. (2014) Consensus for the diagnosis, prognosis and treatment of canine mammary tumors - 2013. *Brazilian Journal of Veterinary Pathology* **7**, 38–69.

CELGENE (2015) Thalidomid REMS. http://www.thalomidrems.com/. Accessed May 10th, 2016.

CHAMBERLAIN, P.P., LOPEZ-GIRONA, A., MILLER, K., CARMEL, G., PAGARIGAN, B., CHIE-LEON, B., and others. (2014) Structure of the human Cereblon-DDB1-lenalidomide complex reveals basis for responsiveness to thalidomide analogs. *Nature structural & molecular biology* **21**, 803–810.

DE SOUZA, C.M., ARAUJO E SILVA, A.C., DE JESUS FERRACIOLLI, C., MOREIRA, G.V., CAMPOS, L.C., DOS REIS, D.C., and others. (2014) Combination therapy with carboplatin and thalidomide suppresses tumor growth and metastasis in 4T1 murine breast cancer model. *Biomedicine and Pharmacotherapy* **68**, 51–57.

DE SOUZA, C.M., FONSECA DE CARVALHO, L., DA SILVA VIEIRA, T., CÂNDIDA ARAÚJO E SILVA, A., TERESA PAZ LOPES, M., ALVES NEVES DINIZ FERREIRA, M., and others. (2012) Thalidomide attenuates mammary cancer associated-inflammation, angiogenesis and tumor growth in mice. *Biomedicine and Pharmacotherapy* **66**, 491–498.

DELFORGE, M., BLADE, J., DIMOPOULOS, M.A., FACON, T., KROPFF, M., LUDWIG, H., and others. (2010) Treatment-related peripheral neuropathy in multiple myeloma: the challenge continues. *The Lancet Oncology* **11**, 1086–1095.

DIMOPOULOS, M.A. and ELEUTHERAKIS-PAPAIAKOVOU, V. (2004) Adverse effects of thalidomide administration in patients with neoplastic diseases. *American Journal of Medicine* **117**, 508–515.

DOS REIS, D.C., SOUZA, C.M. De, CAMPOS, L.C., SILVA, A.C.A., LOPES, M.T.P., CASSALI, G.D., and others. (2014) Thalidomide promotes leukocytosis in mice inoculated with 4T1 mammary carcinoma. *Journal Brasileiro de Patologia e Medicina Laboratorial* **50**, 64–66.

ESCUDIER, B., LASSAU, N., COUANET, D., ANGEVIN, E., MESRATI, F., LEBORGNE, S., and others. (2002) Phase II trial of thalidomide in renal-cell carcinoma. *Annals of Oncology* **13**, 1029–1035.

FLORENCE, A.L. (1960) Is Thalidomide to Blame? *BMJ: British Medical Journal* **2**, 1954.

ITO, T., ANDO, H., SUZUKI, T., OGURA, T., HOTTA, K., IMAMURA, Y., and others. (2010) Identification of a primary target of thalidomide teratogenicity. *Science* **327**, 1345–1350.

JANKOWSKI, M., FULTON, L., SHEAFOR, S., PRESCOTT, D. and KHANNA, C. (1999) Ongoing evaluation of single agent thalidomide in dogs with measurable cancer. In Veterinary Cancer Society 19th Annual Meeting.

KARAYANNOPOULOU, M., KALDRYMIDOU, E., CONSTANTINIDIS, T.C. and DESSIRIS, A. (2001) Adjuvant Post-operative Chemotherapy in Bitches with Mammary Cancer. *Journal of Veterinary Medicine Series A* **48**, 85–96.

LANA, S.E. and DOBSON, J.M. (2011) Principles of Chemotherapy. In BSAVA Manual of canine and feline oncology. Eds J.M. Dobson and B.D.X. Lascelles. 3rd ed. British Small Animal Veterinary Association. pp 67–79.

LAVALLE, G.E., DE CAMPOS, C.B., BERTAGNOLLI, A.C. and CASSALI, G.D. (2012) Canine malignant mammary gland neoplasms with advanced clinical staging treated with carboplatin and cyclooxygenase inhibitors. *In Vivo* **26**, 375–379.

LENZ, W. (1988) A short history of thalidomide embryopathy. *Teratology* **38**, 203–215.

MARCONATO, L., BUCHHOLZ, J., KELLER, M., BETTINI, G., VALENTI, P. and KASER-HOTZ, B. (2013) Multimodal therapeutic approach and interdisciplinary challenge for the treatment of unresectable head and neck squamous cell carcinoma in six cats: A pilot study. *Veterinary and Comparative Oncology* **11**, 101–112.

MCBRIDE, W.G. (1961) Thalidomide and congenital abnormalities. *Public Health* **278**, 1358.

MCBRIDE, W.G. (1977) Thalidomide embryopathy. *Teratology* **16**, 79–82.

NOVOSAD, C.A. (2003) Principles of treatment for mammary gland tumors. *Clinical techniques in small animal practice* **18**, 107–109.

OWEN, L.N. (1980) TNM Classification of tumors in domestic animals. 1st ed. World Health Organization. pp 1-53.

SINGHAL, S. and MEHTA, J. (2002) Thalidomide in cancer. *Biomedicine & Pharmacotherapy* **56**, 4–12.

SORENMO, K.U. (2003) Canine mammary gland tumors. *The Veterinary clinics of North America. Small animal practice* **33**, 573–596.

SORENMO, K.U., WORLEY, D.R. and GOLDSCHMIDT, M.H. (2013) Tumors of the mammary gland. In Withrow & MacEwen's Small Animal Clinical Oncology. Eds S.J. Withrow and D.M. Vail. 5th ed. W. B. Saunders Company. pp 538–556.

STEINS, M.B., BIEKER, R., PADRO, T., KESSLER, T., KIENAST, J., BERDEL, W.E., and others. (2003) Thalidomide for the treatment of acute myeloid leukemia. *Leukemia & Lymphoma* **44**, 1489–1493.

STEWART, A.K. (2014) How thalidomide works against cancer. *Science* **343**, 256–257.

TEO, S., EVANS, M., EHRHART, J., BROCKMAN, M., ALLEN, D., MORGAN, M., and others. (2000) Lack of peripheral neuropathy in Beagle dogs after 53 weeks oral administration of thalidomide capsules. *Human & Experimental Toxicology* **19**, 615–622.

TEO, S.K., EVANS, M.G., BROCKMAN, M.J., EHRHART, J., MORGAN, J.M., STIRLING, D.I. and THOMAS, S.D. (2001) Safety Profile of Thalidomide after 53 Weeks of Oral Administration in Beagle Dogs. *Toxicological Sciences* **59**, 160–168.

TEO, S.K., COLBURN, W.A., TRACEWELL, W.G., KOOK, K.A., STIRLING, D.I., JAWORSKY, M.S., SCHEFFLER, M.A., THOMAS, S.D. and LASKIN, O.L. (2004) Clinical pharmacokinetics of thalidomide. *Clinical Pharmacokinetics* **43**, 311–327.

VETERINARY CO-OPERATIVE ONCOLOGY GROUP (2004) Veterinary cooperative oncology group - common terminology criteria for adverse events (VCOG-CTCAE) following chemotherapy or biological antineoplastic therapy in dogs and cats v1.1. *Veterinary and Comparative Oncology* **2**, 194–213.

WOODS, J.P., MATHEWS, K.A. and BINNINGTON, A.G. (2004) Thalidomide for the treatment of hemangiosarcoma in dogs. *Veterinary and Comparative Oncology* **2**, 108–109.

CAPÍTULO 3 – THALIDOMIDE TREATMENT IN AN ADVANCED STAGE CANINE MAMMARY GLAND CARCINOSARCOMA

ABSTRACT - Carcinosarcomas are uncommon in the dog and present an unfavorable prognosis. Thalidomide has been used in the investigational treatment of several diseases due to its known immunomodulatory and anti-angiogenic properties. A female dog underwent radical unilateral mastectomy, which enabled the diagnosis of a stage III and grade III carcinosarcoma, followed by chemotherapy treatment with doxorubicin and carboplatin. Twelve months after the mastectomy, thoracic radiographs revealed the presence of multiple nodules in the lung, and thalidomide administration was initiated at 20 mg/kg/day during three months and then 10 mg/kg/day without discontinuation. The patient did not present any adverse events related to the thalidomide administration and thoracic radiographs demonstrated stable metastatic disease. The patient was euthanized presenting metastasis in several other organs and overall survival was considered 963 days. The progression of distant metastasis in the studied patient was considered to be hindered by thalidomide.

Key-words: Adjuvant Therapy, Dog, Metastasis, Neoplasm, Survival

INTRODUCTION

Mammary gland neoplasms are the most common neoplasm in intact bitches[1]. The biological behavior of these tumors varies considerably, and prognosis is influenced by several factors, such as: age, histological type, clinical stage, tumor size, clinical behavior of the tumor, mitotic index, histologic grade, regional or distant metastasis, microvessel density, and molecular markers[2,3]. All malignant mammary gland neoplasms have the potential to metastasize, however, histological type and several clinical prognostic factors influence the metastatic risk[3]. An increase in clinical stage worsens prognosis, and distant metastasis is associated to an accelerated demise of the affected animal[4].

Surgical excision of canine mammary gland neoplasms is considered the standard treatment modality. However, dogs with more undifferentiated and advanced tumors may require adjuvant therapy[3,5]. Chemotherapy should be recommended when the histotype of the primary neoplasm is associated with poor prognosis, i.e., solid carcinomas, micropapillary carcinomas, anaplastic carcinomas and carcinosarcomas, or when patients present metastasis[2,6]. Chemotherapy may also be used in the adjuvant setting and in dogs with gross metastatic disease[3].

Despite thalidomide's tragic history, thalidomide has been used in the investigational treatment of a myriad of diseases[7]. In humans, therapeutic activity of thalidomide has been described for non-neoplastic diseases, e.g. erythema nodosum leprosum, and neoplastic diseases, e.g. multiple myeloma[8,9]. The drug is usually given as a single daily dose in the evening, due to somnolence and orthostatic hypotension, and teratogenicity is the most serious adverse effect of thalidomide[10]. The known immunomodulatory and anti-angiogenic properties of thalidomide and its analogues provided the impetus to investigate these agents in the treatment of both hematologic malignancies and in solid tumors. Ongoing trials, either in combination with chemotherapy or as single agents, have been initiated in neoplastic and non-neoplastic diseases[11].

Currently, there are insufficient clinical trials concerning the use of thalidomide in veterinary medicine. Therefore, the aim of this paper is to report a case of thalidomide induced inhibition of progression of distant metastasis of a canine mammary gland carcinosarcoma.

CASE REPORT

An eleven-year-old schnauzer presenting mammary gland tumors was admitted at the Veterinary Hospital of the Federal University of Minas Gerais, Brazil. Thoracic radiographs (TR), including right and left lateral recumbent (RLR and LLR) and ventrodorsal (VD) projections and abdominal ultrasound (AU) did not demonstrate distant metastasis. The patient was classified as clinical stage III ($T_3N_0M_0$)[12] and underwent radical unilateral mastectomy.

Mammary gland tumors were fixed in 10% neutral buffered formalin and embedded in paraffin and 4-µm thick histologic sections were stained with hematoxylin and eosin and classified according to WHO's Histological Classification for canine and feline mammary tumors[13]. Macroscopic evaluation of the primary neoplasm demonstrated a 5.0 x 3.0 x 1.5 cm nodule in the right cranial abdominal mammary gland. Microscopic analysis revealed epithelial cells arranged in a predominantly tubular pattern associated with spindle-shaped cells. Epithelial cells were characterized by prominent nucleoli and moderate cellular pleomorfism, with an average of two mitoses per high power field (400x). Spindle cells presented scattered mitotic figures, moderate cellular pleomorfism, marked anisocytosis and anisokaryosis, nuclear hypercromasia, and occasional multinucleated cells. Based on histologic findings the neoplasm was diagnosed as a carcinosarcoma (Fig. 01). Histological grade was established according to the Nottingham system[14], and the primary neoplasm was considered grade III. In addition, the patient presented a 0.4 cm nodule in the cranial thoracic mammary gland, diagnosed as a carcinoma in mixed tumor, and the inguinal lymph node was negative for metastasis.

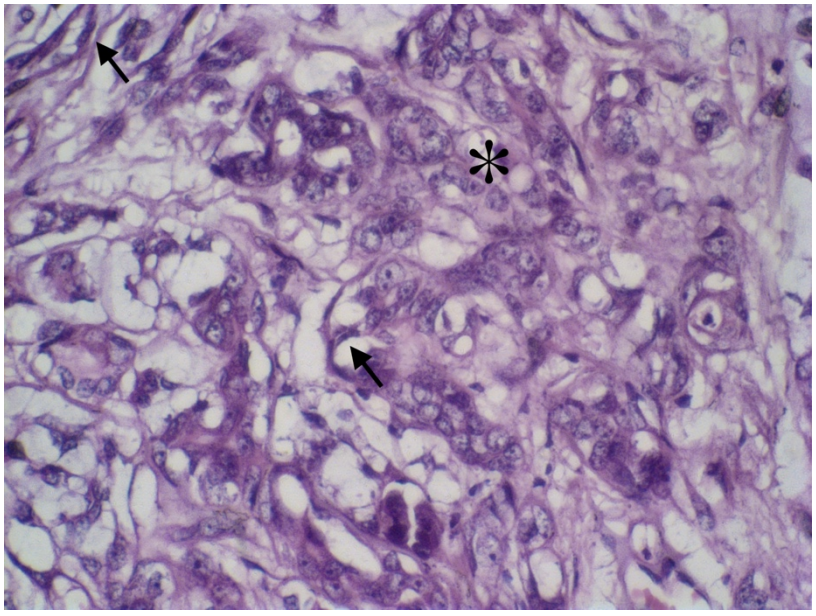

Figure 01. Canine mammary gland presenting a carcinosarcoma presenting neoplastic epithelial cells (asterisk) and spindle cells (arrows). Hematoxylin and eosin, 600x.

Due to the histological diagnosis, surgical excision was followed by chemotherapy, consisted by four cycles of an alternating combination of doxorubicin at 30 mg/m^2 and carboplatin at 300 mg/m^2, given intravenously every 21 days, and follow-up was subsequently performed every three months with clinical evaluation, TR, and AU of the patient.

Twelve months after the mastectomy, TR revealed the presence of multiple nodules in the lung, not associated with respiratory clinical signs. Thalidomide administration was initiated at 20 mg/kg, given orally every 24 hours at night time, during three months. Afterwards, thalidomide was given orally at 10 mg/kg every 24 hours at night time, without discontinuation. The patient did not present any adverse events related to the thalidomide administration.

TR analysis demonstrated a slow progression of the metastatic lung disease, classified as a stable disease (increase lower than 25% in tumor maximum diameter) (Fig. 02). After fifteen months of the thalidomide treatment, an AU demonstrated the presence of a hyperechogenic splenic nodule of 1.65 x 1.66 cm and the animal was submitted to a splenectomy. Microscopic analysis of the splenic nodule enabled the diagnosis of a splenic metastasis of the primary carcinosarcoma.

Twenty months after the diagnosis of the pulmonary nodules and the beginning of the thalidomide treatment, the patient was euthanized due to a progressive peripheral vestibular syndrome. Overall survival was considered 32 months (963 days), defined as the period between the date of surgical removal of the tumor and death caused by the disease.

In addition, the dog was submitted to post-mortem evaluation and gross metastasis were revealed in the lung, mediastinal and mesenteric lymph nodes, liver, pancreas, and adrenal glands, as well as neoplastic emboli within central nervous system. Metastasis were subsequently confirmed through microscopic analysis, with cells presenting similar histologic features to those observed in the primary tumor (Fig. 03).

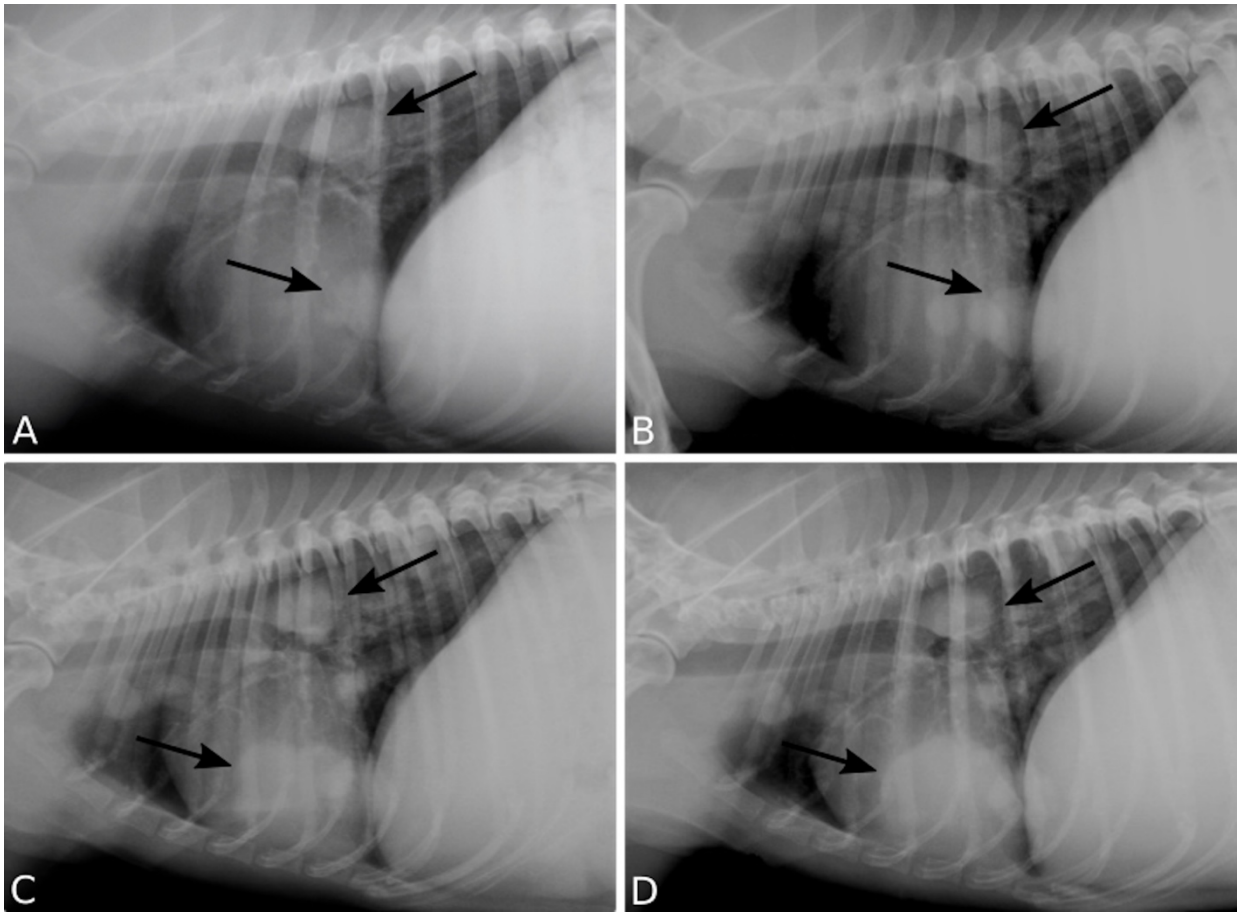

Figure 02. Pulmonary metastasis of a canine mammary gland carcinosarcoma. Right lateral recumbency thoracic radiographs (TR) presenting multiple nodules (arrows). TR performed 12 (A), 18 (B), 24 (C), 30 (D) months following surgical excision of the primary neoplasm.

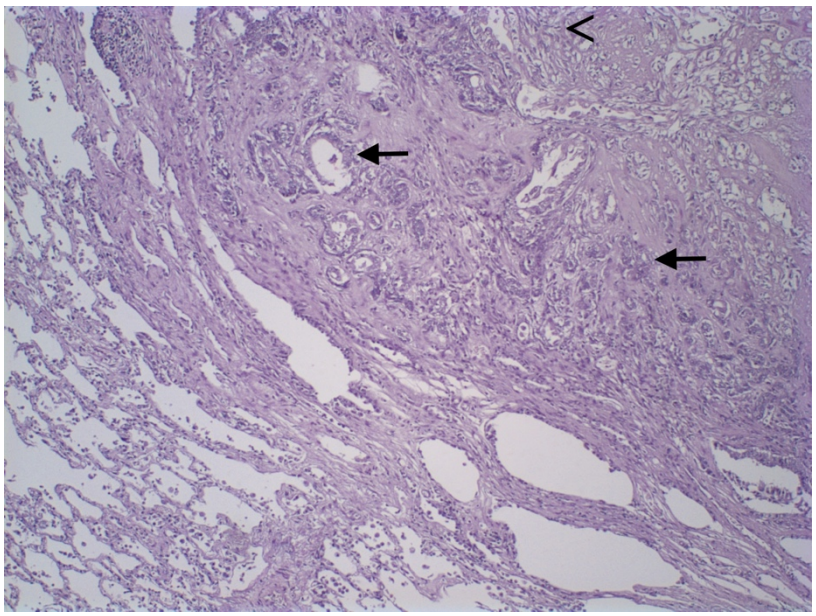

Figure 03. Lung tissue presenting a malignant epithelial (arrows) and spindle cells (arrowhead). Hematoxylin and eosin, 200x.

Morphometric analysis of the inflammatory infiltrate was performed according to Estrela-Lima and colleagues[15]. Inflammation within the primary neoplasm and the pulmonary distant metastasis were considered multifocal and intense, with similar quantification of inflammatory cells, presenting an intense lymphocytic component.

Three µm-thick histologic sections from the primary neoplasm and lung metastasis were obtained for immunohistochemistry. Slides were incubated following the details provided in table 1. A polymeric-based detection system (Novolink Polymer Detection System, Novocastra, Newcastle, UK) was used for detection of antigen-antibody reaction and 3,3'-Diaminobenzidine was used as chromogen. Slides were subsequently counterstained using Harris hematoxylin. Sections from a canine mammary carcinomas known to express Ki-67, ER and PR, COX-2, and CD31 were used as positive controls. Negative controls were assessed using normal serum (Ultra V Block, Laboratory Vision) as the primary antibody.

Table 1. Target antigen and clone, dilution, antigen retrieval method, and incubation time and temperature for immunohistochemical staining for Ki-67, Estrogen Receptor (ER), Progesterone Receptor (PR), Cyclooxigenase-2 (Cox-2), and CD31.

Antigen	Clone	Dilution	Antigen Retrieval Method / Buffer	Incubation Time (h) / Temperature	IHC Evaluation
Ki-67	MIB-1	1:50	PH (125°C/2min) / Citrate	16 / 4°C	Dutra and colleagues[16]
ER	1D5	1:20	PH (125°C/2min) / EDTA	1 / RT	Hammond and colleagues[17]
PR	HPRA2	1:20	PH (125°C/2min) / EDTA	1 / RT	Hammond and colleagues[17]
Cox-2	SP21	1:80	WB (98°C/20min) / Citrate	1 / RT	Lavalle and colleagues[18]
CD31	JC70A	1:100	PH (125°C/2min) / Citrate	16 / 4°C	Weidner and colleagues[19]

PH: Pressurized Heat; WB: Water Bath; RT: Room Temperature; h: hours

The primary neoplasm presented a proliferation index of 18.1%, was positive for estrogen and progesterone receptors, Cox-2 score 2, and a microvessel density of 33/200x field. Lung metastasis presented a proliferation index of 9.4% and a microvessel density of 61/200x field.

DISCUSSION

The lungs are the most common site for distant metastasis in dogs with malignant mammary gland tumors[3]. The absence of salient clinical abnormalities associated with pulmonary metastatic disease leads to considerable attention on thoracic radiography as a diagnostic, prognostic, and staging tool[20]. However, additional staging tests, including abdominal ultrasonography and abdominal and skeletal radiographs, may be indicated[3,21].

Three view TR are necessary for all dogs with malignant mammary gland neoplasms, which remains as the standard diagnostic method for the evaluation of thoracic metastatic disease in veterinary medicine. Conventional radiography can detect lesions ranging from 6-9 mm in diameter[3,22]. Radiographic detection of lung masses depends on several intrinsic (location, size, mass opacity, normal thoracic and extrathoraric structures) and radiographic technique factors, as well as the interpretation of radiographs[23]. The RLR projection is the most sensitive for detection of lung metastasis, followed by the LLR and the VD projection. The sensitivity for the three-view combination is nearly 100%, regardless of the number of readers [23].

There are no established guidelines for the treatment of malignant mammary gland neoplasms beyond surgery. However, treatment recommendations generally intensify with advancing clinical stage and increasing seriousness of the prognostic factors[3]. Carcinosarcomas present extremely variable histological characteristics[24] and are uncommon in the dog[13]. Von Euler[25] reports that carcinosarcomas are always very aggressive and present an unfavorable prognosis. The lungs are the most frequent site of metastasis, followed by regional lymph nodes. Carcinosarcoma metastasis are characterized by carcinomatous, sarcomatous, or mixed components. Other sites less frequently affected are heart, kidneys, liver, adrenal glands, brain, ovaries, hypophysis, bones, spleen, and pleura. The average time between detection and death in seven dogs was 18 months[24].

Thalidomide was described as a potent angiogenesis inhibitor in vivo[26]. The discovery of thalidomide's antiangiogenic properties coincided with the emerging importance of angiogenesis in tumor growth and progression[27]. The anti-angiogenic activity of thalidomide suggest that the drug may present potential clinical benefit in the

treatment of malignancies in which neoangiogenesis is present, including solid tumors[11,26,28]. Antiangiogenic therapy is an appealing strategy for targeting resistant disease, and accumulating evidence suggests that the combination of cytotoxic chemotherapy and antiangiogenic therapy has greater antitumor effects than either strategy alone[27]. Therefore, a novel treatment protocol option for advanced canine mammary gland tumors may consist of surgery, chemotherapy and thalidomide in association, although clinical trials are necessary to verify the clinical benefit of the proposed therapeutic protocol.

Thalidomide and its analogs exhibit a multitude of biologic effects on cytokine and cell-mediated responses[11]. The effects of thalidomide on immune function are incompletely understood; however, anti-inflammatory and immunomodulatory activities have been described[28].

The antiangiogenic and immunomodulatory properties of thalidomide are implicated in the major activity of the drug in patients with multiple myeloma and holds promise in the treatment of other hematologic malignancies. Results in solid tumors are less encouraging, but patients with certain tumors, such as glioma, renal cell cancer, and prostate cancer, may benefit[27]. Our results demonstrated an increase in the microvessel density at the metastatic site when compared to the primary neoplasm. The inflammatory infiltrate was similar in the primary and metastatic neoplasms. Therefore, the antiangiogenic and immunomodulatory properties of the drug were not demonstrated. However, the proliferative index of the primary neoplasm was higher than the metastatic proliferative index, which may suggest the antitumor effect of the drug.

In canines, the safety profile of thalidomide was evaluated and daily oral administration was considered well tolerated. The NOAEL (no-observed-adverse-effect levels) was 200 mg/kg[29]. Two studies began to evaluate the clinical benefit of thalidomide in canine malignancies; however, the clinical trials were not completed[30,31]. The present case report suggests that the drug provided clinical benefit in the treatment of distant metastasis. In addition, the patient maintained adequate quality of life during the thalidomide treatment, with an absence of adverse effects.

Gross metastatic disease at the time of diagnosis was associated to a poorer prognosis, with a median post-operative survival of 5 months, when compared to 28

months for animals diagnosed with mammary gland neoplasms that lacked evidence of metastasis at diagnosis[32]. Another study found a 13.6% survival rates one year after mastectomy for animals diagnosed with distant metastasis [4]. In veterinary oncology, there are insufficient options for the treatment of distant metastasis. However, the 20-month overall survival after the diagnosis of pulmonary nodules in the present case report was considered satisfying.

The progression of distant metastasis in the studied patient was considered to be hindered by thalidomide. However, clinical trials are necessary in order to confirm the benefit of thalidomide in canine mammary gland neoplasms, as well as in other malignant neoplasms in veterinary medicine.

ACKNOWLEDGEMENTS

This research was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP Grant 2014/01329-9) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq Grant 302449/2013-2).

REFERENCES

1. Brodey RS, Goldschmidt MH, Roszel JR. Canine mammary gland neoplasms. J Am Anim Hosp Assoc. 1983;19:61–90.
2. Cassali GD, Lavalle GE, Ferreira E, Estrela-Lima A, Nardi AB De, Ghever C, et al. Consensus for the diagnosis, prognosis and treatment of canine mammary tumors - 2013. Brazilian J Vet Pathol. 2014;7(2):38–69.
3. Sorenmo KU. Canine mammary gland tumors. Vet Clin North Am Small Anim Pract. 2003;33:573–596.
4. Yamagami T, Kobayashi T, Takahashi K, Sugiyama M. Prognosis for canine malignant mammary tumors based on TNM and histologic classification. J Vet Med Sci. 1996;58(11):1079–1083.
5. Novosad CA. Principles of treatment for mammary gland tumors. Clin Tech Small Anim Pract. 2003;18(2):107–109.

6. Nunes FC. Diagnóstico, prognóstico e tratamento dos carcinomas de glândulas mamárias de cadelas atendidas no hospital veterinário da UFMG – Estudo retrospectivo. Federal University of Minas Gerais; 2015.
7. Paravar T, Lee DJ. Thalidomide: mechanisms of action. Int Rev Immunol. 2008;27(3):111–135.
8. Singhal S, Mehta J. Thalidomide in cancer. Biomed Pharmacother. 2002;56:4–12.
9. Dimopoulos MA, Eleutherakis-Papaiakovou V. Adverse effects of thalidomide administration in patients with neoplastic diseases. Am J Med. 2004;117(7):508–515.
10. Adlard JW. Thalidomide in the treatment of cancer. Anti-Cancer Drug. 2000;11:787–791.
11. Melchert M, List A. The thalidomide saga. Int J Biochem Cell Biol. 2007;39(7-8):1489–1499.
12. Sorenmo KU, Worley DR, Goldschmidt MH. Tumors of the Mammary Gland. In: Withrow SJ, Vail DM, Page RL, editors. Withrow & MacEwen's Small Animal Clinical Oncology. 5th ed. Saint Louis: Elsevier Saunders; 2013. p. 538–556.
13. Misdorp W, Else RW, Hellmén E, Lipscomb TP. Histological classification of mammary tumors of the dog and the cat. 2nd Series. Washington DC: Armed Forces Institute of Pathology. American Registry of Pathology and the World Health Organization Collaborating Center for Worldwide Reference on Comparative Oncology; 1999. p. 1-59.
14. Elston CW, Ellis IO. Assessment of histological grade. In: Elston CW, Ellis IO, editors. Systemic Pathology: The breast. 3rd ed. London: Churchill Livingstone; 1998. p. 365–384.
15. Estrela-Lima A, Araujo MS, Costa-Neto JM, Teixeira-Carvalho A, Barrouin-Melo SM, Cardoso S V, et al. Immunophenotypic features of tumor infiltrating lymphocytes from mammary carcinomas in female dogs associated with prognostic factors and survival rates. BMC Cancer. 2010;10(1):256–269.
16. Dutra AP, Granja NVM, Schmitt FC, Cassali GD. C-erbB-2 expression and nuclear pleomorphism in canine mammary tumors. Brazilian J Med Biol Res. 2004;37(11):1673–1681.

17. Hammond MEH, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American society of clinical oncology/college of American pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer (unabridged version). Arch Pathol Lab Med. 2010;134(7):48–72.
18. Lavalle GE, De Campos CB, Bertagnolli AC, Cassali GD. Canine malignant mammary gland neoplasms with advanced clinical staging treated with carboplatin and cyclooxygenase inhibitors. In Vivo. 2012;26:375–379.
19. Weidner N. Intratumor microvessel density as a prognostic factor in cancer. Am J Pathol. 1995;147(1):9–19.
20. Miles KG, Lattimer JC, Jergens AE, Krause GF. A retrospective evaluation of the radiographic evidence of pulmonary metastatic disease on initial presentation in the dog. Vet Radiol. 1990;31(2):79–82.
21. Sorenmo KU, Rasotto R, Zappulli V, Goldschmidt MH. Development, anatomy, histology, lymphatic drainage, clinical features, and cell differentiation markers of canine mammary gland neoplasms. Vet Pathol. 2011;48(1):85–97.
22. Nemanic S, London CCA, Wisner ER. Comparison of thoracic radiographs and single breath-hold helical CT for detection of pulmonary nodules in dogs with metastatic neoplasia. J Vet Intern Med. 2006;20(3):508–515.
23. Lang J, Wortman JA, Glickman LT, Biery DN, Rhodes WH. Sensitivity of radiographic detection of lung metastases in the dog. Vet Radiol. 1986;27(3):74–78.
24. Misdorp W, Cotchin E, Hampe JF, Jabara AG, von Sandersleben J. Canine Malignant Mammary Tumors. III. Special Types of Carcinomas, Malignant Mixed Tumors. Vet Pathol. 1973;10:241–256.
25. von Euler H. Tumors of the mammary gland. In: Dobson JM, Lascelles B, editors. BSAVA Manual of canine and feline oncology. 3rd ed. Gloucester: British Small Animal Veterinary Association; 2011. p. 237–247.
26. D'Amato RJ, Loughnan MS, Flynn E, Folkman J. Thalidomide is an inhibitor of angiogenesis. Proc Natl Acad Sci U S A. 1994;91(9):4082–4085.
27. Richardson P, Hideshima T, Anderson K. Thalidomide: Emerging Role in Cancer Medicine. Annu Rev Med. 2002;53:629–657.

28. Calabrese L, Fleischer AB. Thalidomide: Current and Potential Clinical Applications. Am J Med. 2000;108:487–495.
29. Teo SK, Evans MG, Brockman MJ, Ehrhart J, Morgan JM, Stirling DI, et al. Safety Profile of Thalidomide after 53 Weeks of Oral Administration in Beagle Dogs. Toxicol Sci. 2001;59:160–168.
30. Jankowski M, Fulton L, Sheafor S, Prescott D, Khanna C. Ongoing evaluation of single agent thalidomide in dogs with measurable cancer. In: Veterinary Cancer Society 19th Annual Meeting. Wood's Hole, MA; 1999.
31. Woods JP, Mathews KA, Binnington AG. Thalidomide for the treatment of hemangiosarcoma in dogs. Vet Comp Oncol. 2004;2(2):108–109.
32. Philibert JC, Snyder PW, Glickman N, Glickman LT, Knapp DW, Waters DJ. Influence of Host Factors on Survival in Dogs with Malignant Mammary Gland Tumors. J Vet Intern Med. 2003;17(1):102–106.

CAPÍTULO 4 – THALIDOMIDE AND METRONOMIC CHEMOTHERAPY IN ADDITION TO SURGERY AND CHEMOTHERAPY FOR THE TREATMENT OF CANINE MALIGNANT MAMMARY GLAND NEOPLASMS

ABSTRACT - The aim of the present study was to evaluate a multimodal approach for the treatment of canine malignant mammary gland neoplasms, including surgery, chemotherapy, thalidomide, and metronomic chemotherapy. Fifty-eight bitches were included, 11 animals were submitted solely to the surgical treatment, 15 to surgery and chemotherapy, 23 to surgery, chemotherapy and thalidomide, and 9 to surgery, chemotherapy and metronomic chemotherapy. In patients subjected to the thalidomide therapy, no statistical difference was found regarding the proliferative index and microvessel densitiy of primary neoplasms and distant metastasis. Diffuse intense inflammatory infiltrate was predominant in primary tumors and diffuse moderate inflammatory infiltrate in metastatic lesions. No statistically significant difference was observed in median survival time (MST) of all four treatment groups when including all clinical stages ($p=0.3177$). However, animals diagnosed with distant metastasis treated with surgery, chemotherapy associated to thalidomide or metronomic chemotherapy presented longer MST when compared to the other two treatment groups ($p<0.0001$). The proposed multimodal therapy protocols that included antiangiogenic therapies contributed towards a clinical benefit for patients presenting distant metastasis.

Key-words: Angiogenesis, Dog, Metastasis, Neoplasia, Therapy, Survival Time

INTRODUCTION

Mammary gland neoplasms are the most common neoplasm that affect female dogs (Brodey et al., 1983). Although the optimal extent of the surgical excision remains unclear (Sorenmo et al., 2013), surgery is considered the most effective treatment for

canine mammary gland neoplasms. Adjuvant therapies, such as chemotherapy, may be required for more high risk, undifferentiated and advanced neoplasms (Novosad, 2003; Sorenmo, 2003), although the efficacy of systemic therapies remains uncertain.

In solid tumors, angiogenesis has been clearly associated to metastasis and disease progression (Raje and Anderson, 2002). The combination of antiangiogenic strategies with conventional antineoplastic chemotherapy protocols will likely be beneficial for the treatment of malignant tumors (Pierini et al., 2012).

In the late 1950s, thalidomide was marketed worldwide as a sedative and a hypnotic, and most importantly as an anti-emetic drug in early pregnancy (McBride, 1977). Thalidomide was associated to congenital deformities and peripheral neuropathy and the drug was withdrawn from the market in the early 1960s (McBride 1977; Lenz 1988). Since then, thalidomide has been used experimentally in various diseases (Adlard, 2000; Teo, 2005) due of its antiangiogenic and immunomodulatory properties (Raje and Anderson, 2002). The antiangiogenic properties are presumably important in the apparent efficacy reported for several cancers, while the immunomodulatory effects of thalidomide depend on the disease, the immunologic status, the type of immune cell that is activated, and the type of stimulus the cell receives (Teo, 2005).

In 2000, metronomic regimens of cytotoxic drugs were established as promising antiangiogenic therapies (Hanahan et al., 2000). Although metronomic chemotherapy lacks significant direct cytotoxic properties against neoplastic cells, it is able to modify the tumor microenvironment mainly hindering tumor stromal angiogenesis. Metronomic chemotherapy aims to eliminate or minimize the interval between chemotherapy administrations, limiting cellular repair and replication and altering the tumor microenvironment (Pierini et al., 2012).

Single agent treatments have been considered less efficacious than combination therapies, which may include therapies that promote host antitumor defenses (Ehrke, 2003). Therefore, the aim of the present study was to evaluate a multimodal approach for the treatment of canine malignant mammary gland neoplasms including surgery, chemotherapy and thalidomide, compared to surgery, surgery and chemotherapy, and surgery, chemotherapy and metronomic chemotherapy protocols.

MATERIAL AND METHODS

Fifty-eight female dogs admitted at the Veterinary Hospital of the Federal University of Minas Gerais, Brazil, and diagnosed with malignant mammary gland neoplasms were evaluated in a prospective manner. The animals were divided into four groups:

Surgery - Animals submitted solely to surgical treatment;

Surgery + *Chemotherapy* - Animals submitted to surgical excision followed by four cycles of carboplatin at the dose of 300mg/m^2, given intravenously every 21 days;

Surgery + *Chemotherapy* + *Thalidomide* - Animals submitted to surgical excision followed by four cycles of carboplatin at the dose of 300mg/m^2, given intravenously every 21 days. Afterwards, thalidomide administration was initiated at 20 mg/kg, given orally every 24 hours at night time, during three months, followed by 10 mg/kg every 24 hours at night time, during three months. The 10 mg/kg dose of thalidomide was maintained without discontinuation in patients diagnosed with distant metastasis;

Surgery + *Chemotherapy* + *Metronomic Chemotherapy* - Animals submitted to surgical excision followed by four cycles of carboplatin at the dose of 300mg/m^2, given intravenously every 21 days. Afterwards, metronomic chemotherapy administration was initiated with cyclophosphamide at 15 mg/m^2, given orally every 24 hours in the morning and firocoxib at 5 mg/kg, given orally every 24 hours during six months. The treatment was maintained without discontinuation in patients diagnosed with distant metastasis.

The surgical technique chosen for the excision of the primary mammary gland neoplasms aimed to be extensive enough to completely remove the tumors, including simple mastectomy, regional mastectomy, and unilateral chain mastectomy (Sorenmo et al., 2013). All surgeries were performed by the same surgical team. All patients underwent clinical evaluation, thoracic radiographs, abdominal ultrasound prior to the surgical procedure and every three months for follow-up. Clinical response was classified as: complete response, complete disappearance of the tumor and symptoms of disease; partial response, decrease in tumor volume of ≥50% or in tumor maximum diameter of >30%; stable disease, neither an increase nor decrease in tumor size or

symptoms; and progressive disease, increase in tumor volume of >25% or in tumor maximum diameter of >20%, appearance of new lesions (Gustafson and Page, 2013).

Clinical stage was obtained from a modified version of the original staging system established by the World Health Organization for canine mammary tumors, which evaluates: tumor size (T_1: < 3 cm; T_2: 3-5cm; T_3: > 5cm), regional lymph node metastasis (N_0: negative; N_1: positive), and distant metastasis (M_0: negative; M_1: positive). Neoplasms are classified as: stage I ($T_1N_0M_0$), II ($T_2N_0M_0$), III ($T_3N_0M_0$), IV ($T_{1-3}N_1M_0$), and V ($T_{1-3}N_{0-1}M_1$) (Sorenmo et al., 2013).

Neoplasms were collected, fixed for 48 hours in 10% neutral buffered formalin and embedded in paraffin and routinely processed. Histological sections 4 µm-thick were obtained and stained with hematoxylin and eosin. Tumors were classified according to veterinary histological criteria (Cassali et al., 2014; Misdorp et al., 1999). Tumors displaying multiple morphological patterns were classified according to the predominant neoplastic pattern. The primary neoplasm of worst prognosis was chosen for analysis in patients presenting multiple tumors.

Animals included in the study presented advanced clinical staging (stage IV or V) or presented histological types considered to have a poor prognosis, i.e., solid carcinomas, micropapillary carcinomas, anaplastic carcinomas and carcinosarcomas (Cassali et al., 2014).

The neoplasms of the *Surgery* + *Chemotherapy* + *Thalidomide* group were further submitted to histological grade, morphometric analysis of the inflammatory infiltrate and immunohistochemistry using anti-Ki-67 and anti-CD31 antibodies in attempt to better understand the possible therapeutic properties of thalidomide in canines.

Histological grade of all invasive carcinomas was established according to the Nottingham system (Elston and Ellis, 1998), which evaluates tubule formation index, nuclear pleomorphism and mitotic count, classifying the carcinomas as grade I-III.

Immunohistochemistry was performed in 3 µm-thick histological sections using two monoclonal antibodies: anti-Ki-67 (MIB-1 clone, Dako, Carpinteria, CA) at a 1:50 dilution, and anti-CD31 (JC70A clone, Dako, Carpinteria, CA), at a 1:100 dilution. Antigen retrieval was performed by using a pressure chamber (Pascal Pressure Chamber, Dako, Carpinteria, CA) treatment in citrate buffer antigen retrieval and

incubated for 16 hours at 4°C. A polymeric-based detection system (Novolink Polymer Detection System, Novocastra, Newcastle, UK) was used for detection of antigen-antibody reaction and 3,3'-Diaminobenzidine was used as chromogen. Slides were subsequently counterstained using Harris hematoxylin. Sections from a canine mammary carcinomas known to express Ki-67 and CD31 were used as positive controls. Negative controls were assessed using normal serum as the primary antibody.

The proliferation index was accessed through the number of positive nuclei for Ki-67 in hot-spot areas in a total of 1000 neoplastic cells at 400x magnification (Dutra et al., 2008). The intratumor microvessel density was obtained through the identification of one 200x magnification field containing the highest density of positive CD31 endothelial cells, mainly observed in the tumor margins. Individual endothelial cells or endothelial cell groups clearly separated from adjacent microvessels, tumor cells, and other connective tissue elements was considered a microvessel (Weidner, 1995).

The analysis of the inflammatory infiltrate was performed in 4 µm-thick histological sections stained with hematoxylin and eosin. The distribution of the inflammatory infiltrate was classified as: focal, 1-3 inflammatory foci; multifocal, more than 3 inflammatory foci; diffuse, inflammatory cells evenly distributed through the tumor. The intensity of the inflammatory reaction was based on the morphometric analysis of the total inflammatory infiltrate performed in eight hot spots in 1000x magnification fields with immersion oil in order to obtain the number of inflammatory cells. The inflammatory infiltrate was classified as: discrete, less than 500 inflammatory cells; moderate, 500-1000 inflammatory cells; and intense, more than 1000 inflammatory cells. Neutrophils, macrophages, lymphocytes, plasma cells, eosinophils, and mast cells were identified based on morphological features and were quantified. The total number of cells was obtained through adding the eight analyzed fields. The intensity of the lymphocytic infiltrate was divided into: discrete + moderate, less than 600 lymphocytes; and intense, more than 600 lymphocytes (adapted from Estrela-Lima et al., 2010).

Survival time was defined as the period (in days) between the surgical removal of the tumor and death caused by the disease. Animals that died from unknown causes

or causes unrelated to the tumor were censored. When authorized by the pet owner, the animal was submitted to post-mortem evaluation and pulmonary metastases were collected for morphological (inflammatory infiltrate) and immunohistochemical analysis.

Statistical analyses were performed with D'Agostino & Pearson omnibus normality test, with data presenting normal distribution expressed as mean ± standard deviation and otherwise as median (range), Student's t-test and Mann-Whitney U test. Median survival time (MST) curves were calculated by the Kaplan-Meier method and compared by the log-rank test. Spearman's correlation was performed to investigate the relationship between therapeutic response to thalidomide and clinical stage, histological type and grade, inflammatory infiltrate, and immunostaining for CD31 and Ki-67 of the primary neoplasm. Therapeutic response was divided into patients presenting progressive disease and patients presenting stable disease or partial and complete responses. Results were considered significant when $p \leq 0.05$.

For patients included in the surgery, chemotherapy and thalidomide group, pet owners were given individual prescriptions for a 28-day period following a signed informed consent form acknowledging that the administration of thalidomide was exclusively for the canine enrolled in the study. Pet owners were instructed to administer the medication using disposable gloves and to be careful while collecting and disposing of faeces, urine and other bodily fluids of the animals. All procedures were performed with the approval of the Ethics Committee for Animal Experimentation of the Federal University of Minas Gerais (CETEA/UFMG) (protocol number 132/2011) and the Ethics Committee for Animal Experimentation of the São Paulo State University (Jaboticabal Campus) (protocol number 021846/14).

RESULTS

Fifty-eight bitches were evaluated. The average age at the time of diagnosis was 10.61±2.72 years and the average time of development of the neoplasm reported by the owner was 251±237.7 days. Forty patients presented advanced clinical staging, 32/54 (59.26%) stage IV and 8/54 (14.81%) stage V, and 14 patients presented initial clinical staging, 3/54 (5.56%) stage I, 3/54 (5.56%) stage II, and 8/54 (14.81%) stage

III. Neoplasm size was smaller than 3 cm in 11/53 (20.75%), 3-5 cm in 12/53 (22.64%), and larger than 5 cm in 30/53 (56.61%).

Twenty (34.48%) patients were submitted to regional mastectomy, 28/58 (48.28%) to chain mastectomy, and the type of surgery was not specified or varied, e.g. simple mastectomy, in 10/58 (17.24%) of cases. Regarding the different treatment groups, 11/58 (18.97%) animals were submitted solely to the surgical treatment, 15/58 (25.86%) to surgery and chemotherapy, 23/58 (39.65%) to surgery, chemotherapy and thalidomide, and 9/58 (15.52%) to surgery, chemotherapy and metronomic chemotherapy.

The primary neoplasms were diagnosed as 15/58 (25.86%) carcinomas in mixed tumors, 15/58 (25.86%) solid carcinomas, 8/58 (13.80%) micropapillary carcinomas, 8/58 (13.80%) carcinosarcomas, 4/58 (6.90%) tubular carcinomas, 2/58 (3.45%) papillary carcinomas, 2/58 (3.45%) malignant adenomyoepiteliomas, 1/58 (1.72%) lipid-rich carcinoma, 1/58 (1.72%) pleomorphic lobular carcinoma, 1/58 (1.72%) osteosarcoma, and 1/58 (1.72%) sarcoma in mixed tumor.

Necropsy was performed in ten animals of the *Surgery + Chemotherapy + Thalidomide* group, and 9/10 (90%) of these animals died due to the progression of the mammary gland neoplasm and presented pulmonary metastases. The mean proliferative index was 26.61% ± 23.08% in primary tumors and 10.05% ± 8.33% in pulmonary metastasis. Median microvessel density was 65.50 (33-179) in primary tumors and 86 (61-145) in pulmonary metastasis. No statistical significant difference was found among Ki-67 and CD31 immunolabeling in primary and metastatic neoplasms (p=0.0859 and p=0.1695, respectively).

Clinical staging, histological type and grade, inflammatory infiltrate, and immunostaining for CD31 were not associated to the therapeutic response or associated to differences in MST in patients treated with thalidomide. However, a positive moderate correlation was found between progressive disease and high Ki-67 immunostaining (higher than the 26.61% mean) and stable disease or partial and complete responses and low Ki-67 immunostaining (lower than the 26.61% mean) (r=0.6351; p=0.05). In addition, the MST analysis of low Ki-67 immunostaining of the primary neoplasm of patients treated with surgery, chemotherapy and the addition of

thalidomide presented a significantly longer survival (MST 1052 days) when compared to high Ki-67 neoplasms (MST 276 days) ($p<0.0001$) (Fig. 01).

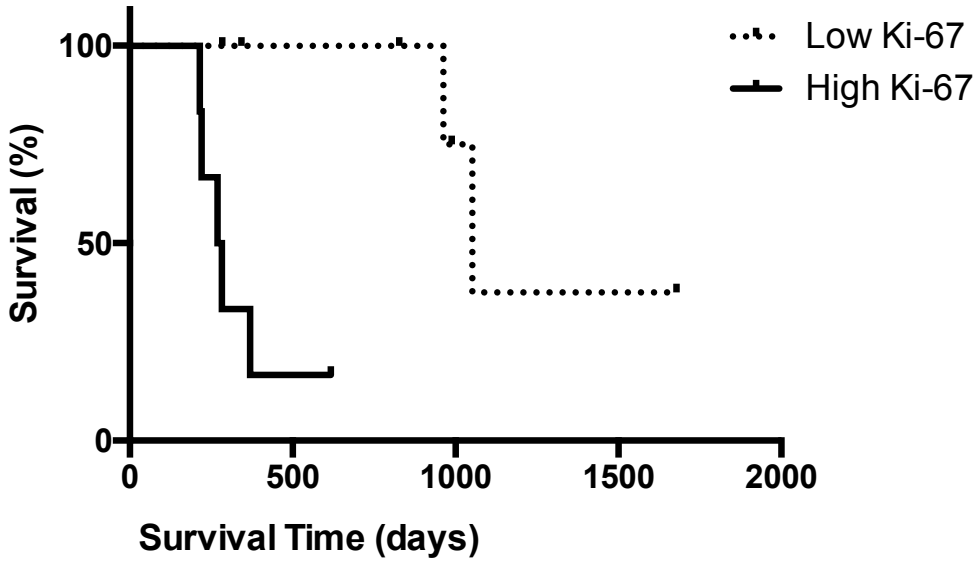

Figure 01. Kaplan-Meier survival curve of 13 bitches diagnosed with high and low Ki-67 immunostaining of malignant mammary gland neoplasms treated with surgery, chemotherapy and thalidomide ($p=0.0029$).

Analysis of the inflammatory infiltrate in patients treated with surgery, chemotherapy and thalidomide demonstrated a predominance in diffuse inflammatory infiltrate in 60% of primary and in 44.45% of metastatic lesions. There was an absence of discrete inflammatory reactions in primary and metastatic lesions, with a prevalence of intense reactions in 95% of primary neoplasms and moderate reactions in 55.55% of metastatic neoplasms. Intense lymphocytic infiltrate prevailed in 70% of primary neoplasms, while discrete+moderate lymphocytic infiltrate prevailed in 88.89% of metastatic neoplasms. The distribution of the inflammatory cells in the primary and metastatic neoplasms are demonstrated in table 01.

Table 01. Analysis of the inflammatory infiltrate in primary neoplasms and pulmonary metastases in animals treated with surgery, chemotherapy and thalidomide.

Inflammatory Cells	**Primary Neoplasm**	**Pulmonary Metastasis**	
Neutrophils	18 (2-388)	27 (5-191)	p=0.2195
Macrophages	278±209.5	453±186.7	p=0.0407*
Lymphocytes	1097 (291-2847)	300 (177-1105)	p=0.0024*
Plasma Cells	185 (8-770)	8 (4-88)	p=0.0002*
Eosinophils	3 (0-112)	0 (0-0)	p=0.0011*
Mast Cells	0 (0-26)	0 (0-0)	p=0.28
Total	1747±663.6	958.2±305.5	p=0.0022*

No statistically significant difference was observed in MST of all four treatment groups when including all clinical stages. Patients treated with surgery presented a MST of 245 days, 242 days for patients treated with surgery and chemotherapy, 845 days for patients treated with surgery, chemotherapy and thalidomide, and 431 days for patients treated with surgery, chemotherapy and metronomic chemotherapy (p=0.3177) (Fig. 02). However, when analyzing the MST of the four treatment groups including only animals that presented distant metastases before or during treatment, animals treated with surgery, chemotherapy and the addition of thalidomide (MST 463 days) or metronomic chemotherapy (MST 376.5 days) presented a significantly longer survival when compared to animals treated solely with surgery (MST 150 days) and surgery and chemotherapy (MST 148 days) (p<0.0001) (Fig. 03). Therapeutic evidence of thalidomide administration was also demonstrated by the evaluation of pulmonary metastases through thoracic radiographs. The metastatic lesions were mostly classified as stable disease, demonstrated by a slow progression of the lung metastasis. One patient presented a partial response of the metastatic lesion during thalidomide administration (Fig. 04).

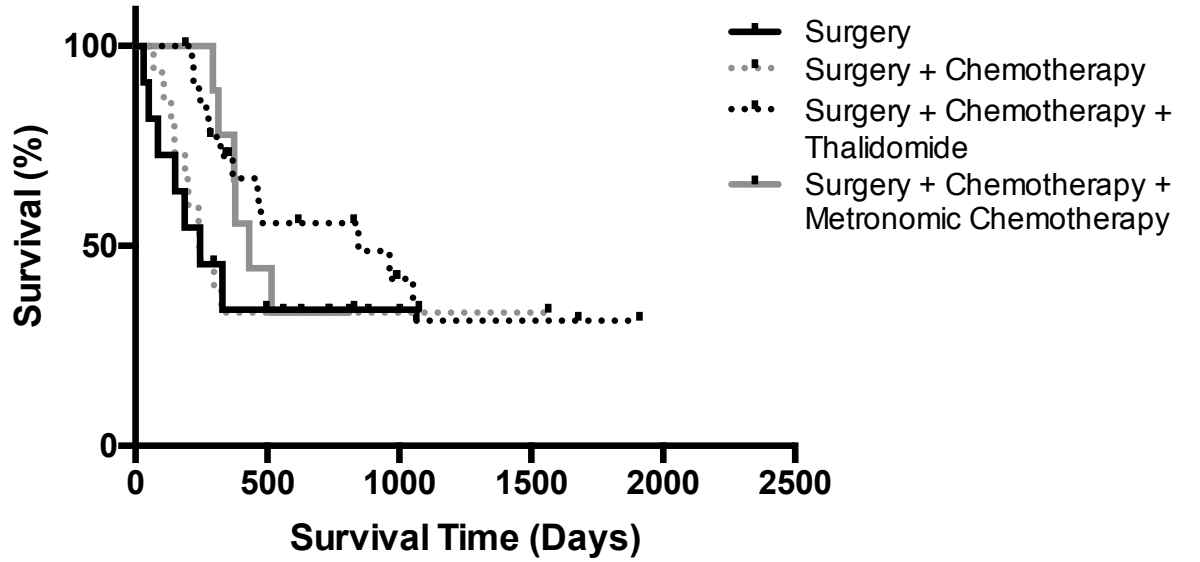

Figure 02. Kaplan-Meier survival curve of 58 bitches diagnosed with malignant mammary gland neoplasms treated with surgery, surgery and chemotherapy, surgery, chemotherapy and thalidomide, and surgery, chemotherapy and metronomic chemotherapy (p=0.3177).

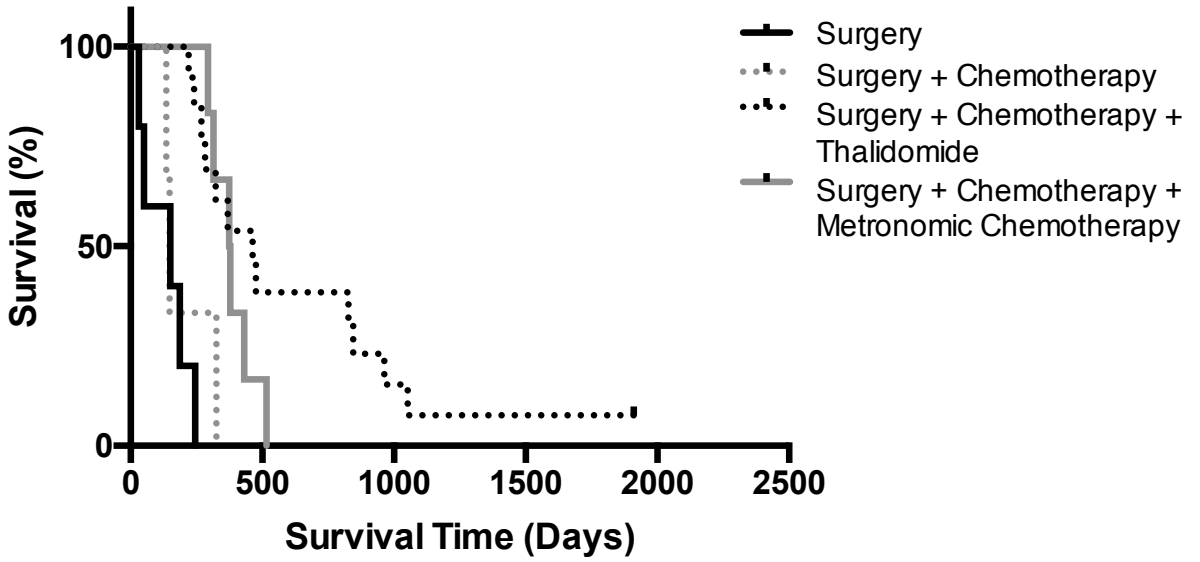

Figure 03. Kaplan-Meier survival curve of 27 bitches diagnosed with malignant mammary gland neoplasms presenting distant metastasis treated with surgery, surgery and chemotherapy, surgery, chemotherapy and thalidomide, and surgery, chemotherapy and metronomic chemotherapy (p<0.0001).

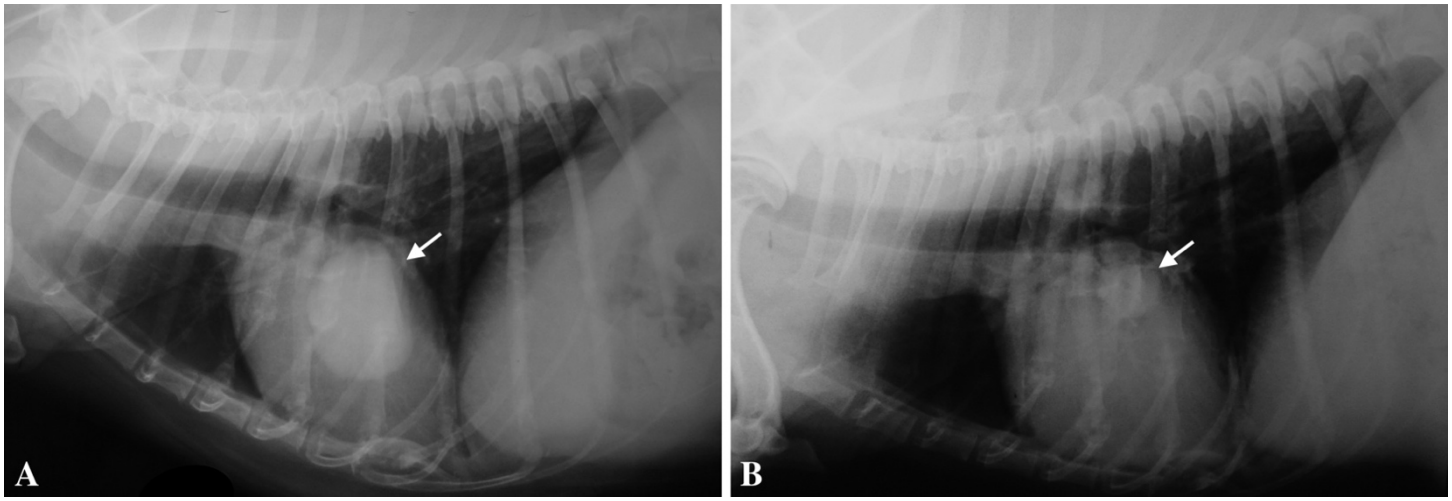

Figure 04. Right lateral recumbency thoracic radiographs demonstrating a partial response of metastatic nodules during the thalidomide treatment (arrow). **A.** Image at the beginning of the thalidomide administration. **B.** Image after 14 months of the thalidomide treatment.

All treatments were well tolerated. The 20 mg/kg dose of thalidomide was mainly associated to an excessive somnolence in some patients with symptom improvement following dose reduction to 10 mg/kg. Metronomic chemotherapy was associated to cyclophosphamide-induced sterile hemorrhagic cystitis in 4/9 (44.44%) patients. However, the interruption of the metronomic chemotherapy followed by treatment with prednisone at the dose of 1 mg/kg, given orally for 10 days was enough to improve all symptoms and metronomic chemotherapy was resumed.

DISCUSSION

In our study, the addition of thalidomide and metronomic chemotherapy to surgery and chemotherapy with carboplatin presented clinical benefit with an increase in survival in bitches diagnosed with mammary gland neoplasms presenting distant metastasis. In addition, higher proliferative index in primary neoplasms were associated to poorer therapeutic responses and shorter survival times in patients treated with surgery, chemotherapy and thalidomide.

Currently, there are no standard guidelines for treatment of canine mammary gland neoplasms. Surgical excision wide enough to completely remove the tumor for single mammary gland neoplasms is considered adequate, while multiple mammary

gland neoplasms may require more extensive resections, such as regional or unilateral chain mastectomy. However, tumors that are large, present metastatic lymph nodes, and aggressive histology are not treated effectively with surgery alone (Sorenmo et al., 2013). The studied population of the present study presented a predominance of advanced clinical stage (IV and V), tumors larger than 5 cm, and several histotypes associated to poor prognosis. Shorter MST were described for micropapillary carcinomas, solid carcinomas, tubular carcinomas, and carcinosarcomas when compared to carcinomas in mixed tumors and papillary carcinomas (Nunes, 2015; Nunes et al., 2014). The high frequency of carcinomas in mixed tumors was expected due to its high prevalence in canine mammary gland neoplasms (Nunes et al., 2014; Toríbio et al., 2012).

Although the available evidence concerning the efficacy of adjuvant chemotherapy is limited, chemotherapy is often suggested for mammary gland neoplasms that present metastasis or recurrence risks (Sorenmo, 2003; Sorenmo et al., 2013). Cassali et al. (2014) recommend chemotherapy when the histotype of the primary neoplasm is associated with poor prognosis, i.e., solid carcinomas, micropapillary carcinomas, anaplastic carcinomas and carcinosarcomas, or when patients present regional or distant metastasis.

Chemotherapy causes DNA damage, interfering with DNA replication in proliferating cells (Hanahan et al., 2000), resulting in antiproliferative and cytotoxic actions (Ehrke, 2003). “Maximum tolerated doses” chemotherapy protocols aim towards the maximum cytotoxicity possible for tumor cells and require an interval for the recovery of normal tissues. These protocols are frequently initially efficacious and result in tumor regression or stabilization, prolonged survival, and may cure the disease. However, responses may be short-lived, with relapses resulting in resistance to the cytotoxic drug and more aggressive cancers (Hanahan et al., 2000). The present study was unable to demonstrate clinical benefit of adjuvant chemotherapy without the association of antiangiogenic therapies, possibly due to a momentary tumor response that was not maintained.

Folkman (1971) proposed that tumor cells need the perfusion induced by new capillaries in order to exceed a diameter of 2-3 mm. If angiogenesis is inhibited, the tumor will remain in a nonvascularized dormant state, hindering metastasis and

increasing the susceptibility of tumor cells to cell-mediated immunologic attack. Therefore, the inhibition of angiogenesis may also synergize immunotherapy (Folkman, 1971). Acceptance of immunomodulatory therapies in the clinic may be improved if they are combined with other therapies of proven clinical utility. However, possible combinations should consider that conventional antitumor therapies may suppress host antitumor defense mechanisms and may therefore not be effective (Ehrke, 2003).

Thalidomide and its analogues inhibit several cytokines, such as interleukin-6, tumor necrosis factor-α, vascular endothelial growth factor and basic fibroblast growth factor, resulting in inhibition of tumor cell growth, survival, migration, drug resistance, and angiogenesis. Furthermore, interleukin-2 and interferon-γ may be stimulated, promoting antitumor immunity (Raje and Anderson, 2002). The mean proliferative index of the pulmonary metastases was lower than the primary tumors and the difference between them presented a trend towards statistical significant difference. Although the evaluation of distant metastasis samples without the thalidomide treatment for comparison was not possible, such findings suggests the antitumor properties of thalidomide and are sustained by the predominant slow progression of the pulmonary metastases of animals during the thalidomide treatment. Furthermore, the proliferative index of the primary neoplasm was found to be correlated to the therapeutic response to thalidomide and influenced in the MST of the patients and should be further studied as a predictive marker for thalidomide response.

The morphometric analysis of the inflammatory infiltrates demonstrated several differences between the primary neoplasms and the pulmonary metastases. Primary neoplasms demonstrated a prevalence of intense infiltrates, while moderate infiltrates prevailed in metastatic lesions. Metastases presented higher counts of macrophages and lower counts of lymphocytes, plasma cells, and eosinophils and lower total cell counts. In addition, lymphocytic infiltrates were predominantly intense in primary neoplasms and discrete+moderate in metastatic lesions. Intense lymphocytic infiltrate was described as an independent prognostic factor associated to lower survival rates in canine mammary gland neoplasms (Estrela-Lima et al., 2010) and the thalidomide treatment in the present study may possibly reduce the intensity of lymphocytic infiltrates. Tumor-associated macrophages may be associated to tumor-promoting

functions or contribute towards the efficacy of anticancer strategies (Mantovani and Allavena, 2015). Thalidomide was found to potentially reduce the exaggerated pro-inflammatory effect of alveolar macrophages in a pneumonia mice model (Kumar et al., 2010), inhibit polarization of M2 macrophages in an animal model of allergic asthma (Lee et al., 2015), and the drug demonstrated immunomodulatory and potent anti-inflammatory properties in an acute lung infection mice model (Kumar and Chhibber, 2008). Thalidomide was responsible for the inhibition of leukocyte recruitment in the murine mammary 4T1 primary tumor and pulmonary metastatic tumors (De Souza et al., 2012). The anti-inflammatory and macrophage increase in the present study may be partially responsible for the observed clinical benefit associated to the thalidomide treatment. Additional studies including macrophage phenotyping in M1 (anti-tumor) and M2 (pro-tumor) will contribute towards a better understanding of the immunomodulatory effects of thalidomide in cancer.

Microvessel density has been described as a prognostic factor for various neoplasms, and a decrease in microvessel density is observed during treatment with an antiangiogenic therapy suggests activity of the treatment (Hlatky et al., 2002). In the present study, no statistical difference was observed between the CD31 immunolabeling in primary neoplasms and the pulmonary metastases. However, microvessel density does not measure angiogenic activity or dependence of a tumor and should not be used as a predictive marker for antiangiogenic treatments or as a marker to demonstrate therapeutic response of an antiangiogenic treatment (Hlatky et al., 2002).

A 4-8 mg/kg/day dose of thalidomide is used aiming towards the maintenance of various neoplastic diseases in humans (Teo, 2005). In canines, the safety profile of thalidomide was evaluated in healthy beagles and a 200 mg/kg dose was considered the NOAEL (no-observed-adverse effect levels) (Teo et al., 2001). We previously reported the adverse events of the thalidomide therapy in canine patients diagnosed with mammary gland neoplasms in advanced clinical staging. The drug was considered well tolerated, and the 20 mg/kg and the 10 mg/kg dose did not impair the activities of daily living. However, the lower dose may be considered when excessive somnolence is found (de Campos et al., 2016). Two clinical trials started to evaluate the treatment of canine hemangiosarcomas (Woods et al., 2004) and various

malignant neoplasms in dogs (Jankowski et al., 1999) with thalidomide but were not completed. A dog diagnosed with a malignant and metastatic Leydig cell tumor was treated with surgery, and metronomic chemotherapy with cyclophosphamide and thalidomide (Togni et al., 2015), and the association of metronomic chemotherapy with cyclophosphamide and thalidomide with maximum tolerated dose chemotherapy was reported to increase survival in canine hemangiosarcomas (Finotello et al., 2016). In felines, Marconato et al. (2013) proposed a multimodal approach, including neoadjuvant and adjuvant bleomycin, piroxican, and thalidomide associated to radiotherapy and surgery for the treatment of unresectable head and neck squamous cell carcinoma. However, the authors affirm that the study does not validate the routine clinical use of thalidomide.

Since thalidomide was withdrawn from the market, several countries have established regulations for the prescription and disposal of thalidomide for humans, e.g. the THALIDOMID REMS^{TM} program of the United States of America (Celgene, 2015). A legal and reliable source of thalidomide for veterinarians worldwide is a challenge. Therefore, metronomic chemotherapy represents an alternative available and affordable treatment that also is characterized by antiangiogenic and immunomodulatory properties.

The priority in veterinary oncology is to maintain patient quality of life, and therefore new chemotherapy strategies should aim to minimize side effects (Pierini et al., 2012). The continuous treatment schedule of metronomic chemotherapy protocols requires a significant dose reduction of the cytotoxic agent (Mutsaers, 2013). Metronomic chemotherapy protocols are appealing because they are generally well tolerated, and present low toxicity, easy administration, and low cost (Mutsaers, 2013; Pierini et al., 2012). However, clinical studies on metronomic chemotherapy in veterinary medicine are still in an early stage (Mutsaers, 2013). Metronomic chemotherapy alters the approach to therapy, not emphasizing the neoplastic cells, and therefore offers potential for the treatment of refractory neoplasms with drugs that have previously failed or the use of drugs that were considered ineffective for a certain neoplasm (Hanahan et al., 2000).

Several clinical trials involving metronomic chemotherapy for various malignant neoplasms have been performed: lomustine was administered in various primary and

metastatic neoplasms (Tripp et al., 2011), chlorambucil for various malignant neoplasms (Leach et al., 2012) and for urinary bladder transitional cell carcinoma (Schrempp et al., 2013), cyclophosphamide associated to toceranib for various malignant neoplasms (Mitchell et al., 2012), cyclophosphamide, etoposide, and piroxican for splenic hemangiosarcomas (Lana et al., 2007), and cyclophosphamide associated to piroxican in soft tissue sarcomas, which was considered very effective (Elmslie et al., 2008). Several drugs may be combined with metronomic chemotherapy, including nonsteroidal anti-inflammatory drugs (NSAIDs) and tyrosine kinase inhibitors (Pierini et al., 2012). In the present study, firocoxib was chosen as the NSAIDs. Lavalle et al. (2012) reported the association of piroxican and firocoxib to chemotherapy and surgery. The authors suggest caution for the use of piroxican due to possible gastrointestinal adverse events, while firoxocib presented an increased toxicity. Metronomic cyclophosphamide has also been associated to an increase in antitumor immunity in animals (Ehrke, 2003). Furthermore, Carvalho et al. (2016) demonstrated an association between Cox-2 expression and aggressive biological behavior characteristic, such as shorter survival, lymph node metastasis, increased proliferation, tumoral lymphocyte and macrophage infiltration, and angiogenesis, emphasizing the usefulness of selective Cox-2 inhibitors in the treatment for canine mammary tumors.

The present study failed to observe a statistically significant difference in MST of all four treatment groups composed by patients with different clinical stages. However, the addition of thalidomide was associated to a MST approximately four-times longer than the surgery and the surgery and chemotherapy groups, and a MST two-times longer than the patients treated with metronomic chemotherapy. Therefore, we suggest additional clinical trials to evaluate the therapy with thalidomide in patients in an adjuvant setting. However, significant longer MST were observed when analyzing patients that presented distant metastasis before or during the study in the groups treated with the association of thalidomide and metronomic chemotherapy when compared to the groups treated solely with surgery and surgery and chemotherapy, demonstrating a clear clinical benefit in the addition of antiangiogenic and immunomodulatory therapies for these patients. Survival rates for animals presenting distant metastasis was described as 13.6% one year after mastectomy (Yamagami et

al., 1996). MST for both the metronomic chemotherapy and thalidomide group was over one year, while MST for groups not treated with antiangiogenic therapies was approximately 150 days.

CONCLUSION

The proposed multimodal therapy protocols of surgery, chemotherapy, and thalidomide or metronomic chemotherapy increased the survival time for patients presenting malignant mammary gland neoplasms with distant metastasis and should be proposed for the treatment of these patients. Additional clinical trials are warranted to evaluate the clinical benefits of the addition of thalidomide and metronomic chemotherapy to other canine neoplasms with advanced clinical staging and to better understand the mechanistic principals of these treatments.

ACKNOWLEDGEMENTS

This research was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP Grant 2014/01329-9) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq Grant 302449/2013-2).

REFERENCES

Adlard, J.W., 2000. Thalidomide in the treatment of cancer. Anti-Cancer Drug 11, 787–791.

Brodey, R.S., Goldschmidt, M.H., Roszel, J.R., 1983. Canine mammary gland neoplasms. J. Am. Anim. Hosp. Assoc. 19, 61–90.

Carvalho, M.I., Pires, I., Prada, J., Raposo, T.P., Gregório, H., Lobo, L., Queiroga, F.L., 2016. High COX-2 expression is associated with increased angiogenesis, proliferation and tumoural inflammatory infiltrate in canine malignant mammary tumours: A multivariate survival study. Vet. Comp. Oncol. In press.

Cassali, G.D., Lavalle, G.E., Ferreira, E., Estrela-Lima, A., Nardi, A.B. De, Ghever, C., Sobral, R.A., Amorim, R.L., Oliveira, L.O., Sueiro, F.A.R., Beserra, H.E.O., Bertagnolli, A.C., Gamba, C.O., Damasceno, K.A., Campos, C.B., Araujo, M.R., Campos, L.C., Monteiro, L.N., Nunes, F.C., Horta, R.S., Reis, D.C., Luvizotto, M.C.R., Magalhaes, G.M., Raposo, J.B., Ferreira, A.M.R., Tanaka, N.M., Grandi, F., Ubukata, R., Batschinski, K., Terra, E.M., Salvador, R.C.L., Jark, P.C., Delecrodi, J.E.R., Nascimento, N.A., Silva, D.N., Silva, L.P., Ferreira, K.C.R.S., Frehse, M.S., Santis, G.W., Silva, E.O., Guim, T.N., Kerr, B., Cintra, P.P., Silva, F.B.F., Leite, J.S., Mello, M.F. V., Ferreira, M.L.G., Fukumasu, H., Salgado, B., Torres, R., 2014. Consensus for the diagnosis, prognosis and treatment of canine mammary tumors - 2013. Brazilian J. Vet. Pathol. 7, 38–69.

Celgene, 2015. Thalidomid REMS. URL http://www.thalomidrems.com/ (accessed 10.05.16).

de Campos, C. B., Lavalle, G. E., Ligorio, S. F., Nunes, F. C., Carneiro, R. A., Amorim, R. L., Cassali, G D., 2016. Absence of significant adverse events following thalidomide administration in bitches diagnosed with mammary gland carcinomas. Vet. Rec. In press.

De Souza, C.M., Fonseca de Carvalho, L., da Silva Vieira, T., Cândida Araújo e Silva, A., Teresa Paz Lopes, M., Alves Neves Diniz Ferreira, M., Passos Andrade, S., Dantas Cassali, G., 2012. Thalidomide attenuates mammary cancer associated-inflammation, angiogenesis and tumor growth in mice. Biomed. Pharmacother. 66, 491–498.

Dutra, A.P., Azevedo Júnior, G.M., Schmitt, F.C., Cassali, G.D., 2008. Assessment of cell proliferation and prognostic factors in canine mammary gland tumors. Arq. Bras. Med. Veterinária e Zootec. 60, 1403–1412.

Ehrke, M.J., 2003. Immunomodulation in Cancer Therapeutics. Int. Immunopharmacol. 3, 1105–1119.

Elmslie, R.E., Glawe, P., Dow, S.W., 2008. Metronomic therapy with cyclophosphamide and piroxicam effectively delays tumor recurrence in dogs with incompletely resected soft tissue sarcomas. J. Vet. Intern. Med. 22, 1373–1379.

Elston, C.W., Ellis, I.O., 1998. Assessment of histological grade, in: Elston, C.W., Ellis, I.O. (Eds.), Systemic Pathology: The Breast. Churchill Livingstone, London, pp. 365–384.

Estrela-Lima, A., Araujo, M.S., Costa-Neto, J.M., Teixeira-Carvalho, A., Barrouin-Melo, S.M., Cardoso, S. V, Martins-Filho, O.A., Serakides, R., Cassali, G.D., 2010. Immunophenotypic features of tumor infiltrating lymphocytes from mammary carcinomas in female dogs associated with prognostic factors and survival rates. BMC Cancer 10, 256–269.

Finotello, R., Henriques, J., Sabattini, S., Stefanello, D., Felisberto, R., Pizzoni, S., Ferrari, R., Marconato, L., 2016. A retrospective analysis of chemotherapy switch suggests improved outcome in surgically removed, biologically aggressive canine haemangiosarcoma. Vet. Comp. Oncol. 9–11.

Folkman, J., 1971. Tumor angiogenesis: therapeutic implications. N. Engl. J. Med. 285, 1182–1186.

Gustafson, D.L., Page, R.L., 2013. Cancer Chemotherapy, in: Withrow, S.J., Vail, D.M., Page, R.L. (Eds.), Withrow & MacEwen's Small Animal Clinical Oncology. Elsevier Saunders, Saint Louis, pp. 157–179.

Hanahan, D., Bergers, G., Bergsland, E., 2000. Less is more, regularly: Metronomic dosing of cytotoxic drugs can target tumor angiogenesis in mice. J. Clin. Invest. 105, 1045–1047.

Hlatky, L., Hahnfeldt, P., Folkman, J., 2002. Clinical application of antiangiogenic therapy: Microvessel density, what it does and doesn't tell us. J. Natl. Cancer Inst. 94, 883–893.

Jankowski, M., Fulton, L., Sheafor, S., Prescott, D., Khanna, C., 1999. Ongoing evaluation of single agent thalidomide in dogs with measurable cancer, in: Veterinary Cancer Society 19th Annual Meeting. Wood's Hole, MA.

Kumar, V., Chhibber, S., 2008. Anti-inflammatory effect of thalidomide alone or in combination with augmentin in Klebsiella pneumoniae B5055 induced acute lung infection in BALB/c mice. Eur. J. Pharmacol. 592, 146–150.

Kumar, V., Harjai, K., Chhibber, S., 2010. Thalidomide treatment modulates macrophage pro-inflammatory function and cytokine levels in Klebsiella pneumoniae B5055 induced pneumonia in BALB/c mice. Int. Immunopharmacol.

10, 777–783.

Lana, S., Lance, U., Plaza, S., Elmslie, R., Gustafson, D., Morley, P., Dow, S., 2007. Continuous Low-Dose Oral Chemotherapy for Adjuvant Therapy of Splenic Hemangiosarcoma in Dogs. J. Vet. Intern. Med. 21, 764–769.

Lavalle, G.E., De Campos, C.B., Bertagnolli, A.C., Cassali, G.D., 2012. Canine malignant mammary gland neoplasms with advanced clinical staging treated with carboplatin and cyclooxygenase inhibitors. In Vivo (Brooklyn). 26, 375–379.

Leach, T.N., Childress, M.O., Greene, S.N., Mohamed, a S., Moore, G.E., Schrempp, D.R., Lahrman, S.R., Knapp, D.W., 2012. Prospective trial of metronomic chlorambucil chemotherapy in dogs with naturally occurring cancer. Vet. Comp. Oncol. 10, 102–112.

Lee, H.S., Kwon, H.S., Park, D.E., Woo, Y.D., Kim, H.Y., Kim, H.R., Cho, S.H., Min, K.U., Kang, H.R., Chang, Y.S., 2015. Thalidomide inhibits alternative activation of macrophages in vivo and in vitro a potential mechanism of anti-asthmatic effect of thalidomide. PLoS One 10, 1–15.

Mantovani, A., Allavena, P., 2015. The interaction of anticancer therapies with tumor-associated macrophages. J. Exp. Med. 212, 435–445. doi:10.1084/jem.20150295

Marconato, L., Buchholz, J., Keller, M., Bettini, G., Valenti, P., Kaser-Hotz, B., 2013. Multimodal therapeutic approach and interdisciplinary challenge for the treatment of unresectable head and neck squamous cell carcinoma in six cats: A pilot study. Vet. Comp. Oncol. 11, 101–112.

McBride, W.G., 1977. Thalidomide embryopathy. Teratology 16, 79–82.

Misdorp, W., Else, R.W., Hellmén, E., Lipscomb, T.P., 1999. Histological classification of mammary tumors of the dog and the cat, 2nd Series. ed. Armed Forces Institute of Pathology. American Registry of Pathology and the World Health Organization Collaborating Center for Worldwide Reference on Comparative Oncology, Washington DC.

Mitchell, L., Thamm, D.H., Biller, B.J., 2012. Clinical and Immunomodulatory Effects of Toceranib Combined with Low-Dose Cyclophosphamide in Dogs with Cancer. J. Vet. Intern. Med. 26, 355–362.

Mutsaers, A.J., 2013. Antiangiogenic and Metronomic Therapy, in: Withrow, S.J., Vail, D.M. (Eds.), Withrow & MacEwen's Small Animal Clinical Oncology. W. B. Saunders Company, Philadelphia, pp. 229–237.

Novosad, C.A., 2003. Principles of treatment for mammary gland tumors. Clin. Tech. Small Anim. Pract. 18, 107–109.

Nunes, F.C., 2015. Diagnóstico, prognóstico e tratamento dos carcinomas de glândulas mamárias de cadelas atendidas no hospital veterinário da UFMG – Estudo retrospectivo. Federal University of Minas Gerais.

Nunes, F.C., Gamba, C.O., Damasceno, K.A., Campos, C.B., Horta, R.S., Araujo, M.R., Monteiro, L.N., Lavalle, G.E., Ferreira, E., Cassali, G.D., 2014. Analisis of clinico-pathological data, therapeutical conduct and overall survival of canine mammary lesions attended at the veterinary hospital of the Federal University of Minas Gerais (UFMG). Brazilian J. Vet. Pathol. 7, 122–126.

Pierini, A., Bocci, G., Giorgi, M., Owen, H., Marchetti, V., 2012. From humans to dogs and back: The translational lesson of metronomic chemotherapy. Am. J. Anim. Vet. Sci. 7, 198–212.

Raje, N., Anderson, K.C., 2002. Thalidomide and immunomodulatory drugs as cancer therapy. Curr. Opin. Oncol. 14, 635–640.

Schrempp, D.R., Childress, M.O., Stewart, J.C., Leach, T.N., Tan, K.M., Abbo, A.H., de Gortari, A.E., Bonney, P.L., Knapp, D.W., 2013. Metronomic administration of chlorambucil for treatment of dogs with urinary bladder transitional cell carcinoma. J. Am. Vet. Med. Assoc. 242, 1534–1538.

Sorenmo, K.U., 2003. Canine mammary gland tumors. Vet. Clin. North Am. Small Anim. Pract. 33, 573–596.

Sorenmo, K.U., Worley, D.R., Goldschmidt, M.H., 2013. Tumors of the Mammary Gland, in: Withrow, S.J., Vail, D.M., Page, R.L. (Eds.), Withrow & MacEwen's Small Animal Clinical Oncology. Elsevier Saunders, Saint Louis, pp. 538–556.

Teo, S.K., 2005. Properties of thalidomide and its analogues: implications for anticancer therapy. AAPS J. 7, 14–19.

Teo, S.K., Evans, M.G., Brockman, M.J., Ehrhart, J., Morgan, J.M., Stirling, D.I., Thomas, S.D., 2001. Safety Profile of Thalidomide after 53 Weeks of Oral Administration in Beagle Dogs. Toxicol. Sci. 59, 160–168.

Togni, A., Rütten, M., Bley, C.R., Hurter, K., 2015. Metastasized Leydig cell tumor in a dog. Schweizer Arch. für Tierheilkd. 157, 111–115.

Toríbio, J.M.D.M.L., Lima, A.E., Martins Filho, E.F., Ribeiro, L.G.R., D'Assis, M.J.M.H., Teixeira, R.G., Damasceno, K.A., Cassali, G.D., Costa Neto, J.M. Da, 2012. Clinical characterization, histopathologic diagnosis and geoprocessing of mammary tumours in bitches from the city of Salvador, Bahia State. Rev. Ceres 59, 427–433.

Tripp, C.D., Fidel, J., Anderson, C.L., Patrick, M., Pratt, C., Sellon, R., Bryan, J.N., 2011. Tolerability of Metronomic Administration of Lomustine in Dogs with Cancer. J. Vet. Intern. Med. 25, 278–284.

Weidner, N., 1995. Intratumor microvessel density as a prognostic factor in cancer. Am. J. Pathol. 147, 9–19.

Woods, J.P., Mathews, K.A., Binnington, A.G., 2004. Thalidomide for the treatment of hemangiosarcoma in dogs. Vet. Comp. Oncol. 2, 108–109.

Yamagami, T., Kobayashi, T., Takahashi, K., Sugiyama, M., 1996. Prognosis for canine malignant mammary tumors based on TNM and histologic classification. J. Vet. Med. Sci. 58, 1079–1083.

CAPÍTULO 5 – CONSIDERAÇÕES FINAIS

A adição da talidomida ao tratamento de neoplasias malignas da glândula mamária canina mostrou-se benéfica para os pacientes do presente estudo, principalmente para os pacientes diagnosticados com metástase à distancia. Atualmente, existe uma carência de opções terapêuticas eficientes para esses pacientes em medicina veterinária. Portanto, o aumento significativo de sobrevida global observado no presente estudo, com manutenção da qualidade de vida e baixo custo, é notável.

Infelizmente, o uso da talidomida é restrito para pacientes da espécie humana, sendo utilizada em animais apenas para pesquisa. Essa limitação nos incentivou a buscar alternativas, sendo que a quimioterapia metronômica, que, semelhante à talidomida, possui efeitos antiangiogênicos e imunomodulatórios, mostrou-se satisfatória.

Diversos estudos ainda são necessários para estabelecer e padronizar as condutas terapêuticas ideais para as neoplasias da glândula mamária canina, porém, o presente estudo demonstrou uma opção pertinente de tratamento. Além disso, futuras pesquisas devem se preocupar com a qualidade do estudo clínico, sempre objetivando o padrão ouro: estudos randomizados, duplo-cego e placebo-controlado, o que ainda é um desafio para as pesquisas brasileiras em medicina veterinária.