UNIVERSIDADE DE BRASÍLIA
FACULDADE DE AGRONOMIA E MEDICINA VETERINÁRIA

AVALIAÇÃO HEMATOLÓGICA E DOS MARCADORES RELACIONADOS AO FERRO EM CANINOS COM DOENÇA MIXOMATOSA DA VALVA MITRAL

NANCI SOUSA NILO BAHIA DINIZ

DISSERTAÇÃO DE MESTRADO EM CIÊNCIAS ANIMAIS

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Dissertação de mestrado submetida ao programa de pós-graduação em Ciências Animais, como parte dos requisitos necessários à obtenção do grau de Mestre em Ciências Animais.

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1. Cardiopatia. 2. Hemoglobina reticulocitária. 3. Cães 4. Anemia.

EPÍGRAFE

Caminante, son tus huellas
el camino y nada más;
Caminante, no hay camino,
se hace camino al andar.
Al andar se hace el camino,
y al volver la vista atrás
se ve la senda que nunca
se ha de volver a pisar.
Caminante no hay camino
sino estelas en la mar.

Antonio Machado (1875 - 1939)

DEDICATÓRIA

Dedico este trabalho aos meus amores: Carlos, João e Clara. Sem vocês tudo seria muito mais difícil.

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RESUMO

A Doença Mixomatosa da Valva Mitral (DMVM) é a cardiopatia adquirida mais comum em cães. Pouca atenção tem sido dada a anemia e a deficiência de ferro no curso da doença, apesar de sua importância na insuficiência cardíaca humana. Este estudo avaliou parâmetros hematológicos e marcadores relacionados à anemia em cães com DMVM, especificamente a hemoglobina reticulocitária (Ret-Hb). 121 cães foram selecionados, amostras de sangue coletadas e analisadas para hemograma, ferro sérico, ferritina e Ret-Hb. Hemoglobina, hematócrito e Ret-Hb diminuíram conforme a doença progrediu, embora não tenha havido diferença significativa entre os grupos. Bastonetes aumentaram significativamente nos estágios avançados da MMVD, sugerindo uma associação da progressão da doença e inflamação. Este estudo conclui que, embora a anemia e a deficiência de ferro não sejam proeminentes em cães com MMVD, a resposta inflamatória provavelmente está presente conforme a progressão da doença.

Palavras-chave: reticulócitos, ferro, doença cardíaca, cardiopatia.

ABSTRACT

Myxomatous Mitral Valve Disease (MMVD) is the most common acquired heart disease in dogs. However, little attention has been given to the potential role of anemia and iron deficiency in the course of the disease despite its significance in human heart failure. This study evaluated hematologic parameters and iron-related markers in dogs with MMVD, specific reticulocyte hemoglobin (Ret-Hb). One hundred twenty-one dogs were selected, and blood samples were collected and analyzed for complete blood count, serum iron, ferritin, and Ret-Hb levels. Hemoglobin, hematocrit, and Ret-Hb levels decreased as the disease progressed, though there was no significant difference among the groups. Notably, band neutrophils increased significantly in the advanced stages of MMVD, suggesting an association with disease progression and inflammation. This study concludes that while anemia and iron deficiency are not prominent in dogs with MMVD, the inflammatory response may exist as the disease progresses.

Keywords: reticulocytes, iron, heart disease, cardiopathy.

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LISTA DE ABREVIAÇÕES

ACVIM - Colégio Americano de Medicina Interna Veterinária/American College of Veterinary Internal Medicine

CHCM - concentração da hemoglobina corpuscular média

CHr - Conteúdo da Hemoglobina Reticulocitária/Reticulocity Hemoglobin Content

CKD - chronic kidney disease

DDVM - doença mixomatosa da valva mitral

EPO - eritropoietina

kg - Kilograma/Kilogram

MCH - Mean Corpuscular Hemoglobin

MCHC - Mean Corpuscular Hemoglobin Concentration

MCV - Mean Corpuscular Volume

mm - Milímetro/Milimeter

MMVD - Myxomatous Mitral Valve Disease

NT-Pro BNP - Pró-Hormônio Peptídeo Natriurético Cerebral

PCV - Packed Cell Volume

pg - Picograma/Picogram

RBCs - Red Blood Cells

Ret-Hb - Hemoglobina Reticulocitária/Reticulocity Hemoglobin

TIBC - capacidade de ligação do ferro/total iron biding capacity

VCM - volume corpuscular médio

CAPÍTULO I

1. INTRODUÇÃO

Na medicina humana a anemia é uma comorbidade frequente em pacientes com insuficiência cardíaca e comumente associada a piora clínica dos sintomas progredindo para a caquexia cardíaca. Adicionalmente, a anemia pode ser constatada em até 40% dos doentes cardíacos (Besarab, 2010; Sutil-Vega; Rizzo; Martínez-Rubio, 2019). Os pacientes humanos anêmicos com insuficiência cardíaca têm o dobro de chance de morrer em comparação aos não anêmicos e com insuficiência cardíaca (Van Veldhuisen et al., 2011).

Para o estabelecimento de parâmetros biológicos relacionáveis nos cães, em especial, aqueles relacionados com a anemia, destacamos a Doença Mixomatosa da Valva Mitral (DMVM) ou endocardiose. Esta cardiopatia é a de maior prevalência nos cães de pequeno porte (Keene et al., 2019), sendo desta forma um dos principais modelos de insuficiência cardíaca (IC).

A DMVM é uma doença progressiva que requer tratamento e acompanhamento de acordo com o estágio em que se encontra. O Colégio Americano de Medicina Interna Veterinária (ACVIM) publicou o consenso da DMVM em que classifica essa doença em quatro estágios que variam de acordo com a evolução. Estes estágios consideram desde predisposição racial até os pacientes refratários ao tratamento clínico (Keene et al., 2019).

O objetivo deste estudo foi avaliar os parâmetros hematológicos e os marcadores relacionados ao ferro em cães portadores da doença mixomatosa da valva mitral, em diferentes estágios da insuficiência cardíaca causada pela DMVM. Para tanto, em diferentes estágios da doença, foi quantificada a hemoglobina reticulocitária, o ferro sérico e a ferritina.

2. FUNDAMENTAÇÃO TEÓRICA

2.1 Morfofisiologia da doença mixomatosa da valva mitral

As valvas atrioventriculares, direita e esquerda, regulam o fluxo sanguíneo entre o átrio e o ventrículo correspondentes. A valva atrioventricular esquerda (valva mitral) é formada pelos folhetos anterior e posterior, o anel mitral, as cordas tendíneas e os músculos papilares. As valvas cardíacas são formadas por 3 camadas de tecido, evidenciando a complexidade anatômica dessas estruturas (Meurs et al., 2018; Alexis et al., 2023; Small et al., 2024). Na

medicina humana o tratamento existente para alterações valvares é cirúrgico, não havendo terapia medicamentosa para retardar a progressão da doença que na maioria dos casos é de cunho genético (Small et al., 2024).

Na veterinária, busca-se o gene responsável pela doença, pois em determinadas raças, como o Cavalier King Charles e o Dachshund a apresentação da doença tem claramente um componente familiar (Meurs et al., 2018). No entanto, alguns autores acreditam que a DMVM está relacionada a uma herança poligênica (O'Brien et al., 2021).

A doença mixomatosa da valva afeta principalmente a valva mitral, mas pode ocorrer em ambas as valvas atrioventriculares, e, muito mais raramente apenas a tricúspide (Keene et al., 2019). Na endocardiose há substituição da matriz extracelular por deposição de glicosaminoglicanos (GAG) e proteoglicanos que leva a formação do tecido mixomatoso que tem aparência espessa (Fox, 2012).

Devido a frequência dessa doença nos cães, o ACVIM a classificou em quatro estágios, como mostra o quadro abaixo, de acordo com a progressão e, ainda orienta quanto ao tratamento a partir do estágio B2 (Keene et al., 2019):

Quadro 1 – Estágios da Doença Mixomatosa da Valva Mitral.

Estágio		Características
A		Cães de raças pequenas predispostas como: Poodle, Dachshund, Cavalier King Charles
B	1	Cães assintomáticos.
	2	Cães assintomáticos, mas com remodelamento cardíaco. A partir desse estágio se inicia o tratamento. Cães no estágio B2 cumprem os critérios: - Relação átrio esquerdo-aorta > 1,6 - Diâmetro interno do ventrículo esquerdo em diástole normalizado pelo peso > 1,7
C		Apresentam sinais de insuficiência cardíaca
D		Pacientes refratários ao tratamento convencional

Fonte: Adaptado de Keene et al. (2019).

2.2 Exames cardiológicos

2.2.1 Eletrocardiograma

O eletrocardiograma (ECG) é um exame não invasivo que registra a atividade elétrica do coração nas fases do ciclo cardíaco. Os cães são mantidos em decúbito lateral direito com os membros paralelos à frente do corpo (Santilli et al., 2019; Ogawa et al., 2022).

Apesar de não ser o melhor método para avaliar tamanho das câmaras, o ECG pode sugerir o aumento das câmaras cardíacas, principalmente aquelas relacionas à DMVM: átrio e ventrículo esquerdos (Ogawa et al., 2022). No caso do átrio esquerdo, o ECG mostra aumento na duração da onda P que se refere a despolarização atrial e pode mostrar sobrecarga ventricular esquerda com aumento na amplitude da onda R ou aumento na duração do complexo QRS (Ogawa et al., 2022).

No estudo retrospectivo feito com 97 cães com estenose da valva pulmonar diagnosticada através de ecocardiograma oriundos de dois centros veterinários na Europa observou-se que a amplitude da onda P apresentou alta especificidade para estenose pulmonar severa, além de evidenciar que 6% dos cães com aumento de duração do complexo QRS tinham associado desvio do eixo a direita (Bini et al., 2022).

Outro estudo com 34 cães no grupo controle e 155 divididos em estágio B1 (42%), B2 (23%) e C (35%), segundo o consenso da DMVM, mostrou que os cães doentes tinham mais possibilidade de apresentar entalhe no QRS no eletrocardiograma quando comparados com cães que não tinham a doença, pois a fragmentação da onda R pôde ser justificada pelo remodelamento ventricular (Baisan et al., 2021).

2.2.2 Ecocardiograma

Atualmente, o padrão ouro para avaliação morfológica do coração é o ecocardiograma. Há diversas modalidades do ecocardiograma, sendo os mais rotineiros o modo bidimensional (modo B), em que a partir da imagem obtida integra-se outros componentes, o modo M, que gera um gráfico de movimentação temporal e o Doppler, cuja análise depende do ângulo de insonação e transforma a imagem obtida em gráficos. É importante haver o eletrocardiograma acoplado para ter certeza do momento correto de mensuração (Mitchell et al., 2019).

O exame ecocardiográfico gera inúmeras avaliações cardíacas. No entanto, para o diagnóstico da DMVM, há duas medidas fundamentais para o estadiamento da doença: a relação átrio esquerdo-aorta que sugere remodelamento atrial quando a relação é maior que 1,6. E, a segunda medida é o diâmetro interno do ventrículo esquerdo em diástole normalizado pelo peso que sugere o remodelamento ventricular a partir de 1,7 (Keene et al., 2019).

Outros parâmetros do exame fornecem subsídio para o acompanhamento ecocardiográfico seriado do paciente, como a onda L no fluxo transmitral, o grau de regurgitação mitral avaliado no Doppler colorido e mais recentemente o speckle tracking ou rastreamento por pontos (Morgan et al., 2020; Hamabe et al., 2021; Vereb et al., 2024)

2.2.3 Pro-Hormônio Peptídeo Natriurético Cerebral (NT-Pro BNP)

NT-Pro BnP é um hormônio neuroendócrino que é estimulado quando há estiramento dos miócitos, sendo usado como biomarcador cardíaco. O aumento nos valores do NT-Pro BNP indicam sobrecarga, uma consequência da DMVM (Zhao et al., 2022). O NT-Pro BNP é rotineiramente usado na medicina humana, para diferenciar dispneia cardiogênica de não cardiogênica, visto que esse biomarcador só está alterado na dispneia de origem cardíaca (Oyama et al., 2013).

O NT-Pro BNP, juntamente com outros biomarcadores cardíacos como a troponina I, foi avaliado em outros estudos indicando que cães com DMVM apresentavam os biomarcadores mais elevados que os cães sadios (Häggström et al., 2000; Chanmongkolpanit et al., 2024).

Esse biomarcador pode ser usado como método de triagem, reduzindo os custos para determinar a necessidade de realizar o ecocardiograma, conforme mostrou o estudo realizado com 119 Dobermanns que usou o NT-Pro BNP para determinar a necessidade de realizar o ecocardiograma para escanear cardiomiopatia dilatada (Dukes-Mcewan et al., 2022).

2.3 Anemia e seus biomarcadores

2.3.1 Anemia

A anemia é uma alteração hematológica importante na medicina humana e que afeta milhões de pacientes em todo mundo podendo apresentar sintomas como fadiga e palidez de mucosas. A ingestão reduzida de ferro ou sangramento gastrointestinal são as principais causas (Garcia-Casal et al., 2023). Tais sintomas podem levantar a suspeita de anemia, mas o diagnóstico laboratorial, que é feito através da mensuração da concentração da hemoglobina, é o padrão ouro (Newhall; Oliver; Lugthart, 2020; Garcia-Casal et al., 2023).

Na medicina veterinária, em especial nos cães, o sangramento gastrointestinal é a causa mais comum de anemia. Isso ocorre porque o consumo de ração industrializada fornece a quantidade de ferro necessária, fazendo com que essa não seja a principal causa de anemia nos animais de pequeno porte (Fuchs et al., 2017a).

A anemia pode ser diagnosticada principalmente pela concentração de hemoglobina, mas outros parâmetros subsidiam o diagnóstico como hematócrito, hemácias, volume corpuscular médio (VCM), contagem de reticulócitos (Garcia-Casal et al., 2023) e a hemoglobina reticulocitária (Campuzano-Maya & Guevara-Arismendy, 2015).

A principal função da hemoglobina é o transporte de oxigênio. Ela é formada de quatro subunidades (alfa e beta). Cada subunidade contém um grupo heme que tem o íon ferro na sua estrutura, sendo o responsável de fazer a ligação com o oxigênio (Safo et al., 2011).

Nos cães, as hemácias duram aproximadamente 100 dias, isso significa que até que o volume corpuscular médio (VCM) e a concentração da hemoglobina corpuscular média (CHCM) comecem a reduzir, um tempo considerado já passou. Os reticulócitos, por outro lado, circulam por dois dias até que amadurecem em eritrócitos. Por isso, avaliar a hemoglobina em células jovens, como os reticulócitos, fornece um diagnóstico precoce da falta ou ausência de disponibilização do ferro, podendo resultar na anemia (Fuchs et al., 2017b).

A anemia da doença inflamatória é outra causa comum de anemia tanto na medicina humana quanto na veterinária. Pode ser uma alteração discreta a moderada, e ocorre na presença de doenças crônicas como neoplasia, doença renal crônica, cardiopatias entre outras (Chikazawa & Dunning, 2016; Garcia-Casal et al., 2023).

Alguns fatores são importantes na anemia da doença inflamatória: ativação dos macrófagos que leva à fagocitose eritrocitária; inibição do metabolismo de ferro pela liberação de hepcidina que promove a retenção do ferro no interior das células que faz com que a síntese de hemoglobina na medula óssea seja prejudicada; e a secreção da eritropoietina que fica prejudicada pela ação de citocinas inflamatórias (Chikazawa & Dunning, 2016).

2.3.2 Reticulócitos

Os reticulócitos são rotineiramente usados para classificar as anemias em regenerativas, quando há aumento dos reticulócitos ou em arregenerativas, quando não há reticulócitos ou estão reduzidos. O padrão ouro para contagem reticulocitária é o manual que é feito no esfregaço de lâmina. No entanto, a contagem automatizada de reticulócitos já é uma realidade e mostrou boa correlação com a contagem manual (Cowgill; Neel; Grindem, 2003). Além disso, a citometria de fluxo vem ganhando cada vez mais espaço, pois tem melhor precisão e acurácia que os métodos manuais, além de não ter a variabilidade do observador que é encontrada no método manual (Chang et al., 2024).

2.3.3 Hemoglobina reticulocitária

A hemoglobina reticulocitária (Ret-Hb) apresentou sensibilidade de 93,3% e de especificidade de 83,2% para o diagnóstico da deficiência de ferro em diversos estudos em medicina humana (Campuzano-Maya; Guevara-Arismendy, 2015). Em um estudo que acompanhou 207 pacientes cardiopatas durante 21 meses, constatou-se que a maioria dos pacientes apresentou anemia (82%) e deficiência de ferro (65%), porém quanto menor o índice de Ret-Hb, pior o prognóstico (Tahara et al., 2022).

Na medicina veterinária o estudo retrospectivo multicêntrico apresentado em um congresso analisou os valores de mais de 10 mil amostras sanguíneas e mostrou alta sensibilidade e especificidade da Ret-Hb para a deficiência de ferro na eritropoiese. O valor de

corte para Ret-Hb foi de 20,9 pg, e os pesquisadores concluíram que a hemoglobina reticulocitária foi uma variável qualitativa para a eritropoiese deficiente em ferro (Fuchs et al., 2017a).

Em outro estudo retrospectivo que avaliou a amostra sanguínea de 833 cães demonstrou que a diminuição da Ret-Hb estava relacionada com a restrição de ferro na eritropoiese. Esse estudo também concluiu a alta sensibilidade e especificidade do Conteúdo da Hemoglobina reticulocitária (CHr), método diferente à obtenção da Ret-Hb, mas que correlaciona os mesmos achados (Steinberg; Olver, 2005).

No trabalho conduzido com cães observou-se que valores inferiores de CHr tinham aumento de ferritina e de CRP (proteína reativa - C) um marcador de inflamação, evidenciando a inflamação nesses animais. Pacientes com inflamação apresentaram mais dificuldade na disponibilização do ferro na eritropoiese, muito possivelmente pelo mecanismo da liberação da hepcidina, uma proteína ligada à regulação de ferro, mas ainda pouco estudada na veterinária (Radakovich; Santangelo; Olver, 2015).

Resultado semelhante foi obtido em um estudo em humanos que comparou a redução da Ret-Hb em indivíduos com deficiência de ferro com o grupo controle. Concluiu-se que a hemoglobina reticulocitária não sofreu influência dos fatores que afetaram a ferritina, como por exemplo a inflamação. A deficiência que a hemoglobina reticulocitária apresentou foi devido a biodisponibilidade do ferro na síntese da hemoglobina das células jovens, isto é, os reticulócitos (Aedh et al., 2023).

Por fim, no estudo que avaliou 182 pacientes, verificou-se que a Ret-Hb além de ser um marcador para o diagnóstico da deficiência de ferro, também foi sensível para avaliar a eficácia do uso oral de ferro no curto prazo. Nesse estudo, os pacientes que receberam 190 mg de sulfato ferroso por dia tiveram um aumento de 7,5% de hemoglobina reticulocitária e de 1,3% de hemoglobina no intervalo de uma semana, evidenciando a eficácia da Ret-Hb como marcador da resposta ao tratamento (Almashjary et al., 2022). Quando a suplementação de ferro por via oral é usada, deve-se reavaliar a hemoglobina dentro de um mês, e quando os valores estiverem dentro normalidade o tratamento ainda deve continuar por três meses (Newhall; Oliver; Lugthart, 2020). Por outro lado, o consenso europeu recomenda o uso de ferro intravenoso em pacientes com insuficiência cardíaca pois há melhora da capacidade de atividade física e da qualidade de vida (McDonagh et al., 2021). Quando a administração de ferro é por via intravenosa, deve ser feita em sistema ambulatorial, pois há maior risco de reação de hipersensibilidade (Newhall et al., 2020).

2.3.4 Ferro

O ferro no organismo se regula como um sistema fechado, em que a ingesta deste elemento na dieta deve suprir as perdas diárias. Uma vez no interior dos enterócitos, o ferro pode trilhar por dois caminhos: se unir à ferritina para permanecer armazenado no interior da célula ou ser liberado pela proteína transmembrana ferroportina para o plasma, onde o ferro se une à transferrina que faz o seu transporte. Os macrófagos podem capturar o ferro e armazená-lo na forma de ferritina, e, quando necessário, liberá-lo novamente ao plasma via ferroportina. Os macrófagos também são os responsáveis pela reciclagem do ferro dos reticulócitos danificados ou senis (Campuzano-Maya; Guevara-Arismendy, 2015).

A transferrina também é a responsável por transportar o ferro para a medula óssea para fazer parte do grupo heme necessário para a produção de hemoglobina dos eritrócitos. Os hepatócitos desempenham importante papel na reserva de ferro, pois captam o ferro unido à transferrina através de dois receptores de membrana, sendo o receptor de transferrina II (TfRII) usado na avaliação da saturação da transferrina (Campuzano-Maya; Guevara-Arismendy, 2015).

A deficiência do ferro no organismo tem como principal característica a anemia, que pode ocorrer por falta absoluta do ferro, ou seja, não há este elemento nos depósitos, ou ainda a anemia por deficiência funcional do ferro. Na segunda causa, a medula óssea demanda mais ferro que o organismo consegue suprir, sendo frequente nas doenças crônicas, infecciosas, inflamatórias e neoplásicas. Nesses casos, os níveis de ferritina sérica se encontram dentro dos valores de referência evidenciando que a reserva de ferro é adequada, contudo não sendo disponibilizado para eritropoiese (Campuzano-Maya; Guevara-Arismendy, 2015).

Na anemia por doença inflamatória os níveis de ferro que deveriam ser disponibilizados para a eritropoiese se encontram abaixo do valor de referência, embora a capacidade de ligação do ferro se mostre aumentada e a ferritina dentro do valor de referência normal ou até mesmo aumentada (Fuchs et al., 2017b).

2.3.5 Hepcidina

Como o ferro não é excretado do organismo, cabe ao hormônio hepcidina inibir a mobilização do ferro dos sítios de armazenamento e a sua absorção pelos enterócitos. Esse hormônio, produzido pelo fígado, tem sua regulação bastante complexa envolvendo vários fatores. Na anemia inflamatória, a hepcidina tem seus níveis plasmáticos elevados reduzindo a absorção de ferro, assim como sua disponibilização pelo sistema monocítico fagocitário (Campuzano-Maya; Guevara-Arismendy, 2015; Fuchs et al., 2017a).

Pacientes da medicina humana com insuficiência cardíaca ou outros processos

inflamatórios têm deficiência de ferro no organismo. Isso ocorre porque citocinas inflamatórias interferem na absorção e biodisponibilidade do ferro levando a deficiência funcional e não uma anemia *per se* (Van Veldhuisen et al., 2011). A hepcidina funciona como mediador na anemia da inflação, pois se liga a ferroportina, ambos são endocitados para o interior da célula ou do macrófago, e, então a ferroportina é degradada no interior, e o ferro fica impedido de sair das células (Chikazawa & Dunning, 2016).

A transcrição da hepcidina é regulada pela interleucina-6 (IL-6), que é liberada por vários tipos celulares, entre os quais as células Kupffer, que desempenham papel imunológico, evidenciando o aumento desse biomarcador durante a inflamação (Roth; Meynard; Coppin, 2019). Por outro lado, em situações de hipóxia, há a liberação de eritropoietina (EPO) para aumentar a produção de hemácias pela medula óssea. A EPO suprime a hepcidina indiretamente através de outra proteína, a eritroferrona (Roth; Meynard; Coppin, 2019; Nemeth; Ganz, 2023).

No estudo conduzido com 86 cães saudáveis de ambos os sexos, a concentração de hepcidina foi a mesma independente de sexo, idade e raça dos cães (Vizi et al., 2023). Esse estudo detectou valores basais de hepcidina no intervalo entre 2,3 a 41,1 ng/ml (Vizi et al., 2020). O mesmo grupo de pesquisadores demonstrou que os cães produziram dois tipos de hepcidina (hepcidina-25α e hepcidina-25β) e ambas ficaram elevadas em inflamação aguda ou crônica (Vizi et al., 2023).

2.3.6 Ferritina

A ferritina é uma proteína que armazena o ferro, sendo encontrada principalmente no fígado. Por outro lado, a ferritina também funciona como uma proteína inflamatória aumentando sua concentração frente a infecção, doenças inflamatórias ou crônicas. O aumento nos valores de ferritina podem significar excesso de ferro, inflamação ou ambas as situações (Garcia-Casal et al., 2021).

A mensuração sérica da ferritina corresponde à quantidade de ferritina encontrada no sangue, mas não informa a quantidade de ferro armazenada em seu interior. Diante disso, os resultados da ferritina sérica para análise de anemia devem ser avaliados com cautela (Plays; Müller; Rodriguez, 2021).

Na medicina humana, durante a pandemia de COVID-19, inúmeros pacientes vieram a óbito por uma intensa resposta inflamatória causada por uma tempestade de citocinas. A ferritina era um dos biomarcadores relacionados a esta importante resposta inflamatória (Plays; Müller; Rodriguez, 2021)

Em 28 cães divididos em 3 grupos (saudáveis, tumor hepático maligno e tumor hepático benigno) avaliou o uso da dosagem da ferritina e da elastografia para diferenciar tumor hepático

benigno do tumor maligno (Huaijantug et al., 2020). Os dados obtidos foram comparados com a biópsia hepática. Concluiu-se que cães com tumor maligno tinham hiperferritinemia mais acentuada que os cães com tumor benigno evidenciando a relação entre a ferritina e doenças crônica como o câncer (Huaijantug et al., 2020).

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CAPÍTULO II

Model of Submission: Research Article, Veterinary Medicine International (Anexo A)

Title of the manuscript: Myxomatous Mitral Valve Disease In Dogs: Should We Worry About Anemia?

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MYXOMATOUS MITRAL VALVE DISEASE IN DOGS: SHOULD WE WORRY ABOUT ANEMIA?

Abstract

Anemia is a common comorbidity in human patients with heart failure and is related to worsening outcomes. This study aimed to evaluate hematologic parameters and iron-related markers in dogs with Myxomatous Mitral Valve Disease (MMVD) and their relationship with the different stages of the disease. The disease is classified in four different stages (A, B, C and D) according to its progression, although stage B is divided in B1 and B2. One hundred twenty-one dogs aged 5 to 17 were prospectively selected and staged for MMVD for nine months during routine attendance at the University of Brasília Veterinary Hospital. The Kruskal-Wallis test was applied, with a significance level of 5%. The results present a decrease in the mean value of hematological parameters of these dogs as the disease progresses; although no significant value: red blood cells (RBCs) (group A - 7,5 µ³; group B1 - 7,4 µ³; group B2 - 7,2 µ³; group C - 6,9 µ³; D - 7,0 µ³), hemoglobin (group A - 16,7 g/dL; group B1 - 16,4 g/dL; group B2 -15,8 g/dL; group C - 15,4 g/dL; group D - 14,8 g/dL), hematocrit (group A - 52,9%; group B1 - 52,2%; group B2 - 50,5%; group C - 49,7%; group D - 47,4%), Reticulocity hemoglobin (Ret-Hb) (group A - 22,6 pg; group B -: 22,6 pg; group B2 - 22,8 pg; group C - 22,4 pg; group D - 22,2 pg), serum iron (group A -153,2; group B1 - 172,9; group B2 - 158,6; group C - 158,7; group D - 125,7), and ferritin (group A - 181,0; group B1- 171,4; group B2 - 178,0; group C - 185,6; group D -184,8). Interestingly, band neutrophils had a statistically significant difference, a 5% significance level (group A - 23,8; group B1 - 14,1; group B2 - 29,2; group C - 174,0; group D - 214,8). Dogs do not have anemia; however, they present a decrease in all hematologic parameters as the disease progresses.

1. Introduction

In human medicine, anemia is a frequent comorbidity in patients with heart failure (1,2). It is commonly associated with clinical worsening of symptoms progressing to cardiac cachexia, and it may be diagnosed in up to 40% of the patients (1,2). Anemic patients with heart failure are twice as likely to die compared to non-anemic patients with heart failure, according to a meta-analysis that evaluated more than 150,000 human patients (3).

In veterinary medicine, the most common laboratory tests used to diagnose anemia are hematological parameters like hemoglobin, packed cell volume, erythrocyte, mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC), along with clinical

parameters (4,5). Nevertheless, other exams, like iron plasma and ferritin, can assess anemia, which shows iron stores (6). Recently, reticulocyte hemoglobin, another biomarker, has been used. It diagnoses iron deficiency outperforming the other traditional biomarkers with high sensitivity and specificity (7). Due to reticulocytes circulating for up to two days before maturing into erythrocytes, measurement of Ret-Hb provides an indirect analysis of the functional iron available for new red blood cell production over the previous 3–4 days (8).

The Myxomatous Mitral Valve Disease is the most common acquired heart disease in dogs. Its prevalence in small-breed dogs made the American College of Veterinary Internal Medicine (ACVIM) publish a consensus guidelines statement in 2019 concerning diagnosis and treatment. MMVD, or endocardiosis, is a progressive disease that can lead to heart failure in the late stage (9). Because of the prevalence of MMVD in dogs, it can be used as a model of heart failure in this specie.

This study aimed to evaluate hematologic parameters and iron-related markers in dogs with myxomatous mitral valve disease (MMVD) and their relationship with the different stages. Specifically, it investigated the presence of anemia and quantified reticulocyte hemoglobin in dogs through blood count.

2. Materials and methods

A total of 132 dogs of various breeds, ages, weights, and sexes were evaluated for the study. Inclusion criteria required a maximum weight of 15 kg and a minimum age of 5 years. All animals had detailed anamnesis, complete physical examination, and cardiac evaluation, including measurement of blood pressure (BP Scan, Inpulse) according to ACVIM guidelines about hypertension in dogs and cats (10), a 12-lead electrocardiography exam (INCardio X, Inpulse) performed as proposed by Santilli (11) and transthoracic echocardiogram (Vivid IQ, GE). The echocardiogram, electrocardiogram, and detailed anamnesis were essential for case delineation, inclusion criteria, and accurate diagnosis. The Animal Research Ethics Committee of the University of Brasília reviewed and approved all studies and procedures under protocol number 23106.064359/2023-27.

Dogs were diagnosed with Myxomatous Mitral Valve Disease according to the ACVIM guidelines classification for this disease through an echocardiogram [9] and measurements following a previous study (12). All echocardiograms were performed with a simultaneously coupled electrocardiogram to ensure the exact moment of heartbeat to be measured. We considered stage A of ACVIM guidelines for dogs with no mitral valve disease independent of the breed, as many animals were mixed breeds. Other stages strictly followed ACVIM

guidelines (9): Stages B1 dogs with no signs of heart enlargement, B2 dogs with cardiac remodeling, C dogs with signs of heart failure, and D dogs refractory to standard treatment.

Blood samples were collected using three different tubes. One tube in an ethylenediamine tetraacetic acid dipotassium salt (EDTA-2K) for complete blood count and reticulocyte profile using an automated hematology analyzer (Mindray BC-780), one tube for iron biomarkers (serum ferritin, and serum iron) analyzed in Cobas C311 (Roche). Both tubes were examined in an external laboratory (Santé Laboratório). The third tube was for the biochemistry profile (ALT, AST, Urea, Creatinine, Protein, Albumin, and Globulin), which was analyzed in Cobas C111 at the clinical pathology laboratory at the university. The samples analyzed at the university were used as exclusion criteria because animals with high liver or renal enzymes and high serum proteins were excluded from the study. The samples evaluated at the external laboratory were used to analyze anemia.

One hundred twenty-one dogs met the inclusion criteria and had their results compiled. Eleven dogs were excluded from the study: three had chronic kidney disease (CKD), two were in treatment for ehrlichiosis, two had leishmaniosis, two were diagnosed with neoplasia, and two had higher liver enzymes.

The data obtained were entered into a Microsoft Excel 2010 spreadsheet and statistically analyzed using Statistica 10.0 software. Shapiro-Wilk normality test was performed on the numerical variables to identify the data distribution. Since most data did not present normality, nonparametric tests were used to compare the groups. The Kruskal-Wallis test compared the groups considering the numerical variables presented through mean and standard deviation. The significance level adopted in the tests was 5%.

3. Results and discussion

Data from 121 animals were evaluated and distributed in 5 groups according to the stage of MMVD. Forty-nine were female, and seventy-two were male. Dogs were, on average, 10 ±3 years old. Table 1 shows the distribution of Dog Breeds by Count and Percentage:

Thirty-two of the dogs, 26.4% were in group A with an average weight of 7.7±2.8 kg, group B1 with 41.3% (n=50) of the animals had an average of 7.8±2.7 kg, 16.5% (n=20) in group B2 with an average weight of 7.4±2.4 kg. The average weight in group C was 7±3.6 with 10.7% (n=13), while 5.0% (n=6) of group D had an average of 6.2±3.1 kg.

Table 1 –.Dog distribution according to breed, sex, age and weight (Mean±Standard Deviation).

Breed	Number	Sex		Age	Weight
		Male	Female		
Chihuahua	1	1	-	11	5.5
Dachshund	7	2	5	8.3±3.0	9.5±1.3
French Bulldog	3	2	1	6±0.6	11.5±0.8
Japanese Spaniel	1	1	-	9	4.6
Lhasa Apso	9	5	4	10.1±2.3	9.8±2.7
Maltese	6	2	4	9.2±2.3	4.5±1.6
Miniature Pinscher	7	6	1	12.3±3.2	3.7±0.8
Mixed Breed	22	14	8	9.9±3.0	9±2.3
Pekingese	3	2	1	8.7±1.5	6.8±1.6
Podengo	1	-	1	7	7
Poodle	3	2	1	10.7±5.1	7.8±1.2
Pug	1	1	-	8	14.3
Samoieda	1	-	1	13	15
Schnauzer	3	-	3	10±2.6	7.7±1.5
Shih Tzu	35	22	13	10.3±3.1	7.5±1.9
Spitz	2	1	1	6	5.4±1.3
Yorkshire	16	11	5	10.9±2.8	5.3±1.7

M (male), F (female). Values of age and weight are in average values.

Hematological parameters (Table 2) commonly used to diagnose anemia, such as red blood cells, hemoglobin, hematocrit, MCV, mean corpuscular hemoglobin (MCH), and MCHC, were all within the reference interval for the species. The additional parameters constituting the study, Ret-Hb and serum iron levels, decrease the mean value as the disease progresses.

Table 2 - Hematological Parameters (Mean±Standard Deviation) in dogs with Myxomatous Mitral Valve Disease at different stages (groups - A, B1, B2, C, and D). The Kruskal-Wallis test was applied, with a significance level of 5%.

Parameters	**Groups**					**Reference value**	***p***
	A n = 32	B1 n = 50	B2 n = 20	C n = 13	D n = 6		
RBC (µ³)	7.5±0.8	7.4±0.9	7.2±0.7	6.9±1.5	7±0.9	5.5-8.5	0.1977
Hemoglobin (g/dL)	16.7±1.8	16.4±1.8	15.8±1.6	15.4±3.2	14.8±2	12-18	0.0878
Hematocrit (%)	52.9±5.3	52.2±6.1	50.5±5	49.7±10.7	47.4±7	37-55	0.1492
Ret-Hb (pg)	22.6±1	22.6±0.8	22.8±1.3	22.4±1.2	22.2±1.4	22.2-28.6	0.6531
MCV (fL)	70.7±3.6	70.4±3	70.5±3.3	72±2.7	67.8±3.9	60-77	0.2447
MCH (pg)	22.3±1.1	22.2±0.9	22.1±1.2	22.3±0.8	21.2±0.8	19-23	0.1905
MCHC (g/dL)	31.5±0.8	31.5±0.7	31.3±0.6	31±0.9	31.4±0.7	31-37	0.3151
RDW (%)	13.9±0.8	14.2±0.8	13.9±0.7	14.2±1.5	15.4±1.4	12-15	0.1181
WBC (mm³)	10045±2956.8	9506.6±2775.1	10619±4293.3	12071.5±4748.3	14815±6465.5	6000-17000	0.0691
Segmented (mm³)	7228±2781.2	6877.5±2274.3	7654.2±3226.3	8727.9±3849.5	10121.3±4579.8	3600-13090	0.2116
Band (mm³)	23.8±51.8	14.1±45.3	29.2±63.5	174±253.1	214.8±526.2	0-510	0.0096
Eosinophil (mm³)	398.8±361.9	455.0±498.8	583.3±507.3	331.5±351.8	800.2±863.8	0-1700	0.4634
Lymphocyte (mm³)	1993.7±1123	1884.4±948.4	1897.9±1260.7	2260.5±11221.1	3096.5±2461.2	720-5100	0.7806
Monocyte (mm³)	335.5±276.7	265±217.7	363.7±265.7	542.8±370.4	582.2±347.1	0-1700	0.1060
Platelets (mm³)	355625±111125.5	394369±104403,6	390800±155257,5	408384,6±206470,2	496833,3±214221,8	175000-500000	0,7219
Plasma protein (g/dL)	7.6±0.6	7.8±0.8	7.6±0.6	7.9±1.1	6.8±1.2	6-8	0.2909
Serum iron (mcg/dL)	153.2±46.4	172.9±58.4	158.6±41.9	158.7±79.3	125.7±38	30-180	0.3854
Ferritin (µg/L)	181±56.2	171.4±55	178±68.1	185.6±48.2	184.8±25.3	80-800	0.7072

RBC (red blood cell), µ³ (cubic micrometers), p (significant level), g/dL (grams per deciliter), Ret-Hb (reticulocyte hemoglobin), pg (picograms), MCV (mean corpuscular volume), fL (femtoliter), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), g/dL (grams per deciliter), RDW (red blood cell distribution width), WBC (white blood cell), mm³ (cubic millimeter), mcg/dL (micrograms per deciliter), and µg/L (microgram per liter).

The decrease in these parameters might occur due to inflammatory cytokines such as TNF-α, interleukins, and, more recently, hepcidin, all of which play a crucial role in cardiac dysfunction (13,14).

Ferritin, in contrast, exhibited a different trend, with mean values increasing in the advanced stages of MMVD, which is expected due to the inflammatory aspect of the disease. In human medicine, ferritin is a biomarker in inflammatory diseases such as neoplasia, chronic kidney disease, and chronic heart failure. In these diseases, ferritin reduction assesses treatment efficacy in high-risk patients (6).

Iron deficiency is reflected when Ret-Hb values are lower than 20.9 pg (15). The lowest value for Ret-Hb in this study was 20.8 pg in stage D, showing that iron deficiency can occur in the late stages of this disease. Because iron is essential for hemoglobin synthesis, anemia typically becomes apparent only in the later stages of chronic disease. This delayed onset of anemia is associated with functional iron deficiency, a condition in which iron stores are adequate, but the bioavailability of iron for heme synthesis is reduced (15,16).

A comparative study (17) evaluated reticulocyte counts in dogs using manual and automated methods, finding that reticulocyte levels appeared elevated when assessed manually but not when measured using a computerized process. On the other hand, another study (18) compared 86 samples of human blood samples using both methods: manually in a new methylene blue-stained smears and an automated process using a hematology analyzer Sysmex XT-2000i to count reticulocytes, and they found no significant statistics. The same conclusion was found in a previous study (18) that compared two different analyzers with manual blood counting from 50 healthy patients and 80 patients affected by various diseases and conditions. The present study analyzed reticulocytes only automatically using BC-780, which analyses through laser flow cytometry. The study (19) performed with the same equipment concluded that the results were in alignment with the Westergren method, the reference method according to the International Council for Standardization in Hematology.

All groups in the present study had normal serum iron, even in advanced disease, as shown in Table 2. Although serum iron is within the standard reference, there is a slight decrease in stage D of MMVD. A study evidenced similar results, although they relate to iron deficiency of 12.5%, showing that a reduction of iron levels can occur without accompanying anemia. However, there was no statistically significant difference among the groups (17), which is consistent with the present study.

Another study identified low serum iron concentration as a potential risk factor for the progression of MMVD in dogs (20). In that study, 18% of the 54 dogs exhibited low serum iron

levels. However, the authors included dogs with chronic kidney disease, which may have contributed to an increased prevalence of low iron in the referenced study. In this study, dogs with CKD were excluded.

Iron lowering is a concern in human medicine because it is linked to a worse prognosis, which is why supplementation is recommended by the European Society of Cardiology in their guideline published in 2021 for diagnosis and treatment of acute and chronic heart failure (21). No dogs in the present study needed iron supplementation.

Ferritin, an indicator of iron storage, was analyzed, and the average of each group was within the reference interval. However, ferritin can serve as an acute inflammatory biomarker, meaning its level increases in response to inflammation. Therefore, using ferritin as a marker of iron status is only reliable in healthy patients (6). Our findings are consistent with a study that evaluated different laboratory techniques to assess iron deficiency in dogs with MMVD (17). This study also found no statistically significant difference in ferritin levels among the groups of dogs.

Although within the reference interval for the species, results showed a difference in the band neutrophils ($p=0.0096$) as the disease progressed. This may be justified because of inflammatory cytokines during severe illness. It is well-established that leukocyte migration occurs in response to molecular events that signal injury or inflammation (22). In the present study, an increase in band neutrophils was observed in dogs with stage C of the disease, rising from a mean of 29.2 mm^3 in stage B2 to 174 mm^3 in stage C, an increase of six times. Patients in stage D showed an even higher level of band neutrophils, with a mean of 214.8 mm^3.

In human medicine, inflammation is related to cardiovascular risk factors (23,24). Neutrophils, and, on some occasion bands, have been implicated in cardiac disease. It has already been demonstrated that there is a link between band neutrophils and ST-Elevation Myocardial Infarction (STEMI), a type of heart attack that is more serious (23).

4. Conclusions

Because mitral valve disease requires periodic monitoring mainly through echocardiography, regularly assessing those hematological parameters alongside echocardiograph re-evaluations is essential, even when hematological values remain within the reference range. Tracking them over time provides valuable insights into the progression of MMVD, which can help veterinarians better understand the disease's evolution, even when iron supplementation is unnecessary to correct anemia.

Although Ret-Hb is stable for up to 48 hours, all samples were analyzed on the same day. However, related to the sample analyzed at the external laboratory, the storage temperature during the transport and movement during transportation could have led to hemolysis, which was observed in some samples.

The present study did not assess additional anemic indicators such as transferrin saturation (%SAT), total iron-binding capacity (TIBC), or hepcidin levels, which could have provided a more comprehensive evaluation of anemia.

This study highlights the importance of paying close attention to the hematological profile, particularly anemia parameters. We conclude that dogs do not have anemia *per se*; still, they present a decrease in all hematologic parameters as the disease progresses.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors declare no conflict of interest.

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CONSIDERAÇÕES FINAIS

Nos cães avaliados, a utilização da hemoglobina reticulocitária como um possível biomarcador para o diagnóstico de deficiência de ferro, não se mostrou promissora. Os parâmetros hematológicos utilizados rotineiramente, como hemoglobina e hematócrito atendem de maneira satisfatória as alterações associadas a ferropenia.

Nesse contexto, apesar dos parâmetros hematológicos, como hemoglobina, hematócrito e o ferro sérico terem apresentado diminuição progressiva com o avanço da doença, podem sugerir que outros fatores atuam para manter o nível sérico de ferro. Em cães, a dieta parece contribuir para o fornecimento adequado do ferro, uma vez que a grande maioria dos cães ingerem ração comercial como base da alimentação. Isso pode explicar os valores de ferro mesmo com a progressão da doença.

O discreto aumento da ferritina no curso da doença, indica o estado inflamatório, mas não havendo associação com a deficiência de ferro. O estado inflamatório do organismo é reforçado pelo aumento dos leucócitos e bastonetes. Destacando relação da inflamação crônica e disfunção cardíaca.

Esses achados sugerem a importância do acompanhamento dos parâmetros hematológicos em cães com DMVM, pois a inflamação pode desempenhar um papel coadjuvante na progressão da doença. Portando, o monitoramento regular desses indicadores pode ser uma ferramenta valiosa para o manejo clínico desses pacientes, contribuindo para o entendimento da patofisiologia da doença e auxiliando na implementação de condutas terapêuticas adequadas.

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