UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA VETERINÁRIA

RETROSPECTIVE ANALYSIS OF 191 DOGS WITH MYXOMATOUS MITRAL VALVE DISEASE BASED ON TOMAZZO ET AL.'S MITRAL INSUFFICIENCY ECHOCARDIOGRAPHIC SCORE

ANDRÉ AVELINO SILVA SANTOS

ORIENTADORA:

DOUTORA ESMERALDA SOFIA DA COSTA DELGADO

CO-ORIENTADOR:

DOUTOR FRANCESCO BIRETTONI

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Resumo ANÁLISE RETROSPETIVA DE 191 CÃES COM DOENÇA MIXOMATOSA DA VÁLVULA MITRAL BASEADA NO MINE SCORE CRIADO POR TOMAZZO ET AL'.

A doença mixomatosa da válvula mitral (DMVM) pode desenvolver-se em cães adultos de qualquer idade, mas a sua prevalência aumenta com a idade, acometendo até 85% dos cães que atingem os 13 anos de idade afetados pela doença.

Não existe um sistema de classificação ecocardiográfica estandardizado e de uso universal, baseado em variáveis ecocardiográficas adquiridas por rotina para classificar a DMVM. O "MINE score" foi proposto por Tomazzo Vezzosi et al. para preencher esta lacuna.

O nosso estudo, de natureza restropetiva teve como objetivo avaliar a reprodutibilidade do "MINE score". Foram incluídos 191 cães com DMVM avaliados ecocardiograficamente no Hospital Veterinário Universitário da "Università Degli Studi di Perugia" entre Setembro de 2017 e Dezembro de 2021. O sistema de classificação foi aplicado e os resultados obtidos comparados com os do estudo "MINE score" original. A contribuição independente das variáveis ecocardiográficas do "MINE score" (rácio LA/Ao, LVIDDn, FS% e E-vel) para diferenciar a severidade da DMVM entre cães do mesmo grupo ACVIM foi avaliada por meio de testes paramétricos para um nível de significância de 0,05.

A amostra teve um rácio macho: fêmea de 1,85, idades compreendidas entre os 4 e os 20 anos (11,3 ± 2,9), peso corporal médio de 7,9 ± 3,7kg e incluiu cães de 18 raças e de raça indefinida.

Foram criados nove subgrupos, distribuindo-se os cães de acordo com a respetiva classificação ACVIM (B1, B2 ou C/D) e as classes de gravidade do "MINE score" (estádio leve, moderado, grave e tardio). Assim, 29% dos cães (n=55) foram classificados como B1 leve, 9% (n=18) como B1 moderado, 2% (n=4) como B2 leve, 9% (n=17) como B2 moderado, 18% (n=35) como B2 grave, 3% (n=5) como B2 em estágio avançado, 1% (n=1) C/D moderado, 18% (n=35) como C/D grave e 11 % (n=21) como estágio tardio de C/D. Na diferenciação de cães B1, contribuições significativas foram dadas pelo rácio LA/Ao (<0,0001), LVIDDn (P-valor <0,05) e o FS% (P-valor de 0,0020). Em cães B2, apenas o rácio LA/Ao (P-valor <0,05) contribuiu em todos os subgrupos. Em cães C/D contribuíram o racio LA/Ao (0,0002), LVIDn (P = 0,0081) e E-vel (P= 0,01).

Todas as variáveis ecocardiográficas contribuíram significativamente para diferenciar a severidade ecocardiográfica em algum dos estágios ACVIM da doença. Mesmo assim, questionou-se o papel global do FS% no "MINE score" e sua especificidade na diferenciação da severidade ecocardiográfica em cães com DMVM. Consequentemente, propomos que sejam realizados estudos subsequentes, substituindo-se o FS% pelo LVDIs normalizado para o peso corporal, potencialmente melhorando a precisão do "MINE score.

Palavras-chave: Doença Mixomatosa da válvula mitral, Insuficiência cardíaca, Cão, Ecocardiografia

Abstract - RETROSPECTIVE ANALYSIS OF 191 DOGS WITH MYXOMATOUS MITRAL VALVE DISEASE BASED ON TOMAZZO ET AL.'S MITRAL INSUFFICIENCY ECHOCARDIOGRAPHIC SCORE

Mitral Myxomatous Valve Disease (MMVD) can develop in adult dogs of any age, but its prevalence increases with age, with up to 85% of dogs reaching 13 years old affected. There is no commonly shared severity score for MMVD based on routinely acquired echocardiographic variables, with the MINE score being proposed by Tomazzo Vezzosi et al. to fill this gap.

This study intended to assess the reproducibility of the MINE score in a different veterinary setup. A retrospective study was conducted, including 191 dogs with MMVD echocardiographically, evaluated at the Veterinary Teaching Hospital of the "Università Degli Studi di Perugia" between September 2017 and December 2021. The MINE score was applied, and the scores obtained were compared with those gathered for the original MINE score study. The contribution of the MINE score echocardiographic variables (LA/Ao ratio, LVIDDn, FS%, and E-vel) to differentiate the echocardiographic severity of the MMVD in dogs from the same ACVIM group was independently evaluated through parametric tests with $p<0,05$ testing for significance.

The studied sample had a male: female ratio of 1,85, with an age range from 4 to 20 years (11,3 ± 2,9) and an average bodyweight of 7,9 ± 3,7kg and dogs from 18 dog breeds or of mixed breed.

Nine subgroups were created by distributing the dogs by ACVIM (American College of Veterinary Internal Medicine) classification B1, B2 and C/D and the MINE score severity classes (mild, moderate, severe, and late-stage). Accordingly, 29% of dogs (n=55) were classified as B1 mild, 9% (n=18) as B1 Moderate, 2% (n=4) as B2 mild, 9% (n=17) as B2 moderate, 18% (n=35) as B2 severe, 3% (n=5) as B2 late-stage, 1% (n=1) C/D moderate, 18% (n=35) as C/D severe and 11% (n=21) as C/D late-stage.

In B1 dogs' differentiation, the contribution was obtained from the LA/Ao ratio (<0,0001), LVIDDn ($P <0,05$) and the FS% ($P=0,0020$). For the severity differentiation of B2 dogs, only the LA/Ao ($P <0,05$) contributed to all subgroups. In C/D dogs the variables contributing were the LA/Ao (0,0002), LVIDDn ($P=0,0081$) and E-vel ($P=0,01$).

All echocardiographic variables significantly contributed to differentiating dog's severity at some ACVIM stage of the disease. Even so, the overall fractional shortening (FS%) role in the MINE score and its specificity at classifying dogs by echocardiographic severity was questioned. Accordingly, we propose further studies based on replacing the FS% for the LVDIs normalized for BW. With those, we intend to fix the ambiguity created by the FS% and enhance the scoring system's accuracy of the MINE score, to which we recognize a remarkable potential.

KEYWORDS: Myxomatous Mitral Valve Disease, Heart Failure, Echocardiography, Dog

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- 2D – Two dimensional
- 3D – Three dimensional
- AV – Atrioventricular
- ACEI - Angiotensin-converting enzyme Inibitor
- AF – Atrial fibrillation
- Ao – Aorta
- A-vel- late diastolic transmitral flow velocity
- BID - Twice a day
- BUN – Blood urea nitrogen
- BW – Body weight
- CFD – Colour flow Doppler
- CKCS - Cavalier King Charles Spaniels
- CS – Clinical Signs
- CT – Computer tomography
- CHF – Cardiac heart failure
- cTnI - Cardiac troponin I
- CRI – Constant rate infusion
- CWD - Continuous-wave Doppler
- ECG – Electrocardiogram /Electrocardiography
- ECM – Extracellular Matrix
- E/IVRT - The ratio between transmitral E velocity and isovolumetric relaxation time
- E-vel - early diastolic transmitral flow velocity
- FS – Fractional shortening
- IC – Intercostal space
- IGF - Insulin-like growth factor
- IV – intravenous
- IVRT - isovolumetric relaxation time
- LA – Left atrium or left atrial
- LAp - Left atrial pressure (s)
- LL - Lateral
- LV – Left ventricle/ Left ventricular

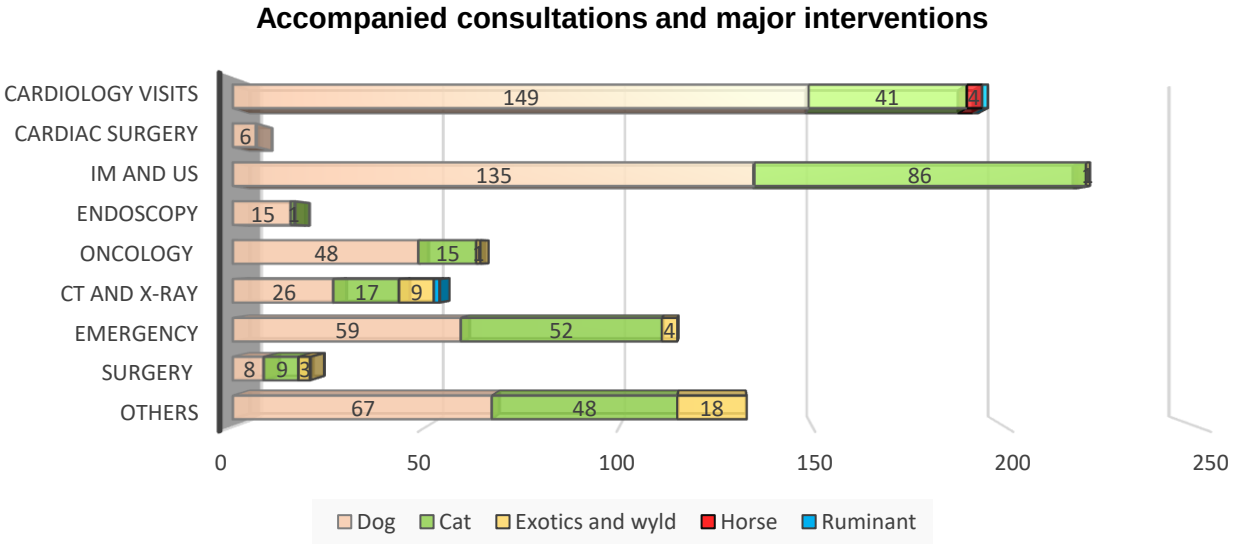
- LVIDDn - Left ventricular internal dimension in diastole, normalized for body weight
- MM – Mucous membranes
- mmHg – Millimeters of mercury
- MMVD – Mitral myxomatous valve disease
- M-mode – One-dimensional time-motion mode
- MR – Mitral regurgitation
- MV – Mitral valve
- m/s – Meters per second
- NT-proBNP - N-terminal pro-B-type natriuretic peptide molecule
- OMSS – Original MINE score study
- PAP - Pulmonary vascular resistance
- PCV - packed cell volume
- PDA - Patent-ductus-arteriosus
- PE – Pulmonary oedema
- PH – Pulmonary hypertension
- PMI - Point of maximal intensity
- PO – Orally
- PVR - Post and precapillary PH simultaneously
- PWD - Pulsed wave Doppler
- RA- Right atrium/right atrial
- RDW - Red blood cell distribution width
- RNA/RNAs - Ribonucleic acid/Ribonucleic acids
- RT2DE - Real-time two-dimensional echocardiography
- RT3DE – Real-time three-dimensional echocardiography
- RPS – Right Parasternal
- RV – Right ventricle/ right ventricular
- SABP - systolic arterial blood pressure
- SID – Once daily
- TID - Three times per day
- TGFβ - Transforming growth factor-beta
- TR – Tricuspid regurgitation
- TV -Tricuspid valve
- US – Ultrasound
- VICs - Valvular interstitial cells
- VHS – Vertebral Heart scale

1. Curricular traineeship in “Ospedale Veterinario Universitário di Perugia”

The curricular internship comprised in the 6º year study plan of the Integrated master’s in veterinary medicine of the Faculty of Veterinary Medicine of the University of Lisbon, took place at the Veterinary Teaching Hospital of the "Università Degli Studi di Perugia" in Italy. Under the guidance of Professor Esmeralda Sofia da Costa Delgado and tutored by Professor Francesco Birettoni.

The internship schedule consisted of 5 daily clinical hours, starting at 9:00 am and ending at 2:00 pm, five days a week. It started on September 20, 2021, and finished on February 25, 2022, in 20 full weeks and 500h.

This internship was a personal challenge for the intern, both due to the Italian language and for its star focus on cardiology and ultrasonography. It allowed the student to enhance and consolidate its clinical knowledge, practical skills and to work in a team. At 9 am, the student could often follow the case’s info being passed from the night shift veterinarians to the one’s from the morning shift. After that it was possible to choose and accompany the appointments and procedures in which to be part. Based on the ongoing appointments, doctor's willingness to communicate and integrate students in the activities, "hands-on" learning opportunities, and the student's interests, the time was used within the activities described in Bar chart 1.



Bar chart 1 - Accompanied consultations and major interventions assisted during the 500h internship in the Veterinary Teaching Hospital of Perugia of the "Università Degli Studi di Perugia".

Clinical cases were followed up with interest in all departments, and there was no shortage of questions whenever pertinent. The student took notes of most clinical cases during the internship, from the anamnesis to its outcome (when available).

1.1. Internal Medicine, abdominal ultrasonography, and endoscopy

In the internal medicine department, the student accompanied all parties involved in the consultation, from the anamnesis, clinical examination, animal restraint, sample collection, complementary diagnostic tests, and therapy institutions.

Within the abdominal ultrasound (US) examinations, the student helped restrain the animals and participated in the interpretation of the Ultrasonographic imaging results. Frequently he had the chance to look for abnormalities within the US probe after the doctor finished the examination. When there were no appointments, and compliant animals were available in the hospital facilities, small groups of students practiced abdominal ultrasonographic examinations. Lung ultrasonographic examinations were also commonly performed by the doctors at this veterinary institution when signs of lower respiratory tract symptomatology were present. With Prof. Birettoni, the student could follow 14 appointments of migrating vegetable foreign bodies in hunting dogs, frequently located in the iliopsoas muscle. Recurring to a linear US probe, the exact location of the grass awn was determined. When considered reachable, an attempt to remove it recurring to grasping forceps, a small incision and local anaesthesia were performed.

Ultrasonographic guided effusion drainage was performed frequently in the IM department. For thoracocentesis, paracentesis and pericardiocentesis, the student could trim the pet's hair and apply the skin disinfection protocol with alcohol and diluted chlorhexidine. Could also prepare the drainage kits and sometimes participate in the effusion drainage contributing to the animal's welfare and collecting a sample for the etiologic diagnosis when necessary. Fine needle aspiration, chiefly US-guided, was commonly performed within the IM department. The student also had the opportunity to help and gain experience with this procedure, from puncture and sampling to smear preparation for cytologic analysis.

In the endoscopy department, the student had the opportunity to follow a rhinoscopy, a bronchoscopy, and multiple esophago-gastroduodenoscopies and colonoscopies. The student could also place catheters, help with bowel cleansing, and perform biopsies collecting samples for histopathology.

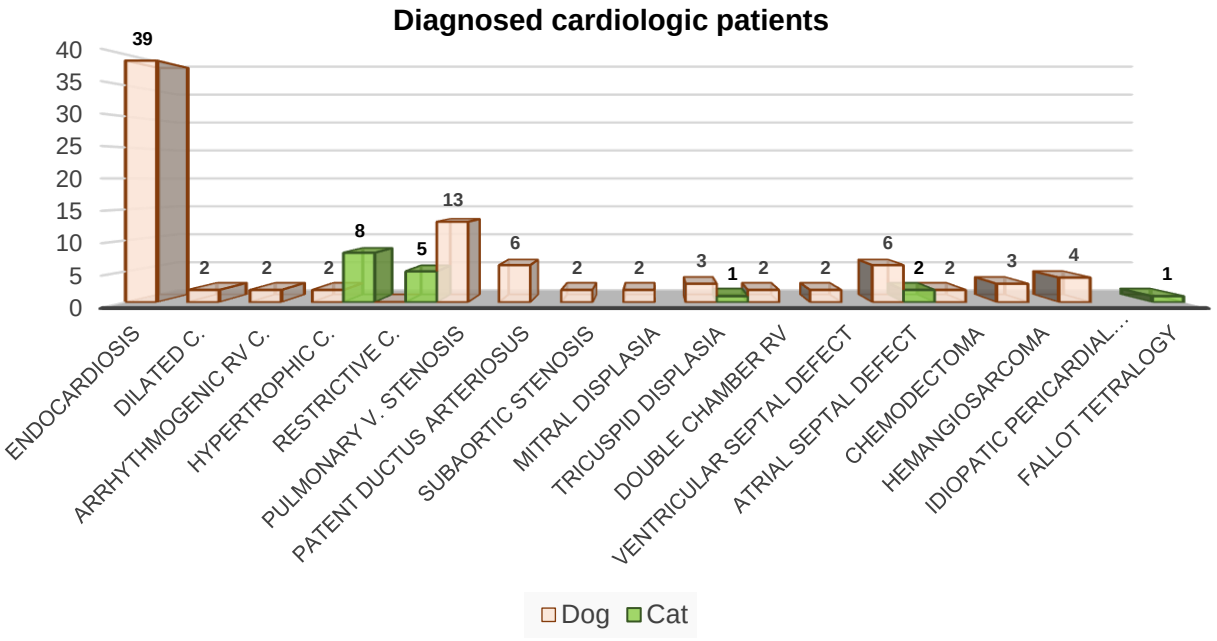
1.1. Cardiology

In the busy cardiology department, the anamnesis was collected with the help of other students and professors (due to the language barrier). The student had the opportunity to perform a

proper physical examination and electrocardiogram (ECG) evaluation on a high number and wide variety of referred cardiac patients and follow their echocardiographic examination. He learned how to perform echocardiographic evaluations, which measurements to collect for each type of condition, and what limits to consider when diagnosing the most common cardiac diseases. After the appointments, the student helped with the patients' reports by performing specific measurements within the echocardiographic machine and communicating them to the cardiologist. Also had the opportunity to practice echocardiographic examinations when complying animals were available, and no consultations were appointed.

The student also participated in a class about electrocardiography (ECG) taught by Professor Birettoni, and together with the day-to-day placement and reading of ECGs and Holter placement in a few dogs of predisposed breeds for dilated and arrhythmogenic cardiomyopathies, the student efficiently enhanced his knowledge about ECG.

Especially due to Professor Porciello's expertise, a large variety of rare cardiology cases arrive in Perugia. This professor is one of the few veterinary surgeons who perform patent-ductus-arteriosus (PDA) closures, valvuloplasties, and pace-maker placements in a US-guided manner instead of using fluoroscopy, as described in the literature. His method allows students to safely follow the operation in real-time, in the same physical space as the minimal-invasive surgical procedure occurs. The intervention needs two assistants, including an excellent cardiac ultrasonographer, in opposition to the classical method, which can be done with only one assistant. During the internship, it was possible to follow 2 PDA closures and 4 valvuloplasties. The student had the opportunity to practice the indirect assessment of pulmonary hypertension (PH) in some of the patients who underwent valvuloplasty.



Bar Chart 2 - Diagnosed cardiologic population of small animal patients, that were subject to a cardiologic visit, followed by the student, from September 20, 2021, to February 24, 2022. Abreviations: C. -Cardiomiopathy; V – valve; RV – Right ventricle.

All patients who were presented and diagnosed with cardiovascular disease and all of those who underwent a check-up following successful treatment were selected from the cardiology visits that occurred during this internship. The frequency of cases is displayed by cardiology disease in bar chart 2.

1.2. Other departments

The remaining hours were spent in the hospital's emergency and intensive care unit, followed by the oncology, radiology, surgery and ophthalmology, exotics, and obstetrics departments.

At the emergency and intensive care unit, it was possible to participate in the team's routine, which included monitoring the clinical evolution of patients. The hospitalized patients were submitted to a clinical examination and the monitoring of basic parameters such as glucose measurements, systemic blood pressure measurements, urinary output determination, and, in addition, several analytical controls. Furthermore, it was also possible to prepare and administer medication, administer food, and take the patients for a walk. On the other hand, there were still several executable tasks under medical supervision, such as placing intravenous catheters, blood and urine collections, cleaning wounds, and applying dressings.

The time in oncology was used to help with the drug administration and learn about the patient's diagnosis, treatment protocols, and prognosis.

In the department of surgery and ophthalmology, it was possible to learn about different procedures, monitor anaesthesia, and follow the patient's outcome while they were in the hospital.

In the radiology department, the student helped with animal positioning, performing X-rays, and reading both X-rays and computer tomography (CT) scans.

The exotic department had a wide variety of wild rescues, such as porcupines, hedgehogs, badgers, foxes, squirrels, birds of prey, tortoises, bats, while pets were less frequent. In this department, it was possible to help administer drugs, do palliative treatments, and follow the patients when moved to other departments for any complementary diagnosis or surgery.

Discussing anaesthesia protocols and monitoring ECG and vital signs, such as heart and respiratory rate, pulse quality, mucous membrane colour, and respiratory pattern, was possible in various departments and activities, such as surgery, CT, endoscopy, and drainage procedures.

2. The myxomatous mitral valve disease, literature review.

2.1. Introduction

Bearing close similarities to chronic degenerative mitral valve (MV) disease in humans (Han et al. 2010), myxomatous mitral valve disease (MMVD) accounts for approximately 75% of all canine heart disease patients in general practice (Keene et al. 2019). It's also the most frequent cause of dog congestive heart failure and cardiac-related mortality (Parker and Kilroy-Glynn 2012). Most affected dogs are clinically asymptomatic for years or even for their lifetime, but approximately 30% will eventually progress to cardiac heart failure (CHF) and might die because of the disease (Borgarelli Michele and Haggstrom 2010; Parker and Kilroy-Glynn 2012).

2.1.1. Definition of MMVD

MMVD is a disease of many designations, being also known as endocardiosis, degenerative or chronic valvular heart disease, myxomatous valvular degeneration, myxomatous transformation, or mucoid degeneration (Francis Smith, Larry Tilley, Mark Oyama 2015; Keene et al. 2019). It's characterized by a slow progressive degeneration of the MV and its apparatus over time, with subsequent left atrial (LA) dilation, ventricular dilation, and all other cardiovascular changes that occur consequently to this degeneration. Although the left atrioventricular (AV) valve is the most commonly affected, tricuspid valve (TV) involvement is seen in nearly 30% (Borgarelli and Buchanan

2012) of affected dogs. Mild semilunar valve degeneration is occasionally seen (Buchanan 1977). The term "myxomatous" refers to the histologic features of this pathologic condition and excludes most if not all other diseases affecting the left AV valve (Ettinger Feldman, Edward C.Feldman 2017).

2.2. The Healthy Mitral Valve Apparatus

In the left side of the heart, allowing the inflow of blood from the left atrium (LA) to the left ventricle (LV) during diastole and unidirectionally pumping blood into the aorta (Ao) during systole, when healthy, is the MV apparatus. Its key structural components are the mitral annulus, the anterior and posterior leaflets, the chordae tendinea, and the papillary muscles attached to the caudal (free) wall of the LV. The harmonious interplay of all these components is essential for the proper functioning of the valve. An imbalance in the mitral structure and function of any component can lead to valvular dysfunction (O'Gara et al. 2008; Dal-Bianco and Levine 2013).

Histologically, the valvular leaflets have a trilaminar structure, composed of 3 distinct layers: *atrialis* layer, spongiosa layer, and ventricular or fibrosa layer. (Goyal et al. 2019). The extracellular matrix (ECM) is a net of macromolecular proteins, with chiefly structural functions, involving valvular and myocardial muscle integrity. The valvular ECM is majorly composed of collagen, proteoglycans, glycosaminoglycans and a variety of proteins.(Theocharis et al. 2016) Besides its structural function, the ECM plays a role in various cellular mechanisms, such as growth, differentiation, and hemostasis (Frantz et al. 2010; Theocharis et al. 2016). Due to dormant interstitial cells and rudimentary vascular and lymphatic systems, the ECM turnover in a healthy MV occurs at a sluggish pace. Neovascularization and remodelling of the ECM and the activation and proliferation of interstitial cells, can be induced by physiological or pathologic-induced stress on the leaflets (Goyal et al. 2019).

2.3. Epidemiology

Around 7,5% of all dogs presented to first opinion veterinary practices have MMVD (Keene et al. 2019). Usually, affected dogs have no congenital abnormalities within the cardiac valves, developing the affection later in life (Whitney 1974). Various reports suggested that MMVD might affect up to 89% of the dogs at some point in their life. Proportions might vary with size, age and breed (Detweiler and Patterson 1965; Buchanan 1977; Kogure 1980; Connell et al. 2012). In terms of sex predisposition, male dogs are the most affected, with a frequently earlier onset of the disease and about three males being diagnosed for every two females (Keene et al. 2019).

Cases of a similar affection in the MV are common in humans, horses, and pigs but rare in cats.(Guarda and Negro 1989; Reef et al. 1998; Connell et al. 2012; Francis Smith, Larry Tilley, Mark Oyama 2015). Endocardiosis is also described in various species, including Gambian pouched rats,

ostriches, and diverse fish species (Müller et al. 2010; Kubba and Al-Azreg 2013; Cooper and Spitsbergen 2016; LaDouceur et al. 2019).

Even though this affection can lead to the death of the animal and smaller dogs tend to live longer lives (Connell et al. 2012), the incidence of MMVD is way higher in small and toy breed dogs (<20 kg), which generally present a slower progression of the disease. In the fewer large dogs affected, the course of the disease occurs typically faster, with more significant myocardial dysfunction and a more reserved prognosis (Keene et al. 2019). Despite the increased risk of MMVD in a subset of breeds, with average adult weights under nine kg (Parker and Kilroy-Glynn 2012), most of those breeds are poorly related phylogenetically, having little in common other than their reduced dimensions (Smith et al. 2001). One hypothesis is that small size may contribute to endocardiosis due to 'crowding' of the thoracic cavity. Alternatively, genes that regulate growth may also be responsible for cardiac development, and thus, genetic recruitment for small size may affect cardiac development (Parker and Kilroy-Glynn 2012).

MMVD evolves progressively, with both prevalence and severity firmly correlated with age. It is estimated that up to 85% of small and toy breed dogs carry valvular lesions by the age of 13 (Buchanan 1977). By then, around 30% of the dogs show clinical evidence of the disease (Borgarelli Michele and Haggstrom 2010; Francis Smith, Larry Tilley, Mark Oyama 2015; Keene et al. 2019). The disease can start from the first third onwards of each dog's life. This was shown with post-mortem evidence in a study performed on 200 randomly selected deceased dogs, where 37% of canines under five years old held valvular lesions, most of which were mild. The study also revealed that lesions of the referred severity were not associated with valvular insufficiency (Whitney 1974). The premature onset of the disease mainly occurs in harshly predisposed breeds. In those, breeding programs should be put in place. Dogs at breeding age (less than 6-8 years) with a murmur or echocardiographic evidence of MMVD should no longer be bred (Birkegård et al. 2016).

In terms of breeding predisposition, the Cavalier King Charles Spaniels (CKCS) takes the lead, with the earlier onset of the MMVD (Keene et al. 2019). In this breed dating back to the 16th century (Knowler et al. 2019), an estimated 50% of the specimens are affected by the age of 6 to 7 years old (Häggström et al. 1992), and by ten years old nearly all of them have it (Madsen et al. 2011). The Dachshund is also particularly affected, with nearly every other dog affected at the age of ten (Pedersen et al. 1996). The previous two breeds, Poodles, Pinchers, Chihuahuas, Cocker spaniels, Whippets (Mattin et al. 2015), and any other small and toy breed dogs, should undergo regular evaluations (yearly auscultation by the general practitioner veterinarian) as part of their routine health care (Keene et al. 2019). MMVD is a serious welfare issue for severely affected dog breeds, and the exact reason behind it is still unknown (Coffman et al. 2021).

2.4. Aetiology

Mitral insufficiency can be caused by diseases other than MMVD, such as endocarditis, congenital malformations, and conditions that lead to the left heart enlargement with dilation of the mitral annulus (eg: dilated cardiomyopathy & PDA) (Francis Smith, Larry Tilley, Mark Oyama 2015).

If a primary cause leading to MMVD exists, it is still to be identified until this date. Nevertheless, rather than a particular cause, it is accepted that endocardiosis results from a complex interaction of chronic mechanical stress, activation of various signaling pathways, altered polygenic expression, together with histological changes and induction of growth factors. The lack of knowledge about its exact aetiology has held up the development of an effective long-term clinical management (Lewis et al. 2011; O'Brien et al. 2021).

2.4.1. Histologic and cytologic changes

Endocardiosis is found in chondrodysplastic dogs with concurrent hereditary disorders such as bronchomalacia and intervertebral disc abnormalities, which suggests that it might be linked to a systemic connective tissue ailment (Hadian et al. 2010; Francis Smith, Larry Tilley, Mark Oyama 2015). Other conditions involving connective tissue dysfunction, such as tracheal collapse and cruciate ligament rupture, are as well frequent in small breeds predisposed to endocardiosis (Aupperle and Disatian 2012). The disease has been consistently described with changes in cellular constituents and ECM of the valve apparatus. Possibly with post-transcriptional regulation from microsatellite ribonucleic acids (RNAs), some valvular interstitial cells (VICs) acquire characteristics of activated myofibroblasts, including the production and secretion of high amounts of matrix metalloproteinases. These enzymes lead to collagen and elastin proteolysis at a faster pace than the producing ability of the remaining "inactivated" VICs (Keene et al. 2019). Studies on the myofibroblast cell membrane showed a vastly increased number of receptors for endothelin, angiotensin, and serotonin, likely to be linked with the MMVD pathogenesis (Oyama and Levy 2010; Cremer et al. 2015; Keene et al. 2019).

The myxomatous degeneration at the matrix level happens with alterations in the collagen composition and disposition within the valvular tissue (Keene et al. 2019). Mild to moderate disease is correlated with a marginal decrease of the overall collagen content in severely affected parts of the valve, accompanied by a rise in immature collagen content. Causing an excessive increase of the ECM components in the spongiosa layer and concurrent destruction of the ventricular layer, the collagen and cellular changes lead to leaflet's mechanical dysfunction (Hadian et al. 2010).

Other described histologic abnormalities at the cardiac level, within the MMVD, include myocardial fibrosis, intramyocardial arteriosclerosis (especially in the papillary muscles) (Falk and Jönsson 2000; Falk et al. 2006), subendothelial thickening, and endothelial cell changes (Keene et al. 2019). Being considered a sterile condition, inflammatory infiltrates are not included in its histologic features (Francis Smith, Larry Tilley, Mark Oyama 2015).

2.4.2. Genetic and hereditary ethology

An inherited background for the disease has been identified in some breeds, and a polygenic component is likely responsible for the disease severity in others (Meurs et al. 2018; Keene et al. 2019). For both the murmur intensity and severity of MMVD, a correlation between the cardiac status of parents and offspring has been shown in various studies. Conclusions of those suggested a polygenic inheritance and a threshold (higher in females) having to be reached before MMVD develops (Madsen et al. 2011). Due to the high prevalence of early disease in the CKCS, this breed represents a consistent resource for research on the genetic background of this pathology (O'Brien et al. 2021). A genome-wide association study performed to identify responsible genes behind the early onset of the disease in this breed found a correlated locus on each chromosome 13 and 14. Further studies may establish a causal relationship between specific mutations and disease development, and with that, genetic tests may become commercially available (Madsen et al. 2011).

Several differences have been identified in gene and microRNA expression between healthy dogs and those mildly or severely affected by the disease. These variations can potentially emphasize the genes and aetiologic pathways behind the MMVD pathogenesis (Oyama and Chittur 2006; Moon et al. 2008; Aupperle et al. 2009; Yang et al. 2019; Markby et al. 2020; O'Brien et al. 2021) Insulin-like growth factor (IGF) has been linked with cardiac development and is also important in the regulation of general growth (Donath et al. 1994). Researchers found a strong correlation between a mutation within the IGF locus on chromosome 15 and overall body size in a study of Portuguese water dogs (Chase et al. 2002). Using single nucleotide polymorphism technology, other studies within the IGF locus demonstrated an intact haplotype in all small breeds examined. Accordingly, most of the small breed dogs are believed to descend from one common ancestor and, given the consistent presence of IGF mutations in the small dog's genome and the fact that it has been linked to cardiac development, it seems plausible that the mutation may have a role in the pathogenesis of MMVD. (Parker and Kilroy-Glynn 2012)

2.4.3. Other causes

Inflammatory mediators have a relevant role in the pathogenesis of MMVD. The MV is subjected to shear stresses throughout its lifetime due to repeated opening and closing. The

subsequent wear and tear induce reparative processes that maintain the valve's integrity and preserve its function by altering its histologic structure, as previously mentioned. Molecular signalling pathways, inflammatory mediators, and chemokines are all involved in these processes(Orton et al. 2012). Both ageing and MMVD have overlapping molecular processes. Still, the lesions of endocardiosis are frequently far more severe and eventually result in valve failure (Connell et al. 2012), likely due to aberrant cellular signalling involved in the pathogenesis of the disease. These signalling pathways include abnormally high levels of transforming growth factor-beta (TGFβ) and serotonin. A hypothesis for MMVD development suggested that predisposed valves show abnormal molecular signature pathways under normal levels of mechanical stress (Orton et al. 2012; Waxman et al. 2012). Tryptophan hydroxylase (TPH-1), a limiting enzyme essential for serotonin synthesis, is up-regulated in dogs with endocardiosis. Various pathways are activated by serotonin, including extracellular signal-regulated kinases, which increases the production of TGFβ. Up-regulated TPH-1, platelet aggregation (which are rich in serotonin) to injured valves, and the succeeding production of TGFβ due to serotonin signalling are critical players in valvular weakening (Orton et al. 2012). Increased serum serotonin levels appear to be genetically determined in Maltese dogs (which present higher serum serotonin levels than other breeds) (Roels et al. 2015). Still, the exact correlation was not found in CKCS, where only increased platelet count influenced the serum serotonin concentrations(Reimann et al. 2021).

Neurohormonal and inflammatory mediators, such as endogenous catecholamines and the renin-angiotensin-aldosterone system, may worsen valvular degeneration and contribute to subsequent myocardial remodelling and ventricular dysfunction..(Han et al. 2008; Menciotti et al. 2017; Yang et al. 2017) The mechanical configuration and function of the MV are suggested to be involved in MMVD's aetiology. According to blood flow, the valvular structure is subject to uneven stress and strain distribution, capable of inducing the upregulation of the signalling pathways that lead to VICs' activation into the pathologic myofibroblast phenotype (Richards et al. 2012).

2.5. Anatomopathological Changes

2.5.1. Gross valvular and Atrial Lesions

Depending on the stage of disease at which the valve is examined, the typical gross lesions may vary. Mild lesions can easily go unnoticed without a watchful examination of the leaflets, which might remain grossly thin and transparent (Of and Dogthe 1996; Aupperle and Disatian 2012). In dogs, in opposition to humans, the anterior leaflet is more often and more harshly affected than its posterior counterpart (Davies et al. 1978; Lester 1995; Terzo et al. 2009).

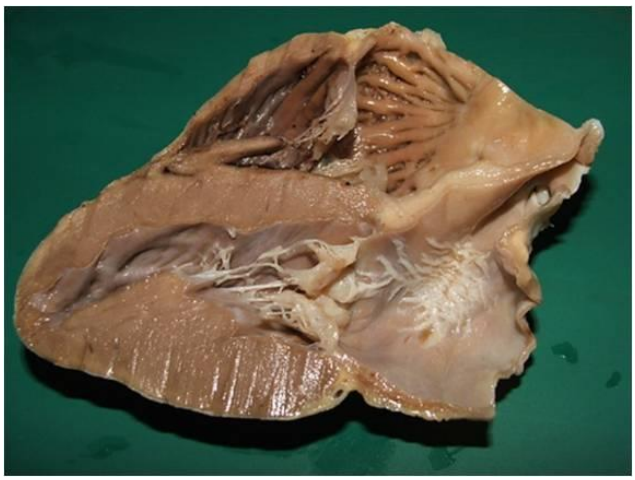


Figure 1 - Fibrotic lesions in the atrial endocardium, opposing the mitral orifice, where the regurgitant jet is directed. (Picture provided by Dr Francesco Birettoni)

First changes usually appear as small nodules at the free margins of the leaflets (Aupperle and Disatian 2012) and are frequently more pronounced within the chordae tendineae insertion points. The nodular lesions increase in size and number throughout the disease and ultimately merge. Grossly, the outer margins of the leaflets acquired thickened, irregular limits, showing bulging/ballooning pointed to the LA side (Whitney 1974; Buchanan 1977; Corcoran et al. 2004; Birkegård et al. 2016). With the progression of the disease, the bulge increases, other parts of the leaflets become affected, and myxomatous degeneration might develop through the chordae tendineae as well. Significant proliferation of fibrous tissue often occurs in severe cases, leading to extensive deformation and thickening of the leaflets and weakening and possibly rupturing the chordae tendineae(Kogure 1980; Beardow and Buchanan 1993; Corcoran et al. 2004). Fibrotic lesions in the atrial endocardium, opposing the mitral orifice (figure 1), where the regurgitant jet is directed, can also be seen. Atrial rupture of various extents might surge in the later stages of the disease (e.g., acquired atrial septal defect or endomyocardial splits) accompanied or not by hemopericardium (Buchanan 1972; Buchanan 1977; Francis Smith, Larry Tilley, Mark Oyama 2015).

2.5.2. Nerve density and aging

Dog MV leaflets have highly developed nerve plexuses (WILLIAMS 1964; Williams et al. 1990) composed of transmitter-specific nerve subpopulations (Han et al. 2010). Experimental studies in dogs showed that autonomic stimulation makes the valve more capable of holding against pressure-induced displacement of its leaflets into the LA (Curtis and Priola 1992). Loss of MV innervation was positively correlated with age in rats (Jew and Williams 1999), but while a significant depletion in MV nerve density was shown in dogs above ten years of age, no differences were found between dogs aged 6–12 months and those with five years (Han et al. 2010).

2.6. Physiopathological changes

The passive initiation of valve closure happens in early systole when LV pressures exceed left atrial pressures (LAp), and the left AV leaflets are forced to close. In healthy dogs, the chordae tendinea tethering effect prevents leaflet prolapse into the LA, while the correct leaflet coaptation precludes significant regurgitation through the valve orifice. The MV then ensures that the entire LV stroke volume is ejected to the systemic circulation through the Ao. In case of mitral insufficiency, a fraction of the stroke volume will be regurgitated to the LA through the mitral orifice during systole (Nishimura et al. 2014; Francis Smith, Larry Tilley, Mark Oyama 2015). Mitral regurgitation (MR) occurs when the MV or respective chordae tendinea have moderate to severe lesions. Gradual stretching and rupture of chordae tendineae worsens MR by causing leaflet prolapse into the LA (Beardow and Buchanan 1993; Pedersen et al. 1996).

The size of the regurgitant orifice called *vena contracta*, together with the gradient between left atrial and left ventricular systolic pressure are the leading factors determining the grade of insufficiency and the regurgitant volume (Mihalatos et al. 2007). The referred gradient depends on intra-atrial pressure, LV function, as well as systemic arterial blood pressure (Ahmed et al. 2009).

When present, regurgitation can be mild, with no consequences to cardiac size and function, or severe, causing progressive changes in those parameters (Mihalatos et al. 2007; Nishimura et al. 2014). MR can be primary when it is solely caused by the degeneration of the MV apparatus or secondary when extreme left-sided cardiac dilation increases the regurgitant orifice. Hence, regurgitation generates regurgitation. Canines with MMVD frequently present a regurgitant jet oriented caudally, likely due to the anterior leaflet's longer dimension and greater mobility compared to the posterior one, which makes the first one more susceptible to prolapse (Ahmed et al. 2009).

When MR is primary and mild, the forward stroke volume is preserved, and the LA easily accommodates the small amount of regurgitant volume. (Nishimura et al. 2014). Severe atrial dilation frequently follows chronic high pressures in the LA, caused by MR. With the MV leaking, the pulmonary venous return is also overloaded by the regurgitant volume, and as a consequence, the LV is filled in diastole with the blood coming from the lungs and the cumulating regurgitant volume (Francis Smith, Larry Tilley, Mark Oyama 2015). The LA enlargement neutralizes the effect of a growing MR volume by maintaining the intra-arterial pressure low and accommodating blood during ventricle systole (Kittleson and Brown 2003; Eriksson et al. 2010), protecting the pulmonary vascular system from hypertension (Kihara et al. 1988).

The MR is generally well tolerated by the myocardium, with many dogs displaying preserved myocardial contractility long after the surge of CHF. Even so, systolic myocardial dysfunction might

arise due to the chronically superimposed volume load on the heart. To compensate for the loss of forward stroke volume caused by the regurgitant MV, the LV increases the end-diastolic volume (Francis Smith, Larry Tilley, Mark Oyama 2015). With severe MR, high-end diastolic volume and increased LV filling pressure lead to the activation of diverse compensatory mechanisms, which ultimately lead to eccentric ventricular hypertrophy, in which the ratio between chamber size and wall thickness remains grossly unchanged (Carabello 2002; Grossman and Paulus 2013; Borgarelli et al. 2015; Francis Smith, Larry Tilley, Mark Oyama 2015). LV remodelling can normalize pressure that tends to increase due to volume overload, thus acting beneficial, maintaining the stroke volume close to normal, until a turning point where the compensatory mechanisms become mostly harmful, causing cellular damage. and myocardial fibrosis (Kittleson et al. 1984; Nishimura et al. 2014).

The increasing LAp is followed by increased venous pressure in the pulmonary system, associated with blood vessel congestion and potentially inducing pulmonary oedema (PE) formation. Frequently, the animal develops clinical signs (CS) at this stage, and it is considered to have reached cardiac CHF (Francis Smith, Larry Tilley, Mark Oyama 2015).

CHF is a syndrome involving all the CS and neuroendocrine activation that results from cardiac dysfunction. Following MMVD, it occurs chiefly due to dramatically increased venous pressures that lead to fluid accumulation in the lungs or a body cavity (congestive or “backwards” heart failure, due to the heart’s inability to drain the venous systems adequately). Within the end-stages, if myocardial dysfunction occurs, CHF can occur due to the compromised ability of the heart to pump blood, to the point that the amount of blood pumped is not enough to match the body’s needs (“forward heart failure”) (Keene et al. 2019). In MMVD, the presence of PE due to left-sided CHF is the most common expression of this syndrome. In the case of right-sided involvement, right-sided CHF might also happen with severe tricuspid insufficiency, which in dogs is commonly displayed as ascites (Francis Smith, Larry Tilley, Mark Oyama 2015).

2.6.1. Complications associated with MMVD

PH is defined as an abnormal increase in pressure on the pulmonary vasculature. Pulmonary arterial pressure (PAP) also called post-capillary PH is a typical complication of severe MMVD. It ultimately leads to echocardiographically measurable PH (Reinero et al. 2020) and depends on pulmonary blood flow, pulmonary vascular resistance (PVR) and pulmonary venous pressure (Schober and Baade 2006; Kellum and Stepien 2007; Kellihan and Stepien 2012). PH initially occurs due to increased LAp, secondary to MR (Kellihan and Stepien 2012; Guazzi and Naeije 2017). However, right ventricular to right atrial (RA) systolic pressure gradients greater than 60mmHg are frequently measured in dogs with MMVD and cannot be explained solely by passive retrograde pressure transfer from the LA to the pulmonary arteries. Such LAp would be incompatible with life,

leading to severe PE and death (Kittleson Kienle, Richard D., 1998; Chiavegato et al. 2009; Ettinger Feldman, Edward C.Feldman 2017). Chronically, postcapillary PH induces reactive vasoconstriction of the pulmonary arteries, causing hypoxia and damaging the pulmonary vasculature, therefore elevating the PVR (Johnson et al. 1999). As a result of MR, postcapillary hypertension can occur isolated or, in later stages, together with high PVR (post and precapillary PH simultaneously) (Vachiéry et al. 2013). Additional factors have been linked with worsening of post-capillary PH, such as the loss of endothelium-dependent vasodilation and the effects of chronic neurohormonal activation (Delgado 2010). The right ventricle (RV) performance is also linked with the degree of PH secondary to left-sided heart disease (Kellihan and Stepien 2012).

Concomitant chronic respiratory diseases, such as tracheal collapse, chronic bronchitis, or interstitial lung disease, commonly affect small breed dogs and might also cause or worsen PH in MMVD patients (Borgarelli et al. 2015).

Involvement of the TV often co-occurs with mitral endocardiosis, either as a primary valvular disease or as a consequence of PH due to severe MR. Degenerative valvular changes present a similar disease course in both AV valves (Francis Smith, Larry Tilley, Mark Oyama 2015). Endocardiosis concerning the TV usually leads to mild to moderate TR, usually well tolerated in the absence of PH (Barbour and Roberts 1986). On the other side, the RV is more sensitive to rises in pressure due to its nature of pumping blood to a low-pressure arterial bed. For this reason, this heart chamber is poorly capable of adapting, and small increases in the afterload might be sufficient to reduce its stroke volume (Wiedeman et al. 1998). In concerning situations, with PH being present, right chamber dilation, secondary to TR can occur, which might expand the tricuspid annulus, worsening the TR (Ettinger Feldman, Edward C.Feldman 2017).

Atrial fibrillation (AF) is the most common supraventricular arrhythmia in dogs and humans. Even though some dogs might develop primary AF, it is more likely for this arrhythmia to be secondary to heart pathologies associated with a disproportionately big LA, such as MMVD (Guglielmini et al. 2000; Menaut et al. 2005; Nishimura et al. 2014; Kirchhof et al. 2016).

MMVD can lead to congestion and tissue hypoperfusion at a systemic level. A study performed on 69 Chihuahuas showed that D/L-lactate and other markers of intestinal mucosal injury have significantly higher values in patients who experienced CHF, compared with healthy dogs and early-stage MMVD patients. Showing a potential relationship between the severity of MMVD morbidity and intestinal mucosal injury in Chihuahuas, as described in humans (Araki et al. 2021).

2.7. ACVIM Staging System for management of MMVD and Heart failure

The staging system for cardiac pathology and CHF was first published in 2009 to effectively relate the severity of the morphological heart chamber changes and CS with appropriate treatment at each stage of the MMVD. It established that affected dogs are expected to progress unidirectionally, from the initial stage to the following, except for patients subjected to corrective surgical treatment, in which the disease course is altered. This classification system comprises four stages: A, B, C, and D, with particular subdivisions (Keene et al. 2019).

Stage A refers to all highly predisposed dogs to MMVD with no signs of morphological or functional changes within the heart. Stage B covers dogs presenting structural heart pathology or a murmur of MR regurgitation, together with evidence of valvular changes, but that never showed any symptomatology associated with the heart disease. Studies showing the benefits of early therapy in asymptomatic dogs with severe morphologic heart changes led to the subdivision of this group into B1 and B2 at the 2019 ACVIM consensus. Stage B1 comprises dogs fulfilling the group B requirements that have no radiograph or echocardiographic evidence of cardiac remodelling due to AV regurgitation and those presenting mild morphologic changes that, according to current clinical trials criteria, do not benefit from early treatment. The B2 group holds the dogs with extensive cardiac remodelling that, according to the same clinical trial criteria, benefit from early medical or surgical treatment to respectively delay or prevent the onset of CHF secondary to MMVD (Keene et al. 2019).

Stage C includes any dog that has or had CS of CHF due to MMVD and presents a positive response to standard treatment. Stage D is the end-stage of this classification system, reserved for symptomatic dogs that are refractory to standard treatment, requiring complex treatment strategies to keep their symptoms nearly controlled. CHF can have an acute presentation in both C and D stages, leading to pet hospitalization and critical care need. This stage is classified as C1 or D1. Chronic staged dogs that can be medicated at home are classified as C2 or D2. (Keene et al. 2019)

2.8. Diagnosis

2.8.1. Clinical Presentation

MMVD is characterized by a long pre-clinical period, with many of the affected dogs dying from causes other than the acquired cardiac disease (Borgarelli et al. 2008; Borgarelli and Buchanan 2012; Vezzosi et al. 2021). A study accessing the survival of MMVD patients involving 302 asymptomatic dogs showed that 70% of these dogs were still alive 6,6 years after being diagnosed with MMVD (Borgarelli et al. 2008). According to ACVIM, stages A and B, which include most of the mild and moderate cases of MR, are not associated with clinical symptomatology. Infrequently, some patients might present a slight exercise intolerance and cough secondary to compression of the left main bronchi associated with LA enlargement (Ettinger Feldman, Edward C.Feldman 2017).

Roughly 30% of patients affected by MMVD ultimately progress to CHF (Borgarelli and Haggstrom 2010). Due to the vague nature of the resulting symptoms, assigning them to MMVD can be challenging and should always be accompanied by at least one thoracic radiograph and clear auscultation of a murmur during the physical exam (Borgarelli and Haggstrom 2010; Francis Smith, Larry Tilley, Mark Oyama 2015). Intense exercise is not part of most dogs' routine, and therefore, lack of stamina, weakness, and exercise intolerance might not be noticed by owners or might be perceived as something natural to the aging pet (Francis Smith, Larry Tilley, Mark Oyama 2015).

Enlargement of the LA followed by increasing pulmonary venous pressure are the main physiopathological events leading to symptomatology in MMVD patients. Together with the left bronchial compression caused by the LA, pulmonary congestion, and oedema due to post-capillary PH, can lead to cough, tachypnea and dyspnea (Ettinger Feldman, Edward C.Feldman 2017; Keene et al. 2019). Within these symptoms, dogs can appear anxious and restless, preferring to sleep in sternal recumbency to decrease any additional pressure on the lungs. Less often, a sufficient forward blood flow cannot be maintained by one or both ventricles, causing patients to experience weakness, reduced stamina, and even syncope (Ettinger Feldman, Edward C.Feldman 2017).

In the few MMVD cases that lead to right-CHF due to a significantly increased right intra-atrial and systemic blood pressure, pleural effusion and enlarged abdomen (caused by ascites, hepatomegaly and splenomegaly) might occur (Ettinger Feldman, Edward C.Feldman 2017). Primary myxomatous degeneration of the TV, in the absence of PH, rarely leads to symptoms. When TR is secondary to PH, besides the congestive signs, the clinical presentation might include exertional fatigue, syncope, and gastrointestinal signs, such as vomit, diarrhea, and cachexia (Johnson et al. 1999; Ettinger Feldman, Edward C.Feldman 2017; Keene et al. 2019).

Even though most MR patients have a mild clinical onset of CHF that worsens with time, some might experience life-threatening acute symptomatology, frequently requiring hospitalization. This occurs most frequently due to complications of the disease, such as severe rupture of chordae tendinea, pericardial effusion, or ventricular tachycardia (Ettinger Feldman, Edward C.Feldman 2017).

Decompensated dogs present decreased activity and might suffer from cardiac cachexia, which negatively impacts their prognosis (Freeman 2009) and, in some cases, might pass unnoticed due to increased fluid retention leading to concurrent maintenance of the bodyweight (Ineson et al. 2019).

Cardiac diseases are among the leading causes of syncope, which some dogs can experience due to MMVD (Kittleson Kienle, Richard D., 1998). Syncopal episodes have a sudden

onset and generally last for a few seconds, ending with spontaneous recovery (Moya et al. 2009). Any cause of reduced cardiac output can lead to syncope, and arrhythmias might be the most frequent cause of cardiac-related syncope (Schaldemose et al. 2014). In MR patients, it might be related to arrhythmias, severe cough bouts while exercising, and exercise in the presence of PH. The frequency might vary from one in a lifetime to multiple episodes in the same day (Ettinger Feldman, Edward C.Feldman 2017). The risk of death is increased in MMVD dogs that experience syncope due to the risk of cerebral hypoperfusion. (Buchanan 1977; Borgarelli et al. 2008).

2.8.2. Physical examination

Especially in small and toy size old dogs, meticulous auscultation should be performed to discard or detect a murmur of MR, present in most cases of MMVD usually surging years before the development of CHF (Keene et al. 2019). A holosystolic band shaped murmur of MR is the most characteristic finding. In some instances, the characteristics of the MR murmur might vary. At the beginning of the disease, the murmur might be protosystolic or midsystolic, and with mitral prolapse, the murmur is frequently better heard in mid to late systole (Häggström et al. 1995; Pedersen, Häggström, et al. 1999; Kvart et al. 2002; Kvart C 2002; Ljungvall et al. 2009; Francis Smith, Larry Tilley, Mark Oyama 2015). At the left apex, near the fifth intercostal space (IC), at the costochondral junction is the point of maximal intensity (PMI) for the auscultation of the MV, where the MR murmur is best heard in most dogs. At the left apex, near the fifth IC, at the costochondral junction is the point of maximal intensity (PMI) for the auscultation of the MV, where the MR murmur is best heard in most dogs. When present, TR may also generate a systolic murmur of variable intensity (Ettinger Feldman, Edward C.Feldman 2017) , likely to be best heard on the right side of the thorax, at the third or fourth IC, which is the PMI for the TV in dogs (Aronson and McCaw 1984). One should know that severe insufficiency of the left AV valve commonly irradiates both dorsally and to the right thorax, challenging a reliable tricuspid regurgitation diagnosis. A soft murmur in old small breed dogs frequently indicates the presence of mild MR. Generally, the progression of the disease leads to an increasing louder murmur, eventually overshadowing the second heart sound (Francis Smith, Larry Tilley, Mark Oyama 2015). Especially in severely affected dogs, a palpable precordial thrill might be present, which classifies the murmur intensity as grade V or higher and is equally correlated with an increased risk of CHF and PH (Ljungvall et al. 2014; Francis Smith, Larry Tilley, Mark Oyama 2015). The subjective classification of the murmur's intensity is done as described in table 1.

Grade	**Murmur characteristics**
Grade I	Soft murmur requiring concentration and silent environment to be heard.
Grade II	A soft murmur that is constantly audible over one valve area, exclusively.
Grade III	Moderate intensity murmur, readily auscultable, radiating to multiple valve locations.

Grade IV	Loud murmur without a precordial thrill, frequently radiating to both sides of the chest.
Grade V	Loud murmur with a precordial thrill (vibration can be palpable on the chest wall).
Grade VI	Loud murmur with a precordial thrill. Audible with the stethoscope away from the chest.

Table 1 – Grading scale of cardiac murmur intensity. (DeFrancesco 2012)

Before reaching CHF, dogs are not expected to present significant respiratory abnormalities secondary to MMVD. Decompensated dogs undergoing CHF might present abnormal lung sounds, such as crackles and increased vesicular murmur (Schober et al. 2011; Ljungvall et al. 2013). Both tracheal collapse and chronic bronchitis are highly prevalent in breeds predisposed to MMVD, challenging the ethologic diagnosis behind these dogs' respiratory signs. Patients with advanced MR are more likely to present poor body condition, a loud heart murmur, and an increased heart rhythm than those affected exclusively by respiratory tract disease (Francis Smith, Larry Tilley, Mark Oyama 2015). A respiratory rate superior to 30 breaths per minute at rest and sleep is expected when pulmonary congestion and oedema occur (Schober et al. 2011; Ljungvall et al. 2013) and from all available diagnostic strategies is considered to have the highest predictive value for imminent clinical decompensation (Keene et al. 2019). Clinicians can encourage owners of dogs with sub-clinical MMVD to routinely monitor their dog's sleeping and resting respiratory rate. This strategy might permit early detection of the clinical status deterioration, allowing a timely intervention by the clinician and improving the dog's welfare (Ljungvall et al. 2013; Ljungvall et al. 2014).

The strength of the femoral arterial pulse is frequently normal in dogs with MR. A diminished pulse strength will accompany a decrease in cardiac output, and pulse variations can be sensed in the case of arrhythmias.(Francis Smith, Larry Tilley, Mark Oyama 2015).

The perfusion time and the colouration of the mucous membranes (MM) should be assessed. An increase in perfusion time above 2 seconds suggests severely low cardiac output, rarely seen even with advanced CHF. Changes in the colouration of MM are also rare. They might happen in very advanced stages of chronic disease or acute cases. A pale MM colour indicates an insufficient stroke volume from the LV, while blueish MM (cyanotic) might result from vast congestion and oedema in the lung parenchyma(Francis Smith, Larry Tilley, Mark Oyama 2015; Reinero et al. 2020).

Abdominal palpation is mostly normal, but in a few patients with TR, extreme disease progression might lead to ascites and organomegaly. A visible jugular pulse may be seen in those dogs. (Francis Smith, Larry Tilley, Mark Oyama 2015; Ettinger Feldman, Edward C.Feldman 2017).

2.8.3. THORACIC RADIOGRAPHY

Radiographing the dog's cardiovascular system can have different indications, such as detecting and staging specific cardiac diseases, assessing the presence of PE, monitoring the heart disease progress, or evaluating the response to a particular therapy. In the case of MMVD, thoracic radiography is frequently the primary complementary diagnostic technique used to approach the disease. A major X-ray issue on cardiac evaluation in dogs is the wide variation of the normal cardiac silhouette appearance in dogs, impeding a precise evaluation of the heart's function and morphology in this species (Thrall , Widmer, William R.,, 2018). Besides the normal interindividual variations, the cardiac silhouette is regular in shape and dimensions in the early MMVD stages. However, when symptoms secondary to MR are present, an enlarged cardiac silhouette is expected on the X-ray image. Even though thoracic radiography has limited capability to delineate the cardiac chambers, significant LA enlargement is generally assessed with certainty. This fact grants value to this imaging technique since the expansion of this chamber predominantly precedes the development of PE. Consequently, a radiologic diagnosis of left-sided cardiogenic PE due to MR can hardly be sustained without radiologic evidence of LA enlargement. (Francis Smith, Larry Tilley, Mark Oyama 2015).

The cardiac silhouette is mainly analysed on a qualitative basis. However, there is a quantitative method, known as the vertebral heart scale (VHS), which takes the effect of body proportions into account when assessing the size of the cardiac silhouette (Buchanan 2000). The VHS method measures the long and short axis of the cardiac silhouette and adds them to estimate its size in terms of the number of vertebral bodies dorsal to it, beginning with T4. The average VHS value is 9,7 for healthy dogs, while 95% of dogs have a VHS value between 8,7 and 10,7. A wide variation is seen between breeds, sex, and individuals, making the VHS relatively ineffective as a diagnostic tool for individual dogs (Birks et al. 2017). In addition, the cardiac and respiratory cycles together can create a variation of up to 1.0 within multiple measurements of the same dog’s heart (Thrall , Widmer, William R.,, 2018). Finally, and following this combination of reasons, there is no evidence of VHS being superior to the subjective radiographic assessment of the heart size.(Lamb et al. 2001) Nonetheless, the VHS can be helpful to monitor the progression of the cardiac disease and the response to a particular treatment by comparing different radiographs of a single patient over time (Hansson et al. 2005; Lord et al. 2010; Boswood et al. 2020). A nonlinear increase of 1,5 VHS over a period of 6 to 12 months has been described as anticipating CHF in dogs with MR by various studies (Lord et al. 2010; P.F. Lord, K. Hansson, C. Carnabuci, C. Kvart 2012; Boswood et al. 2020).

Within the lateral (LL) view, the dorsocaudal aspect of the cardiac silhouette changes in shape, secondary to the LA augmentation. In contrast to the normal curvature of the dorsocaudal cardiac border towards the trachea's bifurcation, this border tends to straighten and run more dorsally or dorsocaudally. LA dilation also leads to dorsal displacement of the tracheal bifurcation, possibly

leading to compression of one (mostly the left) or both bronchi. The compression of the bronchi might lead to narrowing of the anatomical structure, particularly in the case of concomitant bronchomalacia. The dilated LA will more likely contribute to the narrowing of diseased bronchi rather than a normal one, with dynamic airway collapse being a frequent outcome of the synergism of both pathologic processes (Francis Smith, Larry Tilley, Mark Oyama 2015; Thrall , Widmer, William R.,, 2018). When this happens, cough might surge and mislead clinicians into misdiagnosing CHF. In a study involving 206 dogs with MMVD, no statistical correlation was found between PE and cough (Ferasin et al. 2013). Considering this, when coughing is present in a dog with a cardiac murmur, differential diagnostics other than CHF should always be considered (Thrall , Widmer, William R.,, 2018).

In the ventrodorsally and dorsoventral projections, the LA augmentation displacing the bronchi might also be noticeable. Enlarged tracheobronchial lymph nodes should be considered a differential for the displacement of the major bronchi in these projections. A significant difference between these two pathological processes can be observed in the LL projections. In those, the LA moves the tracheal bifurcation dorsally, while the tracheobronchial lymph nodes are located dorsally to the airway, ventrally relocating the bronchi. (Thrall , Widmer, William R.,, 2018)

Radiographic signs of LV enlargement due to MMVD are typically seen jointly with signs of CHF and frequently lead to an overall enlarged cardiac silhouette.(Thrall , Widmer, William R.,, 2018)

Increased post-capillary PH might be perceived radiographically with the presence of pulmonary venous distention. This finding is highly suggestive of venous congestion in a dog with severe MR and typically precedes the surge of PE (Borgarelli et al. 2015; Francis Smith, Larry Tilley, Mark Oyama 2015; Thrall , Widmer, William R.,, 2018), mainly in the peri-hilar region, with a predilection for the right caudal lung lobe. Oedema fills the interstitial lung tissue at its onset, but it will eventually invade the alveolar spaces as the disease progresses (Francis Smith, Larry Tilley, Mark Oyama 2015). When tissue fluid intrudes the alveoli, it creates a contrast with air-filled structures, such as the bronchi, which is described as an air bronchogram (Francis Smith, Larry Tilley, Mark Oyama 2015; Thrall , Widmer, William R.,, 2018). Combined with radiographic evidence of LA enlargement, pulmonary alveolar opacities are diagnostic of left-sided CHF and have almost invariably etiologic responsibility for signs of respiratory distress and the auscultation of crackles during the physical exam (Chiavegato et al. 2009; Borgarelli et al. 2015; Francis Smith, Larry Tilley, Mark Oyama 2015; Thrall , Widmer, William R.,, 2018).

Radiographic signs of RA enlargement are usually hard to identify in dogs. In contrast, a significant right ventricle enlargement might be more accessible to perceive following severe TR due to PH in MMVD patients. The enlargement of this chamber, together with an increasing RA, accompanies a previously enlarged left heart in MMVD patients, leading to a progressively more

evident generalized cardiomegaly. Echocardiography should always be used to get a definitive diagnosis. A broadly enlarged cardiac silhouette can have different grounds, even in dogs with MMVD, such as hemopericardium due to atrial rupture (Thrall , Widmer, William R.,, 2018). Radiographic signs of right-sided CHF might include enlargement of the caudal vena cava, hepatomegaly, splenomegaly, ascites and occasionally pleural effusion (Ettinger Feldman, Edward C.Feldman 2017; Thrall , Widmer, William R.,, 2018; Reinero et al. 2020).

2.8.4. ECHOCARDIOGRAPHY

US can be used to diagnose and monitor MMVD progression (Schober et al. 2010). An echocardiographic technique needs to be adopted and used consistently to obtain the same views within the same sequence in each patient. The image planes are frequently obtained with the transducer on the right parasternal (RPS) window. Views obtained from the opposite recumbency in left apical windows (caudal and cranial left parasternal views) are mostly used for Doppler echocardiography (Francis Smith, Larry Tilley, Mark Oyama 2015).

To enable accurate timing of measurements, simultaneous ECG should be performed together with the echocardiographic examination. More, the simultaneous use of the ECG during echocardiography allows a prolonged time of screening for arrhythmias, which would not be feasible with the classic ECG exam on a paper trace (Francis Smith, Larry Tilley, Mark Oyama 2015).

2.8.4.1. Two-dimensional ultrasonography

In real-time two-dimensional echocardiographic (RT2DE) image formation, a sector-shaped beam of US waves is reflected and refracted by the interfaces of cardiac tissue, hence providing a two-dimensional (2D) cross-sectional (tomographic) image in real-time (Francis Smith, Larry Tilley, Mark Oyama 2015). This echocardiographic method is widely available worldwide and requires little data analysis to evaluate the heart function (Monaghan 2006; Dickson et al. 2017). It accurately identifies valvular features such as thickening, increased echogenicity, ruptured chordae tendineae and systolic protrusion of the leaflets into the LA (Bhave and Lang 2011; Dominique Penninck 2015).

2.8.4.2. Tree-dimensional echocardiography Mode

Dekker et al. were the first to describe three-dimensional echocardiography (Dekker et al. 1974), which was followed by real-time three-dimensional echocardiographic (RT3DE), first reported in 1990 (von Ramm and Smith 1990; SHEIKH et al. 1991). RT3DE is apprised as more accurate than RT2DE in various functions, such as evaluating valvular anatomy in humans (Bhave and Lang 2011) and the LA dimensions and function in dogs. Nonetheless, most veterinarian clinicians performing echocardiographic evaluations do not have access to the expensive and time-consuming technology necessary to analyse the data obtained by three dimensional (3D) echocardiography

(Tidholm et al. 2011; Tidholm et al. 2013; Dickson et al. 2017). Besides that, extra accuracy is not required in most cases since simpler and more efficient methods, such as 2D echocardiography, can successfully differentiate groups of patients by different clinical statuses (Dickson et al. 2017).

2.8.4.3. Motion mode

Differently from the RT2DE, the one-dimensional time-motion (M-mode) echocardiography uses only one narrow beam of US, whose echoes are displayed in the form of a distance-time graph. Superior time resolution and the possibility of displaying multiple cardiac cycles and different cardiac cycle moments in one image are the main advantages of this mode (Francis Smith, Larry Tilley, Mark Oyama 2015). It is mostly used for time-dependent, short-axis measurements of the heart, such as chamber dimensions and motion captured in RPS (Dominique Penninck 2015).

2.8.4.4. Doppler echocardiography

Doppler ultrasonography is based on the interaction of US with moving particles (e.g., moving red blood cells), leading to frequent changes in the echoes received by the probe, with this phenomenon being designated as the “Doppler effect” (Boote 2003; Francis Smith, Larry Tilley, Mark Oyama 2015). Besides providing information about the presence, direction, and velocity of the blood flow (Dominique Penninck 2015), the US Doppler can also characterize myocardial motion (Francis Smith, Larry Tilley, Mark Oyama 2015). Doppler techniques are primarily used in combination with RT2DE and can assess MR severity and elucidate its mechanisms (Zoghbi et al. 2017). In the particular case of MMVD, several Doppler modes are helpful, the most common being Colour Doppler echocardiography, together with the pulsed and continuous wave Doppler for exact measurements (Zoghbi et al. 2017).

2.8.4.4.1. Spectral Doppler echocardiography

Spectral Doppler allows the determination of blood flow velocities by parallel aligning a cursor in a particular region of interest on an RT2DE image, which is then presented as a graphic of blood flow velocity versus time. Pulsed wave, continuous wave and high pulse wave are three variations of spectral Doppler echocardiography (Francis Smith, Larry Tilley, Mark Oyama 2015). A critical factor for a correct assessment of the flow velocities is an optimal alignment that should be parallel or, in the worst-case, within 20 degrees of the flow (Francis Smith, Larry Tilley, Mark Oyama 2015).

2.8.4.4.1.1. Pulsed wave Doppler Echocardiography

In the pulsed wave Doppler (PWD), pulses of US are produced by the transducer, which at different times acts as a receiver or transmitter (Francis Smith, Larry Tilley, Mark Oyama 2015). The round-trip time of the US allows the determination of the tissue from which the measured velocity arises, with the maximum depth determining a specific pulse-repetition frequency (Dominique

Penninck 2015). PWD can only measure accurately until an utmost velocity limit, that when surpassed, leads to the production of a phenomenon called "aliasing", where blood flow will appear both positively and negatively in the display, with the signals wrapped around the baseline (Francis Smith, Larry Tilley, Mark Oyama 2015).

2.8.4.4.1.2. Continuous-wave Doppler Echocardiography

In opposition to PWD, continuous-wave Doppler (CWD) emits and receives US waves simultaneously and continuously and does not suffer from velocity ambiguity. On the negative side, CWD does not allow the determination of tissue depth from where velocities are originating (Dominique Penninck 2015; Francis Smith, Larry Tilley, Mark Oyama 2015). One of the main applications for this mode is the indirect assessment of PH, when TR is present, which can be accessed with two-dimensional (2-D) imaging and colour Doppler transducer (L. Hatle 1964; Ge et al. 1992).

2.8.4.4.2. Colour Flow Doppler Echocardiography

Colour flow Doppler (CFD) is a variation of the PWD mode, in which velocity characteristics are encoded not in a graphic but within a colour scheme. In this mode, the different colours representing the direction and velocity of the blood flow are superimposed on the RT2DE in a user-selected colour display. Blood flow moving away from the transducer is represented in blue, whereas when the movement is towards the probe, the colour red is seen. The most significant advantage of this method is that it enables the ultrasonographer to visualize the source of velocities in the heart and great vessels directly within the anatomical framework of the image (Dominique Penninck 2015; Francis Smith, Larry Tilley, Mark Oyama 2015).

2.8.5. Echocardiographic diagnosis and management of MMVD

The echocardiographic evaluation of MMVD in dogs is similar to that performed in humans (Lancellotti et al. 2013; Zoghbi et al. 2017). The severity of the disease can be assessed through measurements of cardiac remodelling (left side AV enlargement), MR (size of the regurgitation jet by colour-Doppler, effective regurgitant orifice area, proximal isovelocity surface area, vena contracta, and regurgitant fraction), and LV filling pressure (mitral inflow, isovolumetric relaxation time, pulmonary venous flow, regurgitant jet profile, and tissue Doppler echo variables) (Kittleson and Brown 2003; Gouni et al. 2007; Schober et al. 2010; Chetboul and Tissier 2012; Di Marcello et al. 2014; Sargent et al. 2015; Baron Toaldo et al. 2016; Larouche-Lebel et al. 2019). To assess the presence and severity of tricuspid endocardiosis, most of those parameters and techniques can be applied. In case of significant TR, PH can also be indirectly assessed (Francis Smith, Larry Tilley, Mark Oyama 2015).

2.8.5.1. Assessment of left heart enlargement and cardiac remodelling

The LA dimension and volume are commonly assessed by RT2DE. (Tidholm et al. 2011; Ettinger Feldman, Edward C.Feldman 2017). There are several views from which the LA can be examined. However, the preferred view is the RPS short axis showing the aortic root, the LA body and the auricle. From this view, the LA diameter can be compared to the diameter of the aortic root (Ao) in early diastole, which is relatively constant for dogs of the same size, thus providing the LA:Ao ratio that in a healthy or a B1 stage dog should be inferior to 1,60 (Hansson et al. 2002). The LA:Ao ratio increases with disease progression, with most dogs reaching CHF presenting a LA:Ao ratio greater than 2 (Häggström et al. 1994).

LV anatomical dimensions, volume, and function can be assessed subjectively or using various echocardiographic quantitative techniques. Traditional assessments of this chamber are done through M-mode or 2D echocardiography images (Meyer et al. 2013). Presently, LV volume reference ranges were exclusively determined for a few breeds (Kittleson et al. 1984; Lord et al. 2010; Ljungvall et al. 2011), limiting the demonstrated potential of its assessment in dogs with MMVD (Tidholm et al. 2010; Ljungvall et al. 2011). Due to this data limitation, the most common variable used to assess LV dimensions is the left ventricular internal dimension in diastole, normalized for body weight (LVIDDn), where the LV short axis (SAx) is divided by the weight-based aortic root area from M-mode measurements (Dominique Penninck 2015). Reaching an LVIDDn of 1,7 is one of the criteria for dogs with MMVD to be classified as stage B2 (Keene et al. 2019).

Within MMVD progression, the end-systolic short-axis LV internal dimension increases at a low pace, in contrast to the LV end-diastolic short-axis dimension and volume. In the latest, a more significant increase characterizes the disease progression (Kittleson et al. 1984; Lord et al. 2010; Ljungvall et al. 2011). A sphericity index, which can be obtained by dividing the end-diastolic volume by the volume of a sphere, can be used to access changes in LV shape in images obtained by either 2D or 3D echocardiographic (Mannaerts et al. 2004; Ljungvall et al. 2011). Although not routinely used, this index can be helpful to evaluate LV remodelling progression in dogs (Ljungvall et al. 2011).

Throughout the progression of the disease, cardiac remodelling allows the LV to maintain a proper forward stroke volume, regardless of the concomitant reduction in the systolic myocardial function (Kittleson et al. 1984). Identification of systolic dysfunction using echocardiographic modalities is challenging in dogs with MMVD. Most of the variables used to access systolic function, such as fractional shortening, ejection fraction, and mean velocity of circumferential shortening, which are ejection phase indices, are dependent on intrinsic contractility and can be influenced by hemodynamic load and sympathetic stimulation, which can potentially mask substantial myocardial dysfunction in diseased dogs (Bonagura and Schober 2009). Dogs with a mild stage of the disease

have preserved ejection phase indices, but when moderate and severe stages are reached, these values tend to rise to compensate for the volume overload. Consequently, a regular fractional shortening (FS) in a dog with severe endocardiosis indicates diminished myocardial contractility. It has been suggested that the best ultrasonographic accessed parameter to infer systolic dysfunction due to MR is the LV end-systolic dimension or volume, which is inversely proportional to the systolic function (Kittleson et al. 1984).

2.8.5.2. Assessment of mitral regurgitation

With spectral and CFD, valve regurgitation can be detected and quantified. Ideally, the US beam should align with the MR flow, which is generally best accomplished from the left apical four-chamber view. Considering that the flow direction depends on the morphology of the leaflet and the orientation of the regurgitant orifice, which might vary, it can be helpful to use different views to obtain the best alignment possible(Dominique Penninck 2015).

RT2DE plays a significant role in the ultrasonographic assessment of mitral insufficiency by confirming that MMVD is the cause of MR (Dominique Penninck 2015). Ultrasonography allows the evaluation of MR extent with qualitative and semiquantitative techniques and assessments, such as the size of the regurgitant jet, the effective regurgitant orifice area, proximal isovelocity surface area, the regurgitant fraction and the vena contracta (Kittleson and Brown 2003; Dominique Penninck 2015). The easiest method is to access MR through visual inspection using CFD. As an alternative, the ratio between the regurgitant jet area and the LA area can be used to provide a seemingly quantitative estimation of the MR range. Unluckily, the results of these assessments are greatly influenced by the CFD control settings, chamber, jet geometry, and hemodynamic conditions (Dominique Penninck 2015).

The MR Jet narrowest part is known as the vena contracta, which is found at, or just downstream of the orifice of a regurgitant valve, where the blood velocity of the jet is highest (Fehske et al. 1994). Its measurement allows a more straightforward method to assess MR severity. It is measured with CFM, generally in an RT2DE image in the left apical four-chamber view (Sargent et al. 2015)(Gouni et al. 2007). Since the shape of the mitral orifice may vary considerably, 3D colour echocardiography provides better estimates of the effective regurgitant orifice by measuring the vena contracta (Fehske et al. 1994). Another useful parameter that can be calculated is the MR fraction, which is the percentage of total LV stroke volume that enters the LA during systole and can be calculated by dividing the regurgitant stroke volume by the total LV stroke volume (Kittleson and Brown 2003). Increased values of effective regurgitant orifice area, the regurgitant volume and regurgitant fraction are positively correlated with MMVD worsening (Kittleson and Brown 2003; Dominique Penninck 2015; Larouche-Lebel et al. 2019).

CWD can also be used to assess systolic function via regurgitant jet velocity, which is then used to obtain the systolic transmitral pressure gradient. Using the simplified Bernoulli equation allows us to estimate the referred gradient: peak MR gradient= 4x(MR velocity). In dogs with MMVD without significant myocardial failure, the regurgitant jet has an average velocity of 5 to 6 meters per second (m/s). In dogs with systemic hypertension, the velocity is generally increased, while decreased velocities are measured in patients with hypotension, significantly increased LA pressure, and myocardial infarction. Accordingly, dogs with acute fulminant CHF have regurgitant jets that are typically below 5 m/s in velocity (Ettinger Feldman, Edward C.Feldman 2017).

2.8.5.3. Left side diastolic dysfunction

Due to its complexity, no echocardiographic measurement will give a complete overview of the diastolic function. However, a range of specific measurements might be used to assess or clarify certain diastolic features (Francis Smith, Larry Tilley, Mark Oyama 2015). Even though ventricular filling pressures and diastolic function can be assessed with Doppler ultrasonography (Bonagura and Schober 2009), the resulting measurements are ambiguous due to the age-related changes in diastolic function and the influence of the growing preload that accompanies MMVD progression. Transmitral flow velocity, isovolumetric relaxation time (IVRT), and the ratio between early diastolic flow velocity (E-vel) and the IVRT (E/IVRT) are the variables accessing the diastolic function, that best predict left-sided CHF in dogs with MMVD (Schober et al. 2010).

The instant pressure gradient across the MV is reflected through the transmitral flow velocity. This parameter is frequently accessed with PWD in a 4-chamber left apical view (Gabriel and Klein 2009; Dominique Penninck 2015; Francis Smith, Larry Tilley, Mark Oyama 2015). There is a dynamic change of the ventricular filling pattern within MMVD progression. The major downside of accessing this variable alone is the "pseudonormal" stage (generally occurring in patients with moderate MMVD), where the transmitral parameters resemble the ones of a healthy young dog. Therefore, transmitral flow patterns (figure 2) should be accessed together with CS and other echocardiographic measurements (Francis Smith, Larry Tilley, Mark Oyama 2015).

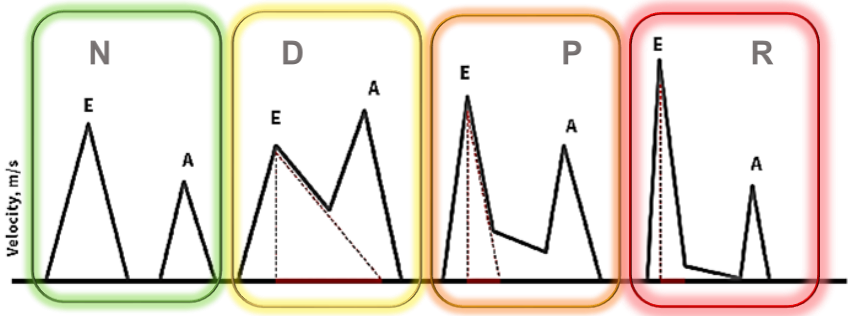


Figure 2 – Transmitral filling patterns (addapted from Fields 2018). Abbreviations: N – normal pattern, D – delayed relaxation pattern, P – pseudo-normal pattern, R – Restrictive pattern.

The interval between the end of aortic ejection and the beginning of ventricular filling is the IVRT. It is measured recurring to simultaneous recording of both the Ao and the mitral flow with spectral Doppler (de Madron 2015). In healthy dogs, the expected values of IVRT decrease with heart rate and increase with age (Schober and Luis Fuentes 2001), while in MMVD, a progressive decrease is normally seen along the course of the disease. The ratio of mitral E wave to IVRT has been correlated with LA pressure and end-diastolic pressure in several studies (Schober, Stern, et al. 2008; Schober, Bonagura, et al. 2008; Eriksson et al. 2010), one of which suggested that an IVRT of less than 45ms and an E/IVRT ratio superior to 2.5 might be indicators of the presence of CHF in dogs with severe MMVD (Eriksson et al. 2010). E: IVRT was also identified as the best Doppler predictor of decreased filling pressures to monitor the treatment response of dogs experiencing CHF (Schober, Bonagura, et al. 2008; Schober, Stern, et al. 2008).

The three main phases of diastolic dysfunction (figure 2) are the delayed relaxation, followed by the pseudoformal stage, and the final phase, where restrictive dysfunction occurs. The E-vel is higher than the late diastolic transmitral flow velocity (A-vel) in a healthy young dog, leading to an E/A ratio higher than 1. When there is delayed relaxation, inversion of the normal parameters occurs. These changes might be impossible to identify in case of fusion of both waves and if AF occurs. IVRT (which increases with delayed relaxation) can be measured in any circumstance, allowing the operator to overcome the ambiguous result. In the stage of pseudo normalization, both the E/A ratio and the IVRT normalize, and more complex echocardiographic measurements and techniques, together with the clinical presentation, should be accessed. In the final diastolic stage, there is restrictive dysfunction associated with a high LAp, further leading to a significant increase in the E-vel. In contrast, the lack of atrial compliance leads to a progressively shorter A wave at this stage. Both contribute to the increase of the E/A ratio value, frequently higher than 2 in the end-stages of MMVD (Yamamoto et al. 1996; Schober and Maerz 2006; Gabriel and Klein 2009). Contrarywise the IVRT decreases with the heightened LAp (Schober, Bonagura, et al. 2008).

2.8.5.4. Echocardiographic assessment of tricuspid valve insufficiency and pulmonary hypertension

The myxomatous lesions on the TV are echocardiographically similar to those described previously for the MV. The diagnosis of PH can be made indirectly when the peak of the TR flow velocity is higher than 3m/s, which corresponds to a TR gradient >36 mmHg (Borgarelli et al. 2015). All the measurements needed to estimate PH can be accessed through short-axis view, with spectral Doppler (Ge et al. 1992)**.** Echocardiography alone is incapable of detecting PH in the absence of TR, which in that case can only be detected through cardiac catheterization, which is generally not

practicable (Ge et al. 1992; Borgarelli et al. 2015)**.** Other echocardiographic findings indicating PH include dilation and hypertrophy of the RV, pulmonary artery dilation and paradoxical motion of the interventricular septum (Borgarelli et al. 2015).

2.8.6. Biomarkers

Substances produced by a specific organ or tissue in proportion to the presence or severity of the damage or disease are called biomarkers. Their clinical use is related to the possibility of obtaining information that is not readily accessible. Changes in ventricular composition, volume, and mass occur during cardiac remodelling, negatively affecting cardiac performance. This remodelling process stimulates the release of particular biomarkers, such as natriuretic peptides and troponins (Oyama et al. 2008; Oyama et al. 2009; Ljungvall et al. 2010).

Natriuretic peptides are increased in the bloodstream of patients with significant cardiac remodelling or ventricular dilation. They can be used to distinguish symptoms due to cardiac disease from symptoms caused by a primary pulmonary condition (Francis Smith, Larry Tilley, Mark Oyama 2015). To determine the cause of CS in dogs with MMVD can be helpful to measure the serum N-terminal pro-B-type natriuretic peptide molecule (NT-proBNP) concentrations through commercially available tests. Dogs with CS due to CHF have higher serum concentrations of NT-proBNP than dogs with CS caused by primary pulmonary disease, although predictive values have not been well established yet. Normal values of NT-proNBP suggest that signs such as exercise intolerance, cough and dyspnea are not caused by CHF (Oyama et al. 2008; Oyama et al. 2009). In MMVD dogs, a significant increase in NT-proBNP generally occurs 3 to 6 months prior to CHF development. Other causes such as renal insufficiency and PH can lead to increased values of NT-ProBNP, and consequently, results of this test should always be used as complementary evidence. Additional uses for the NT-ProBNP biomarker may include monitoring the effectiveness of PH therapy (Francis Smith, Larry Tilley, Mark Oyama 2015).

Troponins are released into the circulation due to myocardial injury. When the release rate exceeds the rate of synthesis, myocardium contractility becomes compromised (Van Der Laarse 2002). As a cardiac biomarker in dogs, cardiac troponin I (cTnI) has been shown to be significantly increased in dogs with cardiac injury of various aetiologies (Schober et al. 2002; Oyama and Sisson 2004; Segev et al. 2008). Its variation with age and sex is also well known and should be considered when analyzing cTnI concentrations. Nevertheless, cTnI holds its potential to improve the understanding of overall cardiac remodelling in dogs with MMVD (Ljungvall et al. 2010), with its clinical use being rather limited within this disease (Francis Smith, Larry Tilley, Mark Oyama 2015).

2.8.7. Electrocardiography

The heart's electrical activity can effectively be recorded from the animal's body surface and represented through the Electrocardiogram (Fye 1994; Werner 2014). Findings correlated with endocardiosis range from normal tracings to marked abnormalities in rate, rhythm, or configuration of complexes. In terms of diagnosis and management of MMVD, the ECG has little importance, having most of its value in the analysis and classification of the various arrhythmias found within the course of the disease (Ettinger Feldman, Edward C.Feldman 2017).

Dogs with mild to moderate MR can usually preserve a sinus arrhythmia. Typically, when canine CHF is reached, sinus arrhythmia is lost, and the development of sinus tachycardia occurs. Supraventricular premature beats are frequent in dogs with endocardiosis (Crosara et al. 2010; Schaldemose et al. 2014), generally with no, to little hemodynamic compromise. Rarely, other arrhythmias, such as AF, paroxysmal supraventricular tachycardia, AV blocks of various degrees, ventricular premature beats, and ventricular tachycardia, might occur. These arrhythmias are primarily found in severely ill MMVD patients, being chiefly linked to a poor prognosis (Ettinger Feldman, Edward C.Feldman 2017).

A recent study comparing electrocardiographic parameters in dogs with different stages of MMVD found that the P wave and certain correct QT intervals (QTc1 and QTc2) were significantly longer in stage C than in staged B dogs. Selecting a cutoff of 43,5 ms for the P wave has shown an accuracy of 65% at detecting CHF, with a specificity (SPT) of 90% and a sensitivity (SSB) of 63%. For QTc1, a cutoff of 307,8ms had a diagnostic accuracy of 78%, while for QTc2, the cutoff selected was 239,2ms, and the diagnostic precision was 77%. The increased duration of the P wave and QT intervals in dogs with MMVD might therefore facilitate the prediction of CHF. The study showed an increased potential of the ECG as a readily available, inexpensive screening tool for predicting CHFCHF (Na et al. 2021).

2.8.8. Laboratory workups and systemic arterial pressure

Laboratory workup such as haematological and biochemical might be altered in cardiovascular disease, especially in CHF, and even though they are not specific, they can be helpful for monitoring and prognostic purposes (Gavazza et al. 2020). From stage C, a basic test including at least packed cell volume (PCV), total serum protein, creatine, Blood-urea-nitrogen (BUN), electrolyte concentrations, and specific gravity should be obtained as soon as possible, since renal failure (of variable degree) frequently accompanies MMVD in older dogs (Keene et al. 2019).

Hypokalemia may occur in advanced cardiac disease due to potassium translocating across the cell membrane (due to sympathetic stimulation) and by renal losses associated with high levels of aldosterone (Virginia Luis Fuentes,Lynelle R. Johnson 2010; DiBartola Elsevier (Amsterdam)., 2012; Gavazza et al. 2020). Oliguria following a decrease in renal function can instead lead to

hyperkalemia and acidosis due to the kidneys' impaired excretion of cations (K+ and H+) (Gavazza et al. 2020). On the other side, advanced congestive CHF with or without concomitant diuretic therapy is the most common cause of moderate to marked hyponatremia in dogs (DiBartola Elsevier (Amsterdam)., 2012; Gavazza et al. 2020).

MMVD dogs frequently have a high azotaemia value (urea and creatinine), which increases with CHF severity. The correlation found between BUN and creatinine values with the ACVIM stages is of primary clinical importance while treating patients with severe heart disease (Ettinger Feldman, Edward C.Feldman 2017). Nonetheless, a relationship between renal function decline and cardiac disease evolution is not established in dogs (Nicolle et al. 2007; Martinelli et al. 2016). A study including 114 dogs with MMVD revealed that PCV and haemoglobin (Hb) amount decrease with the severity of the disease. When present, anaemia was associated with a poor outcome. The severity of the anaemia was also correlated with the degree of CHF and the increase of blood creatinine, leading the authors to suggest the occurrence of a “cardiorenal anaemia syndrome” in dogs, similarly to what occurs in humans (Yu and Huang 2016).

Red blood cell distribution width (RDW) is a quantitative measure of anisocytosis and a widely available marker to predict adverse outcomes of left CHF and PH in humans (Levy et al. 2006; Pocock et al. 2006). A recent study involving 146 dogs with MMVD showed that even within reference intervals, RDW is an independent predictor of poor outcomes in dogs with mitral endocardiosis (Guglielmini et al. 2021). Another study with 162 human patients, 62% with PH, showed a good correlation between RDW and a fatal outcome if severe PH was present, with a superior prognostic value than the use of NT-proBNP (Hampole et al. 2009). An attempt to demonstrate the exact correlation in dogs has failed, with RDW not correctly predicting PH severity (Swann et al. 2014).

Systemic hypertension from concomitant diseases can lead to severe irreversible organ damage in dogs with MMVD (Acierno et al. 2018). The same can happen with hypotension, which may occur within the end-stages of MMVD, associated with circulatory shock from inadequate tissue perfusion and oxygen delivery to the tissues (Petit et al. 2013). Consequently, frequent systolic arterial blood pressure (SABP) measurements are recommended in cardiopathic dogs, including those asymptomatic (Atkins et al. 2009). Falling of SABPs in advanced stages of the disease might occur due to medical treatment (e.g., loop diuretics and vasodilators), flow reduction due to severe MR, hypercontractility due to extent myocardial remodelling, or a combination of those factors (Petit et al. 2013).

2.9. Treatment and prognosis of MMVD

2.9.1. Medical therapy

Medically, the disease cannot be cured. Treatment is primarily directed toward managing the hemodynamic effects of severe MR with left atrial enlargement, congestive CHF, and possibly PH (Gordon et al. 2017).

No medical treatment is recommended for dogs in Stage A or B1 (Keene et al. 2019), while at stage B2 Pimobendan is recommended at a dose of 0,25-0,30 mg/ Kg orally (PO) BID (Boswood et al. 2016; Boswood et al. 2018). Some specialists also recommend using angiotensin-converting enzyme inhibitors (ACEI) for patients in which significant LA enlargement occurs between consecutive monitoring examinations (Kvart et al. 2002; Atkins et al. 2007; Pouchelon et al. 2008). Cough suppressants are also recommended by some specialists when atrial enlargement is thought to be the cause of the symptomatology (Keene et al. 2019).

Stage C includes patients with acute symptomatology requiring hospital-based treatment (C1) and patients requiring chronic, home-based treatment (C2). In acute, hospital-based treatment, furosemide 2 mg/kg IV or IM should be administered hourly until the patient's respiratory rate and effort markedly decrease or a dosage of 8 mg/kg in 4 hours is reached (Keene et al. 2019). After the initial bolus, furosemide can also be administered at constant rate infusion (CRI) at a dosage of 0,66-1mg/kg/hour, in case of life-treating PE (Adin et al. 2003; Adin et al. 2018) . After the onset of diuresis, free access to water is recommended by some specialists.(Keene et al. 2019) Administration of ACEI together with furosemide is recommended in chronic C patients. However, some specialists also use it in the acute stage since it leads to a more significant reduction in capillary wedge pressure than furosemide administration alone (David Sisson et al. 1995). Pimobendan 0,25-0,30 mg/ Kg PO BID should be continued or introduced, knowing that its benefits are maximized with long term administration. Acute patients also benefit from adequate oxygen supplementation, and in case of cavitary congestive signs, mechanical drainage of effusion to relieve respiratory symptomatology. Sedation with opioids alone or combined with anxiolytic drugs can help control anxiety and prevent worsening of respiratory symptomatology. In acute patients that fail to respond to the referred treatment combinations, dobutamine can be administered to boost the left ventricular function, ideally with ECG monitoring. When life-threatening CS are present in C-stage patients, drugs usually reserved for D stage patients are sometimes required. Venodilators, administered CRI, such as sodium nitroprusside, or topical nitroglycerine ointment, can help solve the unresponsive PE.(Sabbah et al. 1993; Nakayama et al. 2007) PO administration of arterial dilators, such as hydralazine and amlodipine, might also be beneficial when venodilators are unavailable (David Sisson et al. 1995).

The home-based treatment for dogs in stage C typically includes PO administration of pimobendan associated with a loop diuretic and an ACEI (Keene et al. 2019). Furosemide is the most used diuretic in this stage, at a dose of 2mg/kg BID, which can be increased if necessary to

ensure respiratory comfort. In scarce cases in which furosemide does not show reasonable symptomatologic control in the acute stage, some specialists prescribe torsemide, which is 10 to 20 times more powerful and can be administered SID (Chetboul et al. 2017). Patients are considered to reach the end-stage of the disease when they chronically need furosemide dosages higher than 8mg/kg per day (or an equally powerful dosage of torsemide) in any administration regime while taking appropriate doses of pimobendane, ACEI, and spironolactone. The use of spironolactone is also recommended as an adjuvant for chronic treatment in stage C dogs, with its primary benefit being the direct antagonism of aldosterone (Bernay et al. 2010; Ames et al. 2017). Treatment with pimobendan should be maintained (Lombard et al. 2006; Boswood et al. 2016). Diltiazem with or without digoxin can control ventricular rate when AF occurs. An average of fewer than 125 beats per minute should be recorded by Holter examination in dogs with well-controlled signs of CHF to confirm antiarrhythmic treatment efficacy. (Gelzer et al. 2009; Gelzer et al. 2015; Pedro et al. 2017). Some specialists recommend cough suppressants and bronchodilators to control persistent CS in stage C MMVD dogs (Keene et al. 2019).

Stage D dogs present CS of CHF when taking the standard treatment for stage C dogs, which, when necessary, may include antiarrhythmic drugs to maintain the mean daily heart rate under 125 bpm (Pedro et al. 2017). Lack of clinical trials accessing drug efficacy in this patient population makes it the most challenging stage to manage, with a wide variety of medications being used. Like the previous stage, stage D management can be segregated into acute hospital-based and chronic home-based treatments (Keene et al. 2019). In either the hospital or at home, higher doses of loop diuretic should be used to decrease respiratory distress in patients whose renal function is not compromised. Torsemide is a common option at this stage since it is more powerful, lasts longer and induces less activation of the renin-angiotensin-aldosterone system than frequent doses of furosemide (Hori et al. 2007).

In the hospital, cavitary centesis should be performed, when necessary, to improve respiratory function and comfort, which can also be boosted with oxygen supplementation and mechanical ventilation (Edwards et al. 2014). In dogs that tolerate significant afterload reduction, arterial vasodilators can be used. In the most severe acute cases that cannot wait for the effect of PO administration, reduction of the afterload with a CRI IV of sodium nitroprusside and ionotropic support with **dobutamine** (especially in hypotensive dogs) are recommended (Sabbah et al. 1993) , if possible, with continuous ECG and arterial pressure monitoring. When PO administration is viable, the previous medication can be replaced (or followed) by administering pimobendan with either hydralazine or amlodipine. This medication should be given together with an ACEI. Even though cardiac output increases typically with the afterload decrease, blood pressure should be monitored to prevent severe and prolonged hypotension. Renal parameters should be accessed 24 to 72 h after introducing these medications (Keene et al. 2019).

In case of significant PH secondary to the left-sided CHF, starting sildenafil at a dose of 1-2mg/kg PO TID is recommended (Reinero et al. 2020). PO administration frequency of Pimobendan might be increased to three times a day, in a dosage of 0,3mg/kg. Sone specialists recommended using bronchodilators at this stage to treat cardiogenic PE as part of the hospital-based treatment (Keene et al. 2019).

In home-based treatments, furosemide administration can be increased in dose and frequency, but this can often be unpractical for the owners, and patients' response to furosemide generally decreases with time. With a more prolonged action time and a better general response, torsemide is the best option for end-stage patients, starting at a dosage of 0,1-0,2mg/kg PO SID, up to a maximum of 0,6mg/kg BID, in the most extreme cases. Spironolactone or Hydrochlorothiazide in combination with any loop diuretic can be used in an attempt to improve diuresis in stage D dogs (Bernay et al. 2010; Keene et al. 2019). Like in acute stages, a third daily dose of 0.3mg/kg of pimobendan might be prescribed off-label, with owner agreement. Hydralazine or amlodipine might provide additional hemodynamic benefits and reduce the cough frequency by reducing the afterload. Digoxin should be introduced or continued (from stage C) if no contraindications are present in patients with AF. According to specialist opinion, it can also benefit stage D patients who maintain a sinus rhythm (Gelzer et al. 2009; Keene et al. 2019). In the case of right-sided CHF and echocardiographic evidence of PH, sildenafil may also be helpful for chronic treatment. Cough suppressants and bronchodilators are recommended to treat persistent cough (Keene et al. 2019).

2.9.2. Dietetic therapy

From stage B2, sodium should be mildly restricted in a highly palatable diet with the correct amount of protein and calories to sustain the optimal body condition (Freeman et al. 2006).

Cardiac cachexia is the loss of muscle mass associated with heart failure, accompanied or not by a significant decrease in body weight (BW). The condition is associated with a poor prognosis, and it is easier to prevent than to treat. For this reason, an adequate diet is crucial to prevent cachexia in stage C dogs (Freeman 2009), which need a highly palatable diet with around 60 Kcal/Kg BW to minimize the weight loss, which usually follows CHF (Freeman et al. 1998; Freeman et al. 2003) . Protein should not be restricted in stage C, except for dogs concurrently affected with severe kidney failure (Freeman 2009). Supplementation with omega-3 fatty acids is recommended in case of decreased appetite, muscle loss, or arrhythmia (Freeman et al. 1998). The chosen diet used in stage C patients should be maintained throughout the rest of their lives, with additional effort to reduce sodium intake in later stages, when refractory fluid accumulation occurs (Keene et al. 2019).

2.9.3. Surgical Treatment

Surgical treatment for MMVD consists of MV repair or valvular replacement. Dogs with severe MR have a poor prognosis even with medical therapy. For those, open surgical MV repair, consisting of circumferential mitral annuloplasty and artificial chordal replacement, might be the best option. This procedure confers durability, improving clinical outcomes with no need for long-term anti-thrombotic therapy (Uechi 2012). After the surgery, anatomical and functional normalisation of the heart occurs, accompanied by a matching decrease in the RAAS activity (Cheng et al. 2022).

Severely degenerated MV can be replaced with mechanical or bioprosthetic valves. In the case of MV replacement, long-term survival is promoted by carefully sizing the prosthetic heart valve to the dog and by intense efforts to prevent thrombosis (Uechi 2012). Bioprosthetic valves are less thrombogenic (Terrier 2007) but more likely to calcify or degenerate over time (Uechi 2012).

Surgical intervention is recommended as early as in advanced Stage B2 patients, in centres demonstrating acceptably low complication rates and effective long-term results. The procedure is also recommended in the further stages of the disease under the same conditions. Higher perioperative mortality and lower overall survival are expected for patients in stage D (Keene et al. 2019).

Despite the recognised benefit of the surgical treatments, these are exclusive of very few sites globally and, consequently, not technically, economically, or ethically possible for the wide majority of canine patients (Keene et al. 2019).

2.9.4. Selective breeding

To a certain extent, MMVD can be prevented by selective breeding, with previous attempts to control it having had variable degrees of success (Lundin and Kvart 2010; Birkegård et al. 2016). Nonetheless, breeding programs should be implemented with caution since selection against one disorder trait without considering other selection criteria could increase the incidence of other genetic disorders unless they are managed concurrently. For instance, previous studies have shown that selection against heart disease in the CKCS has increased the prevalence of otherwise rare conditions, such as syringomyelia (Rusbridge 2005; O'Brien et al. 2021).

2.9.5. Prognosis and average survival time

Outcomes from diverse controlled clinical trials are available regarding the medical treatment of dogs with MMVD both before and after CHF. Studies such as these assess treatment effects and identify other variables with prognostic value for patients reaching CHF. The use of prognostic variables with qualitative and quantitative results from clinical studies may aid clinicians and owners in planning and deciding on the best treatment plan for dogs with MMVD (Häggström et al. 2009).

The type of adjunct therapy influences survival. Increased survival times have been reported with pimobendan and ACE inhibitors such as enalapril and benazepril (Häggström et al. 2009). In a study with 194 dogs evaluating the efficacy of pimobendan with conventional therapy on survival and recurrence of PE in dogs with CHF due to MMVD, a survival time 2,5 times higher in dogs taking pimobendan than the dogs not receiving the drug was reported (Mizuno et al. 2017). Another study with 360 MMVD patients accessing the benefit of using pimobendan in B2 staged dogs concluded that this drug can delay CHF by nearly 15 months (Boswood et al. 2016). Other factors such as increasing maintenance dose of furosemide, increasing exercise intolerance, increasing cardiac size and severity of MR (VHS score and LA/Ao ratio), decreasing serum creatinine concentration (likely due to cachexia), and decreasing systolic function are correlated with decreased survival time in MMVD patients (Häggström et al. 2008; Häggström et al. 2009; Sargent et al. 2015). The breed may also impact the outcome after the onset of CHF. A couple of studies showed significantly higher survival times in CKCS that experienced CHF compared with the prognosis of a group with other dog breeds in the same circumstances (Borgarelli et al. 2008; Häggström et al. 2009). Laboratory findings such as anaemia, high RDW, high white blood cells and elevation of the BUN are additional predictors of an adverse outcome in dogs with MMVD (Ingrid et al. 2017; Guglielmini et al. 2021).

The prognosis of dogs that underwent MV repair or replacement is significantly improved, with reported survival times over three years after the procedure. Other studies reported a 93% survival at 38 months following MV repair (Uechi 2012).

3. RETROSPECTIVE ANALYSIS OF 191 DOGS WITH MYXOMATOUS MITRAL VALVE DISEASE BASED TOMAZZO ET AL.'S MITRAL INSUFFICIENCY ECHOCARDIOGRAPHIC SCORE

3.1. Introduction

Mitral Myxomatous valve disease (MMVD) can develop in adult dogs of any age, but its prevalence increases with age, with up to 85% of dogs reaching 13 years old affected. The presence of degenerative valvular lesions does not imply that the dog will die, nor will it develop symptoms due to this affection. Depending on the rate of valvular disease progression relative to other common pathologic conditions that occur late in life and lead to death, the presence of MMVD without CS may or may not alter the course of affected dogs' lives.

Echocardiographic assessment of progressive left-sided heart enlargement and increasing transmitral E wave blood flow velocities were proven to significantly predict MMVD progression rate, useful to determine dogs at higher risk of developing heart failure and cardiac death. However, a

genuinely predictive (sensitive and specific) and widely used risk stratification scheme based on routinely acquired echocardiographic variables is not yet available. The Mitral Insufficiency Echocardiographic (MINE) score, based on 4 of those echocardiographic variables: left atrium-to-aorta ratio, left ventricular end-diastolic dimension normalized for body weight, fractional shortening, and E-wave transmitral peak velocity was created by Tomazzo Vezzosi et al. to fill this gap. The created score includes 4 severity categories: mild (score: 4-5), moderate (score: 6-7), severe (score: 8-11) and late-stage (score: 12-14). A score >8 was shown to predict cardiac death, while a score predictive of heart failure should soon be determined.

This retrospective study was made to test the echocardiographic score within a different patient population, with data collected by multiple operators in a particular clinical environment, thus validating the universal applicability of the MINE score.

3.2. Study goals

This retrospective study was delineated to contribute to establishing a universal easy-to-use echocardiographic classification scheme of MMVD's severity in dogs based on routinely acquired echocardiographic variables. Additionally, this study aims to characterise echocardiographically the MMVD patient population that underwent a cardiac evaluation at the Perugia Veterinary Teaching Hospital (PVTH) from September 2017 to December 2021. For that, the following objectives were established: (I) bibliographic review of MMVD, (II) Gathering of 191 patients' ultrasonographic data, both from the university database and from the daily appointments that occur during the curricular internship (III) Statistic evaluation of the collected data and severity classification of MMVD using the MINE score, proposed by Tommaso Vezzosi et al. (IV) Comparison of the time-limited obtained results with the ones gathered for the original study proposing the MINE score (V) Statistical assessment of the contribution of each echocardiographic variable to the final score and respective severity classification (VI) Selection of specific data to contribute to the next stage of the MINE score studies, in an attempt to establish CHF predictive scores.

3.3. Materials and methods

An observational and retrospective study was conducted. Dogs were recruited at the Department of Veterinary Medicine of the University of Perugia. During their visit to PVTH, pet owners gave their consent for their pet's data treatment for scientific purposes. Therefore, no further approval was sought to employ the echocardiographic data in our study.

3.3.1. Selection of the clinical cases

Clinical databases of the Veterinary Teaching Hospital of the University of Perugia were reviewed for complete echocardiographic evaluations of dogs diagnosed with MMVD between

September 2017 and December 2021, with 191 dogs fulfilling the inclusion requirements. The first echocardiographic evaluation of each dog at the referred institution was selected.

3.3.2. Inclusion criteria

All selected dogs were subjected to a complete physical examination and echocardiographic evaluation, ensuring the diagnosis of MMVD. Case records were obtained, and information regarding breed, age, sex, BW, ACVIM stage, echocardiographic variables, and baseline treatment was obtained and registered in a database. (Vezzosi et al. 2021)

3.3.3. Exclusion criteria

Dogs weighing more than 20kgs, aged less than three years old and having a fractional shortening (FS) of less than 25%, being pregnant, presenting atrial fibrillation, or with any systemic or cardiac disease other than the MMVD, were excluded from the study, similarly to the original MINE Score study (OMSs) (Vezzosi et al. 2021).

3.3.4. Data collection and clinical records assessment

The echocardiographic data were collected during the appointment or retrieved from the clinical records. When any of the MINE score variables were absent in the clinical records, additional measurements were performed in the echocardiographic image database through the MyLab-Desk software, version: 10.0 by the student with the aid of the responsible clinicians.

The patient's cardiac medication prescribed at the date of the echocardiographic evaluation was assessed either directly after the consultation or through the clinical records.

3.3.5. Main differences to the Original MINE score study

There were a few key differences between the current study and the OMSs. First, in the OMSs, performing at least one thoracic radiography at the date of the selected echocardiographic evaluation was an inclusion criterion for all dogs, while in PVTH, alternative methods were used to assess CHF and no inclusion criteria were employed for that matter.

No updates to the original ACVIM classifications given to the patients by the consulting doctor in the PVTH were made in our study, in opposition to the OMSS. Thus, assessing doctors' compliance with the ACVIM guidelines and limits for the universal applicability of the MINE score.

Survival was not assessed in this study, in opposition to the OMSS, where the MINE score's clinical usefulness was sustained by evaluating its association with survival.

Finally, in this study, an adaptation was made to severity classification by widening the last severity class from a score range of 13 to 14 to a score range of 12 to 14 (table 3). In the OMSS, 12 was considered as severe (8 to 12) rather than late-stage (13 to 14).

In the current study, the prescribed cardiac medication on the day of the echocardiographic consultation was assessed to check its agreement with the ACVIM guidelines. In the OMSS, only the medication history was assessed to show that different protocols were applied in the dog population, which can be seen as a limitation due to potential interference with survival.

3.3.6. Epidemiology

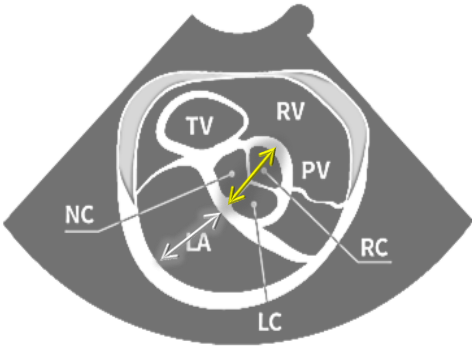
The evaluated population included 190 dogs and was characterized by sex (male and female), age (between 3 years & 9 months until 19 years old), breed (Mixed breed, CKCS, Dachshund, Pincher, Chihuahua, Poodle and thirteen others) and weight (from 2 to 20kg). The cardiac medication taken at the date of the examination was also assessed.

3.3.7. Echocardiographic examination and scoring system

Transthoracic echocardiographic examinations had been carried out by experienced cardiology professors from the PVTH or by other doctors with variable degrees of veterinary cardiology experience, under the supervision of the most experienced cardiology professors. US machines equipped with phased-array transducers and a simultaneous single-lead electrocardiogram were used for the examinations. Dogs were imaged without sedation from the right and left parasternal positions, and standard echocardiographic 2-dimensional, M-mode, and Doppler images were acquired.

Four echocardiographic variables constitute the MINE score: (1) the left atrium-to-aorta ratio (LA/Ao) determined from an RPS short-axis view (Figure 3A&B), (2) the left ventricular end-diastolic dimension normalized for body weight (LVIDDn), figured on the M-mode (Figure 4B&C) and obtained using the RPS short-axis view (Figure 4A), (3) fractional shortening of the left ventricle (FS), determined using the M-Mode (Figure 4B&C) thought the RPS short-axis view (Figure 4A) and the

E-wave transmitral peak velocity (E-vel), measured from the left apical 4-chamber view with PWD (figure 5A&B). The first and second variables quantify the left heart size, and the third and fourth assess the left heart systolic and diastolic functions, respectively.



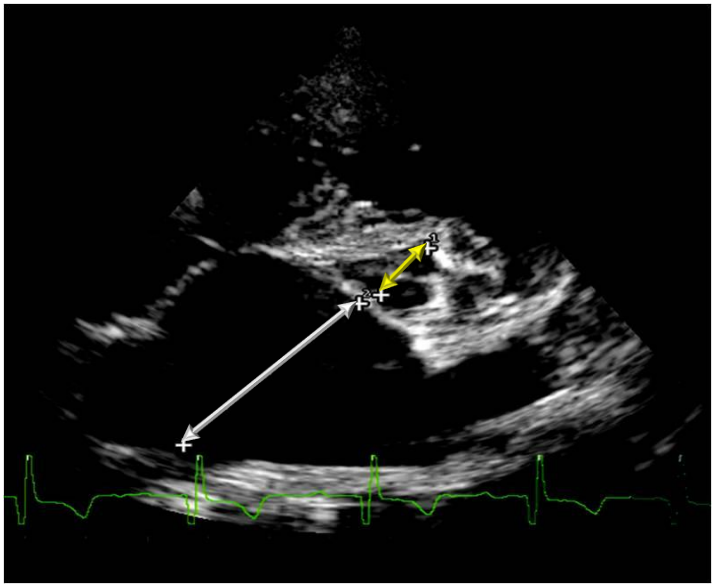
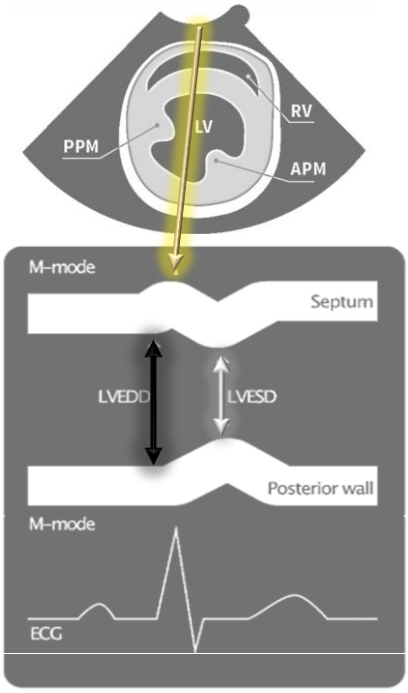


Figure 3A (on the left) – Right-sided short-axis view at the level of the left atrium (LA) and aorta (Ao). Abbreviations: LA = left atrium, LC = left coronary cusp, NC = non-coronary cusp, PV = pulmonic valve, RC = right coronary cusp, RV = right ventricle, TV = tricuspid valve (adapted from Estrada 2017), Yellow arrow – aortic diameter measurement, White arrow – atrial size measurement for LA/Ao ratio. **Figure 3B** (on the right) – RPS short-axis view at the level of the LA and Ao of a dog with MMVD LA/Ao ratio of 3,4. (Image provided by Dr Francesco Birettoni)



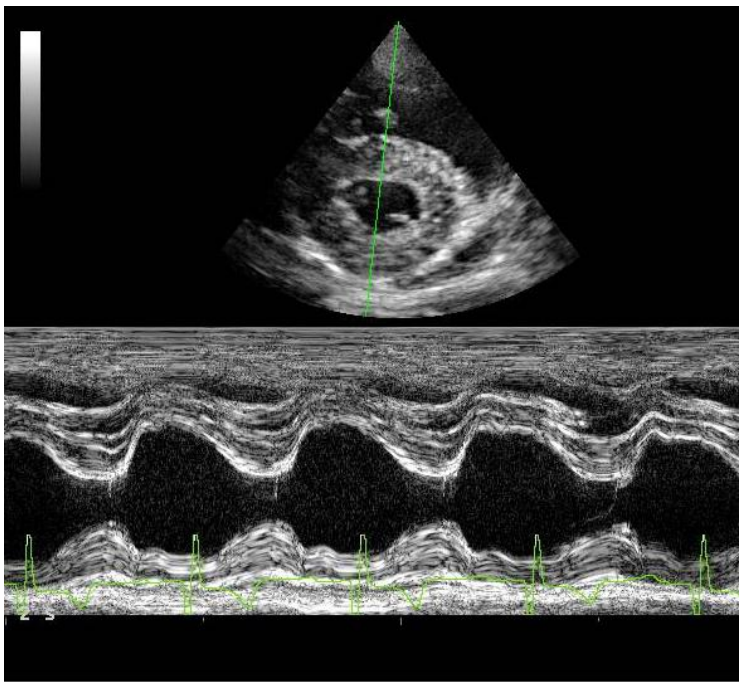


Figure 4A (top left) – Adapted illustration of the right-sided parasternal short-axis view at the level of the papillary muscles (Estrada 2017). Abbreviations: APM = anterior papillary muscle, LV = left ventricle, PPM = posterior papillary muscle, RV = right ventricle**. Figure 4B** (bottom left) - Adapted illustration of the left ventricular measurements using M-mode echocardiography (Estrada 2017). Abbreviations: LVIDs - left ventricular internal dimension in systole, LVIDd - left ventricular internal dimension in diastole. **Figure 4C** (right) – M-Mode thought the RPS short-axis view of a dog with MMVD and an FS of nearly 60%. (Image provided by Dr Francesco Birettoni)

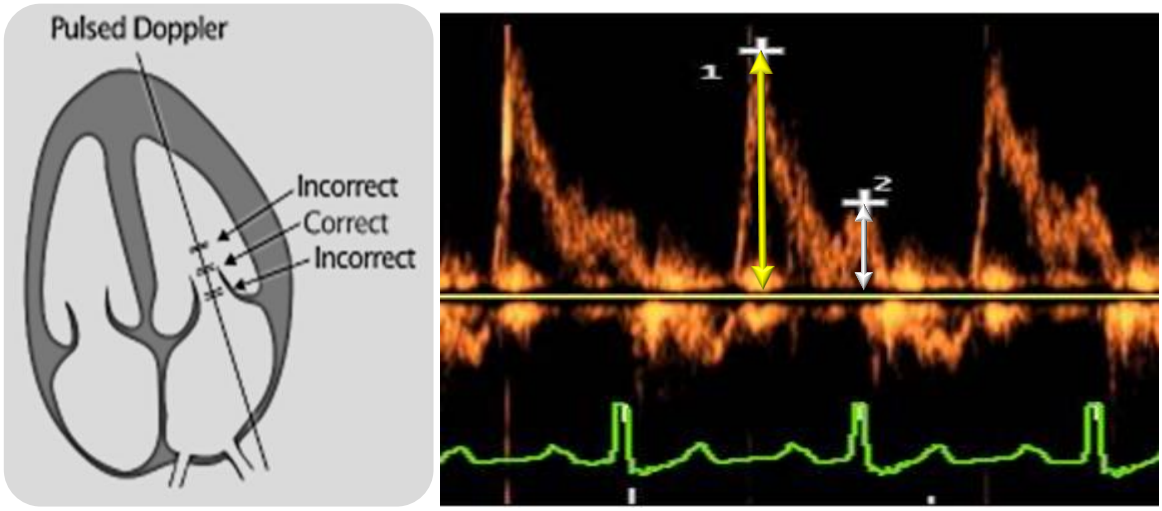


Figure 5A (on the left) – Correct positioning of the pulsed wave Doppler beam in the left apical four-chamber view (DESAI and KLEIN 2007; Nagueh et al. 2016) **Figure 5B** (on the right) - The E-wave and A-wave transmitral peak velocities (image provided by Dr Francesco Birettoni). Measurements are taken from the left apical 4-chamber view through PWD on a dog with MMVD and a restrictive pattern due to an E/A ratio of nearly 2,8. E-vel – Yellow arrow, A-vel – white arrow.

The specific severity cut-offs and the respective scores for the four variables (displayed in table 2) were defined by Tomazzo et al., based on echocardiographic studies on prognosis and severity in dogs with MMVD.

	Score			
	1	**2**	**3**	**4**
LA/Ao	<1.70	1.70-1.90	1.91-2.50	>2.50
LVIDDn	<1.70	1.70-2.00	2.01-2.30	>2.30
FS (%)	<45	45-50	>50	
E-vel (m/s)	<1.20	1.20-1.50	>1.50	

Table 2 - Selected echocardiographic cutoffs and relative scores. Abbreviations: E-vel - E-wave transmitral peak velocity; FS - fractional shortening of the left ventricle; LA/Ao - left atrium-to-aorta ratio; LVIDDn - left ventricular end-diastolic dimension normalized for body weight

Based on the authors' clinical experience, a scoring system was arbitrarily created, and four severity classes were defined. The individual scores of each one of the four echocardiographic variables were summed, and the total scores obtained were distributed by the four severity classes: mild, moderate, severe, and late-stage (as shown in table 3). In opposition to the OMSS, the severity class matching a total score of 12 was changed, being considered "late-stage" rather than "severe" in this study.

Severity classification	Total score
Mild	4-5
Moderate	6-7
Severe	8-11
Late-stage	12-14

Table 3 - Severity classification based on the total score obtained from the summation of the single scores according to Table 2

3.3.8. Statistical Analysis

Descriptive statistical analyses were conducted using Microsoft Office Excel 2016® and GraphPad Prism 5 software for Windows. Results are presented as a minimum, maximum, mean ± standard deviation (SD) for several chosen samples.

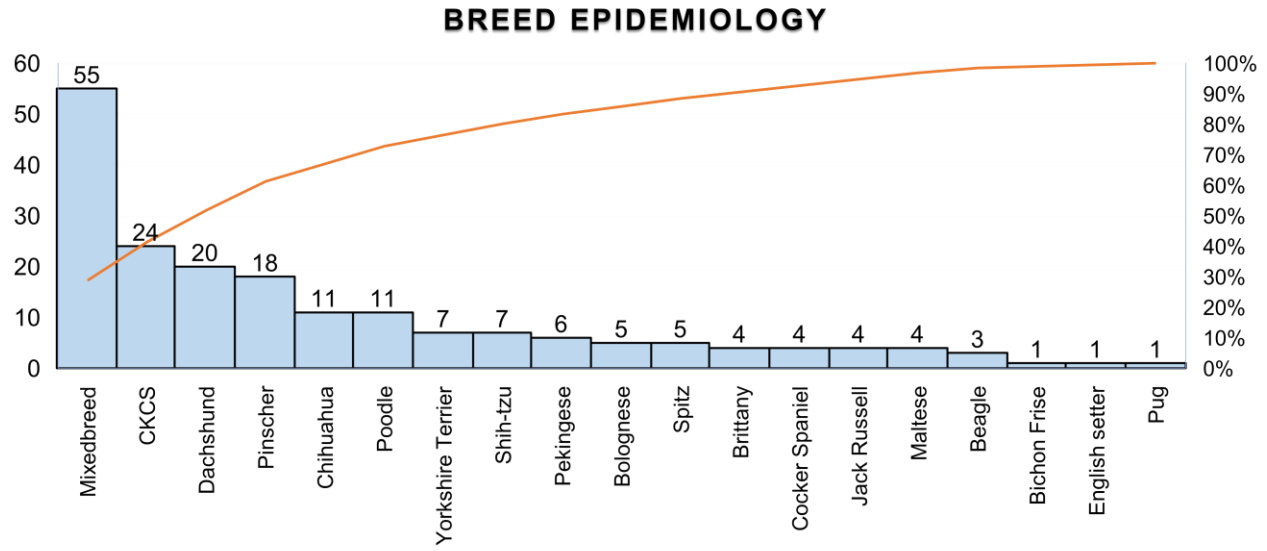
For the statistical analysis, nine subgroups were created by first diving the dogs in ACVIM Classification B1, B2 and C/D and then creating subgroups considering the MINE classes obtained at each ACVIM stage. Descriptive analyses with the Shapiro-Wilk test were conducted in the nine subgroups to determine the normality of the data distribution.

The analysis of each MINE score variable contribution to the final score was conducted by pairing the subgroups formed within the independent ACVIM groups through parametric tests. A t-test was performed to assess significant differences between the mean values of the MINE score subgroups from ACVIM B1 and ACVIM stages C/D. At the same time, an analysis of variance (ANOVA) was used to compare the severity subgroups obtained from ACVIM B2 stage patients. All referred groups were compared, and their differences were recognized as significant when P values <0,05 were obtained.

3.4. Results

3.4.1. Phenotypic characterization

The evaluated population included 191 dogs, with a mean age of 11,3 ± 2,9 years, with a minimum of 3 years & 9 months and a maximum of 20 years old. In terms of gender, there were 67 females (35%) and 124 males (65%), with a ratio of 1,84 males affected for each. The dog's weights varied from 2 to 20kg, with an average of 7,9 ± 3,7kg. The most frequent dog breeds were the CKCS with 24 patients (12,5%), followed by the Dachshund with 20 affected dogs (10,4%). Mixed breed dogs made up the overall majority, with 55 dogs (28,6%) representing other frequent affected breeds were 18 Pinchers, 11 Poodles, 11 Chihuahuas, 7 Shi-Tzus, 7 Yorkshires and 6 Pekingese. The remaining 32 dogs (16,7%) were from 10 other breeds. The breed distribution can be accessed in the following histogram (histogram 3).



Histogram 3 – Breed epidemiology of the current study. All the dog breeds included in the study can be found on the X-axis. The number of dogs is on the left y-axis and their cumulative percentage on the right y-axis.

3.4.2. Phenotypic variation by echocardiographic severity class

When distributing the dogs by the four severity classes within the MINE score (table 4), age showed a rising tendency with the severity classes. Male dogs were prevalent in every severity class, representing 64% of the mild, 64% of moderate, 63% of severe and 73% of late-stage dogs, which is translated by ratios between 1,7 to 2,7 males per female patient by severity class. A negative correlation between weight and severity was not observed in the current study sample, with a mean weight of 8,4 kg for mild dogs, 6,8 kg for moderate, 8,0 for severe and 7,8 for late-stage dogs. In both mild and moderate severity classes, no dogs heavier than 16kg were included, while in the severe and late-stage groups together, six dogs with >16kg were counted.

	Severity Class			
	Mild (n=59)	**Moderate** (n=36)	**Severe** (n=70)	**Late-stage** (n=26)
Sex (M / F)	38 / 21	23 / 13	44 / 26	19 / 7

Age (years)	10,5 ± 3,2	11,1 ± 2,8	11,6 ± 2,7	12,4 ± 2,2
Body Weight (kg)	8,4 (2,2-15,4)	6,8 (2-16)	8,0 (2,4-19,9)	7,8 (3,2-20)

Table 4 - Baseline clinical data of all study dogs (n = 191) according to the severity class of the Mitral INsufficiency Echocardiographic (MINE) score. Note: Data represents mean ± SD, median (min-max)

3.4.3. ACVIM Classification and Medical treatment

Regarding the ACVIM classification (figure 4), 30% (n=73 dogs) were in stage B1, 32% (n=61) in stage B2, and 30% (n=57) in stage ACVIM C-D (figure 6).

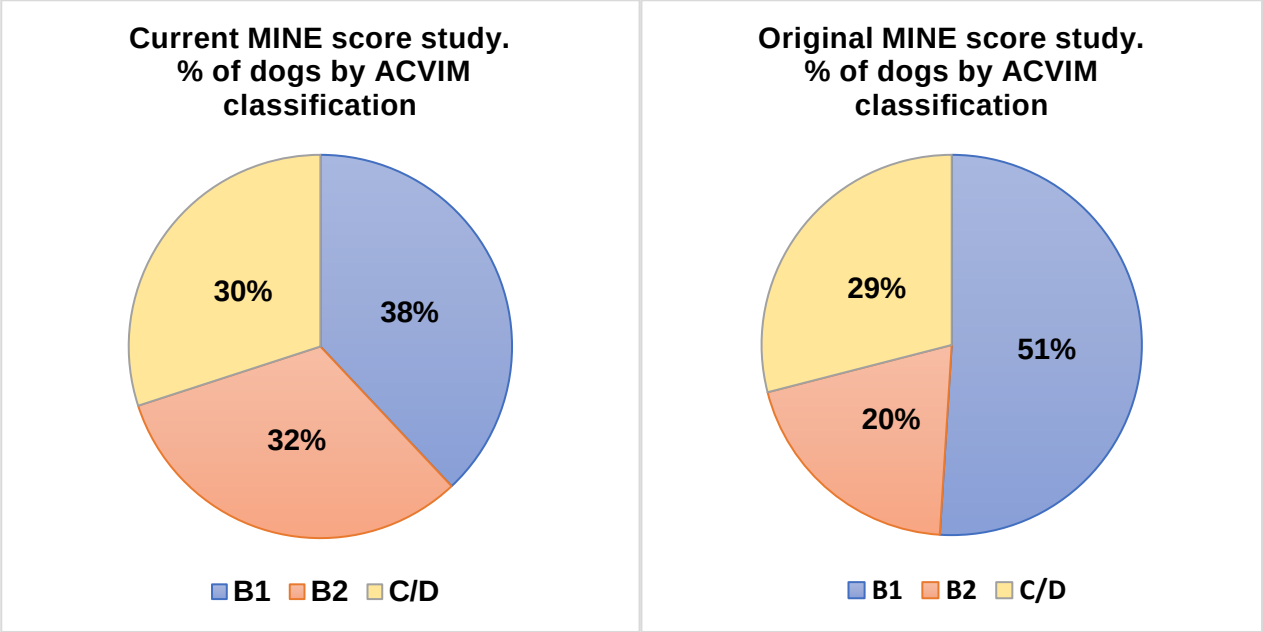


Figure 6 - Pie chart's comparing the percentages of dogs classified by the established study groups, based on ACVIM classification for MMVD: B1, B2 and C/D, between the current and the original MINE score studies.

3.4.3.1. Medical treatment by ACVIM Classification

Concerning treatment from the date of inclusion in the study (Table 5), on what concerns dogs in stage B1, 63 (86%) were not prescribed any drugs, 10 (14%) were taking an ACEI, and 2 (3%) had been prescribed spironolactone. In stage B2, from the total of 61 dogs, only 2 (3%) dogs did not get ant therapy, 57 (93%) were recommended to start or continue pimobendan, 37 (61%) an ACEI, 10 (16%) were medicated with spironolactone and 5(8%) with furosemide. In stage C-D, 55 (96%) dogs were recommended to continue treatment or introduce pimobendan, 57 (100%) an angiotensin-converting enzyme inhibitor, 50 (88%) furosemide, 108 (67%) spironolactone, 7 (12%) torsemide, 7 (12%) Nitroglycerine and 2 (4%) sildenafil and finally 2 (4%) hydrochlorothiazide.

ACVIM B1 Stage (n=73)	ACVIM B2 stage (n=61)	ACVIM C/D stages (n=57)	Total (n=191)

Administered drugs	Pimobendan	-	57 (93%)	55 (96%)	112 (59%)
	ACEI	10 (14%)	37 (61%)	57 (100%)	104 (54%)
	Spironolactone	2 (3%)	10 (16%)	4 (7%)	16 (8%)
	Furosemide	-	5 (8%)	50 (88%)	55 (29%)
	Hydrochlorothiazide	-	-	2 (4%)	2 (1%)
	Torsemide	-	-	7 (12%)	7 (4%)
	Nitroglycerin	-	-	7 (12%)	7 (4%)
	Sildenafil	-	-	2 (4%)	2 (1%)

Table 5 – Number of dogs prescribed cardiac drugs by ACVIM stage.

3.4.4. MINE scoring and severity classification

Nine subgroups were formed by matching the dogs' ACVIM stages (B1, B2 and C/D) and the severity classes corresponding to the dogs' MINE scores. From the nine groups, six were considered representative of their ACVIM stage and were considered to produce consistent, relevant results with the statistical analysis performed. Those were B1 mild with 55 dogs (29%), B1 moderate with 18 dogs (9%), B2 moderate with 17 dogs (9%), B2 Severe with 35 dogs (18%), C/D severe with 35 dogs (18%) and C/D late-stage with 21 dogs. While the smaller, less representative groups were the B2 mild, the B2 late-stage and the C/D moderate, with 4 (2%), 5 (3%) and 1 dog (1%), respectively. Each variable of the echocardiographic MINE score was subjected to a descriptive analysis using the Shapiro-Wilk test to determine the normality of the data distributions among the nine subgroups. The MINE score independent variables' contribution to the final score was assessed by pairing the subgroups formed inside the 3 ACVIM groups (B1, B2 and C/D) through parametric tests.

3.4.4.1. Left Heart Chambers size assessment and scoring

The left heart chambers' size was assessed using the LA/Ao ratio measured in the RPS short-axis view and the LVIDDn, taken from RPS M-mode. Within the MINE Score, significant heart enlargement was considered when the ratio LA/Ao was higher than 1,7, and the same cutoff was applied for the LVIDDn values.

The LA/Ao ratio in the 191-dog sample revealed a mean value of 2,00 ± 0,59, with a range of values from 1,22 to 4,90. From those (table 7), 38% (n=72) showed a ratio <1,7, getting a score of 1 for this variable. A score of 2 was obtained by 14% (n=26) of dogs, with a LA/Ao ratio of 1,7 to 1,9, inclusively. A score of 3 and a LA/Ao ratio higher than 1,9 and up to 2,5 inclusively was obtained by 28% (n=53).

ACVIM Stage		**B1 Stage** (n=73)		**B2 stage** (n=61)				**C/D Stages** (n=57)		
Echocardiographic Severity Classification		**Mild**	**Moderate**	**Mild**	**Moderate**	**Severe**	**Late-stage**	**Moderate**	**Severe**	**Late-stage**
N of dogs		55	18	4*	17	35	5*	1*	35	21
LA/Ao	**Mean**	**1,44**	**1,58**	**1,75**	**1,82**	**2,05**	**2,46**	**1,75**	**2,44**	**3,03**
	Std dev.	0,17	0,22	0,13	0,20	0,27	0,38	-	0,39	0,63
LVIDDn	**Mean**	**1,58**	**1,69**	**1,55**	**1,85**	**1,99**	**2,29**	**1,82**	**2,12**	**2,31**
	Std dev.	0,15	0,21	0,12	0,16	0,24	0,24	-	0,32	0,27
FS (%)	**Mean**	**39**	**49**	**40**	**45**	**45**	**51**	**48**	**47**	**50**
	Std dev.	5,7	9,0	5,0	5,7	8,0	5,3	-	6,9	8,2
E-vel (m/s)	**Mean**	**0,77**	**0,82**	**0,77**	**1,02**	**1,18**	**1,41**	**0,94**	**1,36**	**1,77**
	Std dev.	0,12	0,12	0,13	0,18	0,24	0,21	-	0,34	0,33

Table 6 - Baseline clinical and echocardiographic data of the dogs included in the study (n = 191) according to the nine subgroups formed by crossing the dogs' ACVIM stages (B1, B2 and C/D) and the severity classes corresponding to the dogs' Mitral INsufficiency Echocardiographic (MINE) score. Note: * - subgroups not thoroughly analyzed due to a short sample number. Abbreviations: E-vel, E-wave transmitral peak velocity; FS, fractional shortening of the left ventricle; LA/Ao, left atrium-to-aorta ratio; LVIDDn, left ventricular end-diastolic dimension normalized for body weight; Std dev. – standard deviation.

ACVIM Stage		**B1 Stage** (n=73)		**B2 stage** (n=61)				**C/D Stages** (n=57)		
		Mild	**Moderate**	**Mild**	**Moderate**	**Severe**	**Late-stage**	**Moderate**	**Severe**	**Late-stage**
N of dogs = 191		55	18	4*	17	35	5*	1*	35	21
Variable	**S**	**Number of dogs with each score / variable**								
LA/Ao	**1**	52	13	1	4	2	0	0	0	0
	2	3	3	3	8	7	0	1	1	0
	3	0	2	0	5	24	3	0	20	2

	4	0	0	0	0	2	2	0	14	19
LVIDDn	**1**	42	8	4	3	2	0	0	3	0
	2	13	9	0	12	19	0	0	10	2
	3	0	1	0	2	11	2	1	16	6
	4	0	0	0	0	3	3	0	6	13
FS (%)	**1**	47	4	3	10	15	0	0	16	6
	2	8	5	1	5	14	3	1	11	5
	3	0	9	0	2	6	2	0	8	10
E-vel (m/s)	**1**	55	18	4	14	16	0	1	11	0
	2	0	0	0	3	17	1	0	15	5
	3	0	0	0	0	2	4	0	9	16

Table 7 – The number of dogs from each subgroup versus the score obtained in each independent echocardiographic variable. Note: * - subgroups not thoroughly analyzed due to a short sample number. Abbreviations: E-vel, E-wave transmitral peak velocity; FS, fractional shortening of the left ventricle; LA/Ao, left atrium-to-aorta ratio; LVIDDn, left ventricular end-diastolic dimension normalized for body weight. Colour code – Red – less than 10% of dogs within the subgroup, Yellow – 10 to 20% of dogs. Green – more than 20 to 90% of dogs. Blue – more than 90% of dogs.

Regarding the LVIDDn, the mean value was 1,89, with a minimum value of 1,17 and a maximum of 2,86. The enlargement of the left ventricle (Table 7) was considered absent in 32% of dogs (n=62), receiving a score of 1 for having an LVIDDn value under 1,7. A score of 2 was obtained by 34% (n=65), with values from 1,7 up to 2,00, inclusively. Values of LVIDDN higher than 2,00 and up to 2,3 correspond to an independent score of 3, obtained by 20% (n=39). A score of 4 was obtained by 13% of the dogs (n=25), which had an LVDDI higher than 2,30.

Results from the Shapiro-Wilk normality tests for the variables accessing left heart enlargement were obtained for the six subgroups with more than five dogs. When it comes to the ratio LA/Ao and considering an alfa value of 0,05, the subgroups B1 mild, B1 moderate, B2 moderate, B2 severe and C/D severe passed the normality test, while the C/D late-stage did not. All six subgroups passed the normality test for the LVDDn, considering the value of alfa=0,05.

When considering the main subgroups isolatedly (table 6), B1 mild dogs presented a mean LA/Ao ratio of 1,44 ± 0,17, B1 moderate 1,58 ± 0,22, B2 moderate 1,85 ± 0,16, B2 severe 1,99± 0,24, C/D severe 2,44 ± 0,39 and C/D late-stage 3,03 ± 0,63. Considering LVIDDn, the values mean values obtained within the subgroups were 1,58 ± 0,15 for B1 mild dogs, 1,69 ± 0,21 for B1 moderate, 1,85 ± 0,16 for B2 moderate, 1,99 ± 0,24 for B2 severe, 2,12 ± 0,32 for C/D severe and 2,31 ± 0,27 for C/D late-stage .

3.4.4.2. Systolic function assessment and scoring

The left ventricle systolic function was assessed by the FS measured through the echocardiographic M-mode, taken from the RPS short-axis view.

The FS in the 191-dog sample showed a mean value of 44 ± 11%, with a minimum value of 22% and a maximum of 67%. The 101 dogs (53%) with a FS under 45% (table 7) scored 1. A score of 2 was given to the 53 dogs (28%) that had an FS between 45% and 50%, inclusively. The remaining 37 dogs (19%) presented a highly hyperkinetic left ventricle with an FS higher than 50% and a score of 3.

Results from the Shapiro-Wilk normality tests for the FS were obtained for the six subgroups with more than five dogs, considering an alfa=0,05. The FS B1 mild, FS B1 moderate, FS B2 moderate, FS B2 severe, FS C/D severe and FS C/D late-stage passed the normality test.

When considering the subgroups isolatedly (Table 6), B1 mild dogs presented a mean FS of 39 ± 6%, B1 moderate 49 ± 9%, B2 moderate 45 ± 6%, B2 severe 45 ± 8%, C/D severe 47 ± 7% and finally C/D late-stage 50 ± 8%.

3.4.5. Left heart diastolic function assessment and scoring

The left heart diastolic function was partially assessed through the transmitral E wave velocity, measured with PWD, taken from LPS apical four-chambers long-axis view.

The assessment of the E-wave transmitral peak velocity in the 191-dog sample showed a mean value of 1,11 ± 0,40 m/s, with a minimum value of 0,46m/s and a maximum of 2,30m/s. To the 119 dogs (62%) that had an E wave velocity under 1,20m/s, the score 1 (table 7) was attributed. A score of 2 was given to 41 dogs (21%) that had an E wave velocity between 1,20 and 1,50m/s, inclusively. The remaining 31 dogs (16%) presented an E Wave velocity higher than 1,50m/s and scored 3, based on the MINE score classification.

Results from the Shapiro-Wilk normality tests for the FS were obtained for the six subgroups with more than five dogs, considering an alfa=0,05. The E wave B1 mild, E wave B1 moderate, E wave B2 moderate, E wave B2 severe, E wave C/D severe, and E wave C/D late-stage passed the normality test.

When considering the subgroups isolatedly (table 6), B1 mild dogs presented a mean E wave velocity of 0,77 ± 0,12 B1 moderate 0,82 ± 0,12, B2 moderate 1,02 ± 0,18, B2 severe 1,18 ± 0,24, C/D severe 1,36 ± 0,34 and C/D late-stage 1,77 ± 0,33%.

3.4.6. Independent echocardiographic variable's contribution to the MINE score

According to the resulting MINE scores, the different severity classes obtained in each ACVIM group were compared using parametric tests, evaluating the independent contribution of each echocardiographic variable to the total scores and the resultant severity classes obtained.

A t-test was performed to determine if there was a significant difference between the mean values of the MINE score groups from ACVIM stage B1 mild vs moderate and from the ACVIM stages C/D severe vs late-stage. Differently, an analysis of variance (ANOVA) was used to compare the various MINE score severity groups (mild, moderate, severe, and late-stage) obtained from ACVIM B2 stage patients. All referred groups were paired, and their differences were recognized as significant when P values <0,05 were obtained.

3.4.6.1. Ratio LA/Ao contribution to the MINE score

Mean values of ratio LA/Ao from ACVIM B1 stage dogs with Mild and Moderate scores were paired and considered significantly different due to a P-value <0,0001, effectively contributing to the differentiation of dog's disease by severity groups within this ACVIM stage.

Within the B2 stage, score comparisons were performed, recurring to the ANOVA test, since the MINE score values for dogs of this ACVIM stage were distributed for all four MINE score severity classes. Regarding a P-value <0,05, significant differences were found between dogs with B2 severe vs B2 moderate, which was the more relevant comparison due to the higher prevalence of B2 staged dogs in these two MINE score classes. Differences were also significant for the same p-value between mild vs late-stage dogs and moderate vs late-stage dogs. On the other side, pairing B2 mild vs B2 severe, B2 severe vs B2 late-stage and B2 mild vs B2 moderate did not result in significant differences with P-value <0,05.

Finally, significant differences were found when comparing mean values of La/Ao ratio from C/D stage severe vs late-stage, with a P-Value of 0,0002.

Overall significant differences were found between all the paired groups composed of more than five dogs, regarding LA/Ao as an independent echocardiographic variable.

3.4.6.2. LVIDDn contribution to the MINE score

Unlike the previous variable, when paring ACVIM B1 stage dogs with Mild and Moderate scores, no significant differences were found between LVIDDn values (P-value > 0,05).

In B2 stage dogs, the comparison between B2 moderate vs B2 severe, B2 severe vs B2 late-stage and B2 mild vs B2 moderate did not show any significant differences for this independent variable (P-value > 0,05). On the other hand, comparing more extreme severity classes, such as LVIDDn B2 mild vs LVIDDn B2 severe, LVIDDn B2 mild vs late-stage and LVIDDn B2 moderate vs late-stage, showed significant differences between variables (P-value < 0.05).

A significant difference was found within the ACVIM C/D stages group (P = 0,0081) when comparing LVIDDn C/D severe vs LVIDDn C/D late-stage, suggesting a higher contribution of this echocardiographic variable in dogs that experienced CHF.

3.4.6.3. Fractional shortening contribution to the MINE score

The FS showed significant differences in the comparison of FS B1 mild vs FS B1 moderate, with a P-value of 0,0020. In the case of B2 stage dogs, no significant differences were found (P-value > 0,05) in any of the six pairings done with the four classes. When comparing FS C/D severe vs FS C/D late-stage, no significant differences were found (P > 0,05).

3.4.6.4. Transmitral E wave velocity contribution for the MINE score

Regarding the transmitral E wave velocity, no significant differences were found when comparing B1 mild dogs vs B1 moderate dogs (P > 0,05). In the ACVIM B2 staged dogs, significant differences were found between B2 mild vs B2 severe, B2 mild vs B2 late-stage and B2 moderate vs late-stage ($p < 0.05$). When comparing close severity classes, such as B2 mild vs moderate, B2 moderate vs B2 severe and B2 severe vs B2 late-stage, no significant differences were found (P > 0,05). In the late ACVIM stages C/D, the C/D severe vs C/D late-stage comparison showed significant differences (P= 0,01).

3.5. Discussion

When comparing the results obtained in the current MINE score study with those obtained in the OMSS, the size of the sample and proportions of dogs classified by sex, age, ACVIM stage, and echocardiographic severity varied significantly. The OMSS had a sample of 560 dogs, which is approximately three times larger than the one assessed in our study(Vezzosi et al. 2021).

The current study included a dog sample with a higher mean age than the sample analysed in the OMSS (Vezzosi et al. 2021). MMVD can develop in adult dogs of any age, but its prevalence and severity increase with age, with up to 85% of dogs reaching 13 years old affected by this disease (Buchanan 1977). In the current study, the mean ages were 10,5 ± 3,2, 11,1 ± 2,8, 11,6 ± 2,7 and

12,4 ± 2,2 years old for mild, moderate, severe, and late-stage dogs, respectively. A positive correlation was found between age and the severity of the disease. In the original MINE score study, the dog's average ages were generally lower, being 9,9 ± 3,2 for mild classified dogs, 10,8 ± 2,9 for moderate, 11,6 ± 2,7 for severe and 11,2 ±1,6 for late-stage dogs (Vezzosi et al. 2021). Overall, the average age in this study was 11,3 years old, which is higher than most studies accessing similar MMVD dog samples, presenting mean ages of 10,3 to 10,7 years of age (Ferasin et al. 2013; Dickson et al. 2017; Vezzosi et al. 2021). Since only the first complete echocardiographic evaluation performed in the PVTH was included in the study, the referral nature of numerous appointments might partially explain the higher mean age of the dog sample. Another factor contributing to the higher mean age is that in PVTH, echocardiographic examinations of mildly affected MMVD patients are not compulsively followed by the registration of all the required measurements, nor the echocardiographic images, necessary to obtain the required data to include the dogs in the current study. The most commonly absent variable was the transmitral E-wave velocity within the incomplete examinations, which led to the exclusion of at least 33 dog appointments, otherwise potentially suitable to this study, that could have influenced the results.

In terms of sex predisposition, the male dogs are generally more affected, presenting on average an earlier onset of the disease and approximately three males being diagnosed for every two females (Keene et al. 2019). This study had 65% of male dogs, with a male: female ratio of 1,84, while in the original MINE score study, males represented 57% of the dogs with 1,33 males to each female (Vezzosi et al. 2021). The higher frequency of males observed in this study might be correlated with the significantly higher frequency of severe echocardiographic classifications obtained in the current study when compared to the OMSS, considering that an earlier onset of the disease in males will lead to higher frequencies of males reaching the late stages of the disease. In this study, males and females present a similar mean age.

In our study, dogs presented a mean weight of 7,87kg, significantly lower than the 8,7kg obtained in the OMSS. In the OMSS a decrease of the average weight was observed with severity, with average weights of 9,1kg for mild dogs, 8,0kg for moderate dogs, 7,0kg for severe dogs and 5,8kg for late-stage dogs (Vezzosi et al. 2021). A negative correlation between weight and severity was not observed in the current study sample, with a mean of 8,4 kg for mild dogs, 6,8 kg for moderate, 8,0 for severe and 7,8 for late-stage dogs. In both mild and moderate severity classes, no dogs heavier than 16kg were considered, while in the severe and late-stage groups together, six dogs with >16kg were counted. The moderate severity class in this study was composed of 92% (n=33) of dogs with less than 10kg, 25% of which are Pinchers and toy Poodles. At the first echocardiographic visit, the higher proportions of small and toy companion breeds in the lower mitral insufficiency severity classes might be due to more regular screenings for MMVD in those breeds

(Keene et al. 2019). Dogs weighing from 15 to 20 kg were all from either hunting breeds (55%) or mixed breed (45%). In the fewer larger dog breeds affected by MMVD, the progression of the disease occurs typically faster and is accompanied by a more reserved prognosis (Keene et al. 2019), which might help to explain why the heavier dogs included in the study were mostly classified in severe or late-stage.

When assessing the epidemiological differences by ACVIM staged dogs, the current study has 13% fewer dogs staged as ACVIM B1 than the OMSS. Like in the OMSS, the nature of the veterinary institutions from where the data of both MINE score studies was obtained included a high proportion of referral cases. Two possible reasons were identified that might have led to a lower frequency of B1 staged dogs within this study. One is the misclassification of dogs as B2 when considering the up-to-date ACVIM guidelines for the diagnosis and treatment of MMVD. Accordingly, a dog is classified as B2 when there is evidence of enlargement of both left chambers, with a LA:Ao ratio higher than 1,6 and a LVIDDn of more than 1,7 (Keene et al. 2019). Contrarily to the OMSS, where corrections to the original data were made based on the ACVIM guidelines when necessary (Vezzosi et al. 2021), in the current study, the ACVIM classification initially given in the PVTH was maintained for the statistical analysis. Consequently, 6% (n=11) of dogs were misclassified as ACVIM B2 due to the absence of significant enlargement of one or both left heart chambers. The second reason is the same referred for the age disparities, due to missing echocardiographic data from MMVD patients with normal-sized chambers and classification of ACVIM B1, narrowing the adequate caseload for the study.

In terms of echocardiographic severity, the proportions were also considerably different in both studies, with the current study having a significantly higher proportion of dogs classified as severe and late-stage compared to the OMSS. From the 560 dogs used in the OMSS, there were 43% classified as mild, 20% as moderate, 33 % as severe, and just 4% as late-stage (Vezzosi et al. 2021). From the 191 dogs included in the current study, less than 1/3 were classified as mild, 19% as moderate, 36% as severe and 14% as late-stage. Since only the first complete echocardiographic report of each MMVD patient was included in the MINE score studies, the lack of data from certain recorded appointments at the PVTH, and perhaps a higher proportion of dogs referred in later stages of the disease to this institution might help to explain the different proportions between both studies.

Even though both studies had a similar proportion of dogs that were considered to have experienced CHF (around 30%), which were generally echocardiographically classified as severe or late-stage, the proportions of dogs getting either one of these echocardiographic classifications was 50% in the current study against only 37% on the OMSS (Vezzosi et al. 2021). This 13% difference is even more significant when considering that only 9% of dogs with the last two severity classifications did not experience CHF in the OMSS, while in our study, 21% of the dogs were

classified as severe or late-stage without evidence of CHF. This data might suggest that CHF is either misdiagnosed or underdiagnosed in the dogs included in the OMSS or in the current study, respectively.

The presence of pulmonary oedema (PE) following left-sided CHF is the most commonly congestive expression of CHF, being frequently used as objective criteria for diagnosing this syndrome (Francis Smith, Larry Tilley, Mark Oyama 2015). Thoracic radiography is the clinical standard for its detection in dogs (Baldi et al. 2012; Vezzosi et al. 2017), and for this reason, in the OMSS, performing a thoracic X-ray at the exact date of the echocardiographic evaluation was a criterion for inclusion (Vezzosi et al. 2021). Radiographs were scarcely performed on the dogs included in the current study. Their diagnosis of PE was either made through lung ultrasonography or by home-based monitoring by the tutors of the respiratory rate at rest or during sleep in the dogs signalled for being at higher risk of developing CHF.

In dogs with PE, artefacts can be detected in the lung US, appearing as vertical hyperechoic lines with a narrow base, going from the pleural surface to the edge of the screen and presenting synchronous motion with the pleura (Baldi et al. 2012). These artefacts are called B-lines and define the so-called “pulmonary interstitial syndrome” (Volpicelli et al. 2012). A study with 63 dogs compared the diagnostic agreement of thoracic radiography and lung ultrasonography in detecting PE in all ACVIM stages of MMVD. It showed 90% sensitivity and 93% specificity of the US method for detecting PE, with positive and negative predictive values of 85,7% and 95,2%, respectively, suggesting this method as a noninvasive diagnostic tool for clinical management of MMVD(Vezzosi et al. 2017).

The second strategy used by the clinicians in the PVTH for detection of cardiogenic PE is continuous home-based monitoring of the dog’s RR at sleep and rest by the owners. From all the diagnostic strategies mentioned, this one is considered to have the highest predictive value for imminent clinical decompensation (Keene et al. 2019). A rate higher than 30 breaths per minute is expected when pulmonary congestion and oedema occur (Schober et al. 2011; Ljungvall et al. 2013). Nonetheless, this strategy's efficacy is highly dependent on the owner's ability and motivation to identify the RR increase.

The use of alternative methods to detect CHF instead of the standard thoracic radiography might have contributed to a possible underdiagnosis of the symptomatic syndrome of MMVD within the current study. Another relevant aspect suggesting the underdiagnosis of CHF is the prescription against the ACVIM guidelines of furosemide to 5 dogs classified as B2 in the PVTH. The long-term use of furosemide can lead to diuretic resistance and increased plasma BUN and creatinine concentrations (Hori et al. 2007). Accordingly, and considering the costs of the drug and the

necessary compromise from the owner for its daily administration, prescription of diuretics should only occur with actual evidence of PE or any other congestive signs.

The MINE score was created by selecting four echocardiographic variables of core importance in the characterization of MV insufficiency in dogs with MMVD. Those variables were selected based on their widespread usage in the diagnosis and management of MMVD and low inter-operator and intra-operator variability (<5%-10%). The chosen echocardiographic variables are used to assess left-sided cardiac remodelling (LA/Ao and LVIDDn), left ventricular dynamics (FS%) and left ventricular filling pressure (E-vel) (Schober and Baade 2000; Chetboul et al. 2005; Schober et al. 2010; Höllmer et al. 2016). All chosen variables are considered to have prognostic value in dogs with MMVD (Gouni et al. 2007; Borgarelli et al. 2008; Bonagura and Schober 2009; Schober et al. 2010; Sargent et al. 2015; Vezzosi et al. 2021), and within the current study, their independent contribution to the final score and the corresponding severity classification was evaluated. In opposition to human medicine, well established and commonly shared echocardiographic severity classifications for mitral insufficiency are absent in veterinary medicine. The ones used in human medicine include the evaluation of structural features (left cardiac remodelling and valve morphology), quantitative Doppler assessment (colour flow jet area, flow convergence zone, density, and profile of mitral regurgitation jet), quantitative assessment (effective regurgitant orifice area, regurgitant volume, and regurgitant fraction) and semiqualitative assessment of MR (vena contracta width, pulmonary vein inflow and mitral inflow) (Vezzosi et al. 2021) . Many of the time-consuming methods necessary to obtain these variables require a skilled operator and may even present poor interoperator reproducibility (Gouni et al. 2007; Di Marcello et al. 2014; Baron Toaldo et al. 2016; Müller et al. 2017). In veterinary medicine, other echocardiographic scores have been proposed to classify the severity of MR (Pedersen, Lorentzen, et al. 1999; Wesselowski et al. 2014; Müller et al. 2017), based on LA size, LV size, MV leaflet anatomy, CFD regurgitant jet area, mitral inflow (deceleration time and E wave velocity) and CWD regurgitant jet density. However, in those proposed scores, many of the variables used are based on subjective assessments and in opposition to the OMSS, survival time was not assessed. The MINE score with the four variables chosen by Vezzosi et al. was proposed to overcome these challenges, being easy to use and accurately reproductive, thus preventing significant variability that could negatively influence clinical decision making. In the original study, all variables were shown to independently predict cardiac death after data analysis resulting from assessing the survival time and cause of death of dogs that died of the 560 canines included in the study (Vezzosi et al. 2021). In the current study, survival time or time-to-CHF were not assessed due to the recent nature of our caseload.

As part of the ACVIM guidelines, cardiac remodelling is defined by LA/Ao and LVIDDn increase in dogs with MMVD. However, there are no commonly accepted guidelines in veterinary

medicine to assess MR severity in dogs with MMVD (Vezzosi et al. 2021). The proposed MINE score was matched with the ACVIM classification in both the OMSS and the current study.

According to the current study, all B1 stage dogs were subdivided into only two severity groups, B1 mild and B1 moderate. As expected, most dogs that did not present enlargement of both left cardiac chambers were placed in group B1 with a Mild classification according to the MINE score.

Mild score severity implies that the dogs will have all echocardiographic variables within the normal range, scoring a total of 4 (1 for each normal variable), which corresponds to the minimum score possible, obtained by 53% (n=29) dogs. Alternatively, B1 dogs in this severity category can have a MINE score of 5 due to one variable mildly increased. From all 59 B1 mild dogs, 24% (n=13) had slight enlargement of LVIDDn, 9% (n=5) had slight atrial enlargement and 15% (n=8) had a mild increase in FS. Dogs classified as B1 moderate had generally enlargement of one left cardiac heart chamber (either the ventricle or atrium) or an FS increase. Of those B1 moderate dogs, 55% (n=10) had an FS higher than 50 alone, 28% (n=5) had an FS between 45 and 50%, also contributing to the moderate severity classification, and only three dogs with this classification had a normal FS (lower than 45). Other factors contributing to classifying dogs as “moderate” severity were an enlarged LVIDDn in 55% (n=10) and an enlarged LA/Ao ratio in 28% (n=5). Also, in the B1 moderate, 70% (n=7) of the dogs with enlarged LVIDDn had increased FS, while only 1 (20%) dog with LA/Ao enlargement presented increased FS%. Overall, FS played a major role in differentiating B1 mild dogs, which presented a mean FS of 39% from B2 moderate, with an average FS of 49%. This independent variable gave the most significant contribution to the severity differentiation within this ACVIM stage.

FS is the most used echocardiographic variable to assess LV systolic function, usually ranging from 25% to 40% in healthy dogs. It increases with increased contractibility, increased preload, or decreased afterload. Due to its high load dependence, FS% values are expected to be increased during most of the course of MMVD. Diastolic ventricular stretching due to cardiac remodelling following MR also contributes to the ventricular hyperdynamic motion (Bonagura and Schober 2009; Francis Smith, Larry Tilley, Mark Oyama 2015), expected in dogs with severe MR and conserved ventricular function. FS is generally between 45 to 55% in those dogs, even during most of their life after CHF (Borgarelli et al. 2007; Bonagura and Schober 2009).

Another possible reason potentially increasing FS% in dogs classified as B1 Mild could be a “fight or flight” response (Goldberg et al. 2017) seen in some patients during their restraint, while the echocardiography evaluation is being performed. When fear-based stress occurs, physiologic activation leads to an increased sympathetic nervous tone and parasympathetic withdrawal, which synergically leads to a high rise in the heart rate and contractility. Catecholamines released from the adrenal glands lead to multiple musculoskeletal actions, including enhanced contractility of cardiac

muscle (via beta-1 receptors), tachycardia and increased systemic blood pressure (Melmed Williams, Robert Hardin., 2020). Even though catecholamines typically present a very short half-life (10 to 100 seconds) (Young 2011 Jan 1), during a stress response, their secretion increases, and their depletion reduces due to a simultaneous decrease in hepatic flow (Zipes 2019). On the other side, a study assessing the chronotropic role of catecholamines in conscious dogs showed that plasma levels of catecholamines during physiologic and pathological states in conscious dogs are unlikely to be substantially responsible for the increased myocardial contractility seen in those states (Young et al. 1985).

A significant pathologic increase in FS is expected following eccentric ventricular enlargement, which is not a common feature seen in B1 stage dogs (Francis Smith, Larry Tilley, Mark Oyama 2015). Accounting that in the current study, this echocardiographic variable only played a role in the severity differentiation of B1 dogs, that role, together with its prognostic significance and inclusion in the MINE score, as well as any other factors that might influence FS values, deserve further investigation.

Together with the FS, LA/Ao ratio was the only other MINE score variable providing a significant statistical contribution in differentiating B1 dogs by echocardiographic severity. However, in opposition to the variable assessing ventricular systolic function, the LA/Ao ratio also contributed to the differentiation of all severity classes in the B2 and C/D ACVIM stages.

ACVIM stage B2 represents a heterogeneous group of animals, with some individuals presenting a slow disease progression and others rapidly progressing to CHF. Considering that, the MINE score might be useful in identifying B2 dogs with a higher risk of CHF or cardiac death (Francis Smith, Larry Tilley, Mark Oyama 2015; Vezzosi et al. 2021).

This heterogeneity is clearly expressed in our study, where four B2 subgroups were formed: B2 mild, B2 moderate, B2 severe and B2 end-stage dogs. The first and last groups included only 4 and 5 dogs, respectively. Within B2 dogs, ACVIM impairments were found in all severity groups classification, and the accuracy of certain echocardiographic variables was also questioned in particular cases.

As previously referred, 11 dogs were misclassified as B2 without matching both or even none of the echocardiographic parameters reflecting the enlargement of the left heart chambers. This impairment with the ACVIM consensus led to the creation of the subgroup B2 mild. All four dogs in this subgroup presented at least one variable assessing chamber enlargement within the normal range. For this reason, the group B2 mild in this study has limited value. Nonetheless, it is theoretically possible to have a small percentage of B2 mild dogs, when considering the ACVIM

classification B2, if a dog has a ventricle size between 1,7 and 2,0 and a ratio LA:Ao higher than 1,6 but lower than 1,7, together with the FS and E wave velocity within the normal ranges.

Moderate severity class implies a score of 6 to 7, which according to the ACVIM guidelines, is majorly expected to be achieved due to mild enlargement of both left cardiac chambers, with or without increased FS or E-vel. In opposition to most B1 moderate dogs, only 41% (n=7) of the 17 moderate B2 dogs presented an increased FS. Regarding the E wave velocity, B2 moderate was the first severity subgroup where dogs scored due to increased transmitral E-velocity, with 18% (n=3) of dogs receiving a MINE score of 2 due to E-vel slightly higher than 1,2.

Significant enlargement of both chambers was a norm within the B2 severe and B2 late-stage subgroups, with very few exceptions. Being classified with severe mitral insufficiency, a couple of B2 dogs obtained a score of 1 for LA/Ao ratio due to ratios of 1,46 and 1,48, which do not match the ACVIM classification nor are expected in the current severity classification. Both dogs obtained a total score of 8 due to an LVIDDn score of 3, an FS score of 2 or 3 and one dog presenting increased E-vel.

The prognostic importance of the left atrial size in dogs with MMVD is well established, and the 2-dimensional RPS short-axis LA:Ao ratio is the most common method used to assess atrial enlargement in veterinary medicine (Sargent et al. 2015; Franco et al. 2016). A 2014 study was performed on 60 dogs from ACVIM stages B1, B2 and C to assess the agreement on the detection of LA enlargement between LA/Ao ratio and left atrial volume using biplane area-length method indexed to body weight (LA Vol/BW). Disagreement between both methods was found in several dogs with mild atrial enlargement. The ratio LA/Ao significantly underestimated the atrial size, and LA Vol/BW was shown superior at detecting LA enlargement in those dogs. The results supported an expected enhanced ability of volumetric methods to accurately measure heart chamber dimensions when compared with methods assessing just one dimension, such as the LA/Ao ratio. They might help explain why in our study, a couple of dogs classified as B2 severe presented normal LA/Ao. Nonetheless, the volumetric methods are more complex and time-consuming, and their prognostic value is yet to be assessed in dogs with MMVD (Wesselowski et al. 2014).

As expected, the B2 group was significantly heterogeneous in terms of cardiac remodelling. In this ACVIM group, 82% (n=50) of B2 dogs presented a score of 2 or 3 for LA/Ao, and 72% (n=44) had those scores for LVIDDn. A small percentage of B2 dogs (6% with LA/Ao and 10% with LVIDDn) presented extreme enlargement of the left chambers, scoring 4 for the respective variables, without the presence of CHF being identified. Extreme values assessing cardiac remodelling contributed to form a subgroup of 5 B2 dogs, classified as late-stage. The severity of mitral myxomatous degeneration determines the rising pace of the regurgitant volume, and usually, the slower the disease progresses, the bigger the dimensions potentially reached by the left cardiac chambers,

without the development of PE or congestion (Staub 1974; Vezzosi et al. 2021) Within a slow progression, the oedema is postponed by the development of a more efficient lymphatic drainage of the pulmonary interstitial in response to chronic pulmonary congestion (Staub 1974). This should explain why in B2 dogs, extreme values reflecting the enlargement of the chambers were measured without CHF being detected, even though cases of undiagnosed CHF are likely in B2 dogs within this study, as previously referred. On the other side, in cases where for instance, the rupture of a primary chordae tendinea occurs, an acute progression of the disease follows, with no time for LA remodelling and a drastic elevation of LA and pulmonary capillary pressure, followed by a surge in acute pulmonary congestion and oedema. (Borgarelli et al. 2015). This can explain why some dogs from group C/D presented mild chamber enlargement, with one dog being classified as C/D moderate.

Nearly 45% (n=18) of B2 dogs classified as having severe or late-stage mitral insufficiency showed signs of high LV filling pressures, most of them with an E-vel score of 2 (1,2-1,5m/s), but six dogs presented severe E-vel values (>1,50m/s). The substantially increased E-vel values chosen as “cuff-offs” for the MINE score are generally seen in the last phase (restrictive) of diastolic dysfunction when the increasing E-vel doubles the A-vel. At this stage, the atrial and ventricular pressures are dangerously high, and the E-vel has predictive value for CHF development (Yamamoto et al. 1996; Schober and Maerz 2006; Gabriel and Klein 2009; Francis Smith, Larry Tilley, Mark Oyama 2015). Even though the statistical analysis of this variable only showed significant differences when comparing distant B2 severity groups (such as B2 mild vs B2 severe, or B2 moderate vs B2 late-stage) and C/D groups, its mean values were seen to consistently increase in accordance with the dog's ACVIM stage and echocardiographic severity classification in the current study.

The FS was highly variable within ACVIM B2 and C/D staged dogs, which might interfere with the more significant contribution of the remaining echocardiographic variables to the dog's echocardiographic severity classification. A normal and subnormal FS% is unexpected in dogs presenting MMVD associated with significant ventricular dilation and, when it occurs, is highly suggestive of systolic myocardial dysfunction (Francis Smith, Larry Tilley, Mark Oyama 2015). Even though myocardial dysfunction is more likely accompanied by extreme chamber enlargement and diastolic pressures, this might not always happen. Without all other three variables getting the highest score, dogs can be classified as severe (score of 8 to 11) while having an actual worst prognosis. The FS% relevance to differentiate mitral insufficiency severity was only seen in B1 dogs in this study. Based on these results, a further review of the FS% overall contribution to the MINE score could enhance the scoring system's accuracy.

Secondary to MR, LV remodelling and enlargement leads to an increase in end-diastolic and end-systolic volumes. Parallel to the establishment of the LVIDDn, used in this study, an allometric

relationship between BW and M-mode derived left ventricle end-systolic internal dimension (LVIDs) was found in one of the most accurate and extensive studies about canine cardiac dimensions (McGinley et al. 2007). It was determined within the study that the M-Mode derived LVIDs divided by the dog's BW elevated to the power of 0.315 ($BW^{0.315}$) should be ≤1.26, and that higher values would chiefly reveal an augmented LV. Dogs with MMVD may bear impaired systolic function (Borgarelli et al. 2004; Borgarelli et al. 2007). Still, the systolic function is challenging to assess in dogs suffering from this disease because of the ventricular loading changes. MR imposes a gradual increase in preload as the disease progresses and a predominantly subtle reduction in afterload that can worsen in case of myocardial dysfunction. To some extent, all the non-invasive indices assessing cardiac function are affected by these changes. In the hyperdynamic hearts of affected dogs, both FS and ejection fraction increase, reducing these indices' sensitivity for assessing LV dysfunction. Alternatively, increased end-systolic left ventricular dimensions may indicate decreased systolic function. Given the relationship mentioned above between this measurement and body sizes, an allometrically scaled LVIDs > 1.26 suggests an increase in LVIDs and can indicate LV systolic dysfunction in patients affected by MMVD (Menciotti and Borgarelli 2017). Therefore, we propose replacing the FS for the LVDIs normalized for BW to fix the ambiguity created by the FS and potentially enhance the scoring system's accuracy of the MINE score.

3.6. Limitations

Since one of the main goals of this study was to apply the MINE score to a different dog population in a different clinical setup, to assess the potential of the MINE score to be a commonly used universal echocardiographic method, some aspects differed from the OMSS. In opposition to the OMSS, the ACVIM classifications initially given to the 191 dogs within their first appointment at PVTH were maintained and never corrected, even if against the ACVIM guidelines for comparing purposes. The verified ACVIM impairments might have interfered with a potentially more significant agreement between results from both studies.

Performing thoracic radiography, concurrently with the echocardiographic, was a primary criterion of the OMSS, but not in the current study, which makes the inclusion of dogs with underdiagnosed CHF more likely within this study compared to the OMSS.

In terms of the limitations of the echocardiographic results, numerous variables might have influenced the results of both MINE score studies and their differences, such as the observer technique for image acquisition and measurements, the used instruments, the dog's hemodynamic variations (autonomic balance, preload, afterload, contractibility, heart rate, drugs) and cardiac anatomic variations between breeds and individuals.

In opposition to the OMSS, where all echocardiographic examinations were acquired by a board-certified cardiologist or by a supervised cardiology resident, in our study, the examinations were chiefly performed by professors and doctors from the PVTH, with variable degrees of veterinary cardiology experience, generally under the supervision of the most experienced cardiology professors: Dr Francesco Porciello, Dr Francesco Birettoni and Dr Domenico Caivano. Results are more likely to vary between ultrasonographers with less clinical experience and echocardiographic training.

3.7. Conclusion

With With this retrospective study, it was possible to confirm the importance of the MINE score, which is to be considered as the golden standard easy-to-use echocardiographic classification of the MMVD's severity in dogs. In the original study, the MINE score clinical effectiveness was evaluated by assessing the survival time of the MMVD patients in different MMVD ACVIM stages. The OMMS' results provided prognostic information that can objectively assess MMVD echocardiographically and identify dogs at higher risk of death due to cardiac disease.

Our study sample displayed significant differences from the original MINE study sample in terms of epidemiology. Nonetheless, applying the MINE score to the 191 dogs assessed at the Perugia Teaching Hospital showed a good agreement with the OMSS results. The group ACVIM B2 showed the broadest range of differences between both studies, with many being potentially explained by substantial impairments within the ACVIM guidelines in our study. Accordingly, thoracic radiography was not used to assess the presence of PE in dogs with advanced MMVD. Simultaneously, an odd number of dogs was classified as B2 while presenting severe echocardiographic features, some of which were prescribed loop diuretics. On the other end, dogs were misclassified as B2 without evidence of both chambers being enlarged. Even so, we considered that certain exceptions might occur, where the use of monodimensional variables to assess the size of left heart chambers might underestimate chamber enlargement. However, since more accurate measurements are too complex and time-consuming to use in the daily veterinary practice, the LA/Ao and LIVDDn and their cut-offs, having a well-established prognostic value, should be relied upon when in accordance with the other echocardiographic variables. We also conclude that the use of lung ultrasonography and home-based monitoring of the dog's RR at rest or during sleep to identify cardiac decompensation, in alternative to the use of thoracic radiography (used as a criterion for the inclusion of dogs in the OMSS), might have led to the underdiagnose of CHF in some dogs included in this study.

The dogs' early prescribing of cardiac medications was widely observed in this study. Cardiac drugs are a lifetime commitment in terms of cost and commitment to owners, which can be avoided by following the up-to-date guidelines. Combined with improved timing for drug prescription, a more

objective prognostic for the dog's cardiac status can be transmitted to the owner, based on the OMSS survival assessment, provided that a more accurate ACVIM classification is granted.

In this study, all four echocardiographic variables significantly contributed to differentiating dog's severity at some ACVIM stage of the disease. Even so, the FS% role to the MINE score and its actual specificity to the MMVD severity was questioned. This variable showed a major role in distinguishing the severity class of ACVIM B1 dogs, which are not expected to present substantial ventricular remodelling, which is known to contribute to the FS% upsurge correlated with MMVD severity. In the remaining ACVIM stages, FS% was highly variable, not contributing to differentiate MMVD severity. Finally, the FS% score role in the final stages of the disease should also be rethought due to progressive myocardial decompensation and FS decline that might occur in those stages, potentially leading to severity underestimation. Further studies are needed to clarify these issues. We propose replacing the FS for the LVDIs normalized for BW to fix the ambiguity created by the FS and potentially enhance the scoring system's accuracy of the MINE score.

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