UNIVERSITÀ
DI PARMA

DIPARTIMENTO DI SCIENZE MEDICO-VETERINARIE
Corso di Laurea Magistrale a Ciclo Unico in Medicina Veterinaria

EFFECTS OF PIMOBENDAN IN DOGS WITH MYXOMATOUS MITRAL VALVE DISEASE: A RETROSPECTIVE STUDY ON ACVIM STAGE B2 PATIENTS AFTER A FEW MONTHS OF TREATMENT

EFFETTI DEL PIMOBENDAN IN CANI CON MALATTIA MIXOMATOSA DELLA VALVOLA MITRALE: UNO STUDIO RETROSPETTIVO SU PAZIENTI ACVIM DI STADIO B2 DOPO ALCUNI MESI DI TRATTAMENTO

Relatore:
Chiar.ma Prof.ssa Cecilia QUINTAVALLA

Correlatore:
Dott. Stefano ORICCO

Laureanda:
Carlotta FERRI

ANNO ACCADEMICO 2021 – 2022

Summary

1 Abstract

Myxomatous mitral valve disease (MMVD) is the most common acquired heart disease in dogs. It takes several months or even years for the disease to progress from mild and asymptomatic to severe disease with signs of congestive heart failure.

This observational, retrospective, multicenter study analyzed a population of ACVIM B2 patients referred to Cardiology Unit of the Veterinary Teaching Hospital of Parma University (OVUD), the Centro Veterinario Imperiese and the Veterinary Teaching Hospital of Turin University.
The purpose of this study was to evaluate the effect of Pimobendan administration on selected echocardiographic parameters of left cardiac chambers volume overload and of mitral regurgitation severity in dogs affected by ACVIM B2 MMVD in a mean period of 6 months from the start of Pimobendan.

In the period of time between January 1st 2019 and 31st July 2022, 57 dogs met the inclusion criteria and were included in this study. Several breeds were represented, in particular mixed breed and Cavalier King Charles Spaniel. There was no significant difference between females and males. The median age of the dogs was 132 months, whereas the median weight was 8,8 kg. The echocardiographic measures evaluated revealed that the size of the cardiac volumes remained stable (median LA/Ao at baseline 1.78, control 1.79; median LAV at baseline 20.65, control 20.6), despite an increase in the rate of mitral regurgitation. In fact, the median of the mitral VTI changed from 12.15 to 13.45, and the median of the mitral to aortic VTI ratio changed from 1.1 to 1.26.

After an average of 6 months of Pimobendan use, there are no statistically significant alterations in echocardiographic and clinical markers linked to volumetric overload. As a result, it is reasonable to assume that MMVD B2 patients who begin Pimobendan therapy do not need to be checked for 6 months.

Abstract

La malattia mixomatosa della valvola mitrale (MMVD) è la malattia cardiaca acquisita più comune nei cani. Ci vogliono diversi mesi o addirittura anni perché la malattia progredisca da lieve e asintomatica a grave con segni di insufficienza cardiaca congestizia.

Questo studio osservazionale, retrospettivo e multicentrico ha analizzato una popolazione di pazienti con ACVIM B2 riferiti all'Unità di Cardiologia dell'Ospedale Veterinario Universitario di Parma (OVUD), al Centro Veterinario Imperiese e all'Ospedale Veterinario Universitario di Torino. Lo scopo di questo studio è stato quello di valutare l'effetto della somministrazione di Pimobendan su selezionati parametri ecocardiografici di sovraccarico di volume delle camere cardiache sinistre e di gravità del rigurgito mitralico in cani affetti da MMVD ACVIM B2 in un periodo medio di 6 mesi dall'inizio della somministrazione di Pimobendan.

Nel periodo di tempo compreso tra il 1° gennaio 2019 e il 31 luglio 2022, 57 cani hanno soddisfatto i criteri di inclusione e sono stati inclusi in questo studio. Erano rappresentate diverse razze, in particolare meticci e Cavalier King Charles Spaniel. Non è stata riscontrata alcuna differenza significativa tra femmine e maschi. L'età mediana dei cani era di 132 mesi, mentre il peso mediano era di 8,8 kg.
Le misure ecocardiografiche valutate hanno rivelato che le dimensioni dei volumi cardiaci sono rimaste stabili (LA/Ao mediana al basale 1,78, controllo 1,79; LAV mediana al basale 20,65, controllo 20,6), nonostante un aumento del tasso di rigurgito mitralico. Infatti, la mediana del VTI mitralico è passata da 12,15 a 13,45 e la mediana del rapporto VTI mitralico/aortico è passata da 1,1 a 1,26.

Dopo una media di 6 mesi di utilizzo di Pimobendan, non sono state riscontrate alterazioni statisticamente significative dei marker ecocardiografici e clinici legati al sovraccarico volumetrico. Di conseguenza, è ragionevole ritenere che i pazienti con MMVD B2 che iniziano la terapia con Pimobendan non debbano essere controllati per 6 mesi.

BACKGROUND OF THE STUDY

2 Introduction

2.1 Myxomatous mitral valve disease

Myxomatous mitral valve disease (MMVD) is the most common acquired heart disease in dogs [1][2]. Since its first description in literature, this disease has been defined with different nomenclature (mitral endocardiosis, chronic mitral valve fibrosis, and myxomatous degenerative mitral valve disease) [3].

MMVD is characterized by a slow progressive myxomatous degeneration from the tips of the mitral leaflets onward with subsequent regurgitation of the mitral valve and atrial and left ventricular dilatation [4].

According to Borgarelli in 2022, it takes several months or even years for the disease to progress from mild and asymptomatic mitral myxomatous disease to severe disease with signs of congestive heart failure [5].

Although it has been known for a long time, the etiology and pathophysiology of mitral disease are currently poorly understood [6]. More recent studies underline the genetic origin of MMVD, but at present causative mutations and complete molecular pathogenesis are unknown [2][7]. The most plausible hypothesis is that at the base of the disease there is a hereditary component of a polygenic type [3].

2.2 Epidemiology and etiology

MMVD is the most common acquired cardiac disease in dogs (a similar disease is also observed in humans, horses, and pigs [8]), but the prevalence is highly variable among different breeds [9]. Several studies have estimated that the prevalence of MMVD in dogs varies between 21% and 89%. This extremely wide range depends on the fact that each dog has different clinical characteristics, and factors such as size, age, and breed can make huge differences [10].

MMVD is most prevalent in small to medium-sized dog breeds such as Cavalier King Charles Spaniels, Dachshund, Shih Tzu [11], Miniature Poodle, Maltese, Pomeranian, Yorkshire Terrier, and Chihuahua [7]. As concerns large dogs, MMVD is mainly diagnosed in German Shepherds and Great Danes, a breed often considered to have one of the shortest predicted lifespans of all breeds [12].

The prevalence of MMVD has been found to increase with age and can approach 100% in geriatric populations of high-risk breeds. Males are over-represented in some epidemiological studies, data not confirmed in a more recent large-scale study [13].

Although the etiology of MMVD is currently unknown, a basis of inheritance has been found to exist in the Dachshund and Cavalier King Charles Spaniel breeds, suggesting a polygenic mode of inheritance. Currently, genetic mechanisms remain to be clarified [2].

In particular, Cavalier King Charles spaniel (CKCS) seems to be a breed particularly prone to MMVD [14][15]. Indeed, in CKCS, it has been estimated that 50% of dogs are affected at the age of 6–7 years [16]. In a recent prospective study of a large number of CKCS, parental cardiac status had a marked influence on the prevalence and severity of murmurs typical of MR in 5-year-old offspring [17].

The ACVIM Consensus on mitral valve disease introduced the concept of patients at risk of developing the disease, i.e. who do not have the disease at the time of their assessment but are predisposed (Class A) [18].

Dachshund is another dog breed that is predisposed to the disease, with a prevalence of about 50% at the age of 10 years [19]. Another breed particularly prone to this valvulopathy are Whippets. Several studies have shown a high frequency of MMVD and a relatively early age of onset in some dogs to support its plausible genetic origin [7].

In literature, it is possible to find several genomic studies concerning canine MMVD. There are large hypotheses and often in conflict with each other. Some of the main studies that have contributed to the genetic study of this pathology are describe below:

Madsen et al. found and analyzed two loci on chromosomes 13 and 14 that are weakly associated with the development of MMVD [16]. In contrast, French et al. found no evidence for loci associated with the development of mitral disease. The authors hypothesized that the familial onset of mitral valve murmurs in the CKCS breed is not due to a single major gene effect but to a condition of polygenic predisposition [20]. A great contribution to the genomic study of MMVD was made by Meurs et al. in 2017, who performed genome-wide sequencing of 10 CKCS and 10 dachshunds, both breeds with a high prevalence of MMVD. No variant was found in any of the evaluated genes, but a single coding variant, predicted to be benign, was found in the COL5A1 gene in nine of the 10 affected CKCS [21].

2.3 Pathogenesis

The exact etiology of MMVD has not yet been identified [3], but there is evidence to support the hypothesis of a polygenic inheritance component in some dog breeds [16][19].
The polygenic nature of this pathology was first formulated by Swenson in the study *"Relationship between parental cardiac status in Cavalier King Charles spaniels and prevalence and severity of chronic valvular disease in offspring*", in which he demonstrates how CKCS with more severe mitral disease had an offspring more prone to the aforementioned disease (more intense heart murmur, earlier disease development)[17].

In the following years, this theory was reapplied and reaffirmed in Dachshunds [19]. Currently, the genes involved in the transmission of the disease have not yet been identified. In addition to the hereditary component of heart disease, other factors are involved, such as the anatomy of the mitral apparatus, physical exercise, and obesity [8].

Several studies have contributed to the identification of the different pathological processes that lead a patient to develop MMVD. Some experimental evidence seems to support a possible role of the serotonin signaling pathway triggered by altered mechanical stimuli in the development of the mitral disease: in this hypothesis, the activation of mechanosensors is associated with a local increase in the expression of tryptophan hydroxylase 1 (TPH1), the main enzyme synthesizing serotonin [22][23].

Another accredited hypothesis is that at the base of the pathology there is also a primary disturbance of the metabolism of collagen and of the extracellular matrix [24][25]: a pathological accumulation of proteoglycans, responsible for structural alterations involving the valve cusps and the tendon cords [26].

From a macroscopic point of view, the mitral cusps show different degrees of fibrous and edematous thickening, while at the microscopic level the spongy of the cusps shows an accumulation of acidic mucopolysaccharides and proliferation of fibroblasts; the fibrous, on the other hand, undergo atrophy [26]. Probably, the damage or the loss of the endothelium in the affected areas play an important role in the progression of the disease, given the ability of the endothelial cells to communicate extensively with the subendothelial cells [8].

To better understand the degree of mitral valve degeneration, valve lesions were classified by Whitney and Pomerance into 4 categories [27]:

- Types 1 and 2 lesions are characterized by small nodular thickenings of the more or less extensive valve flaps, without the involvement of the tendon cords.
- Type 3 lesions are characterized by a larger area of valve involved.
- Type 4 lesions involve the chordae tendineae and are frequently associated with rupture of the same [3].

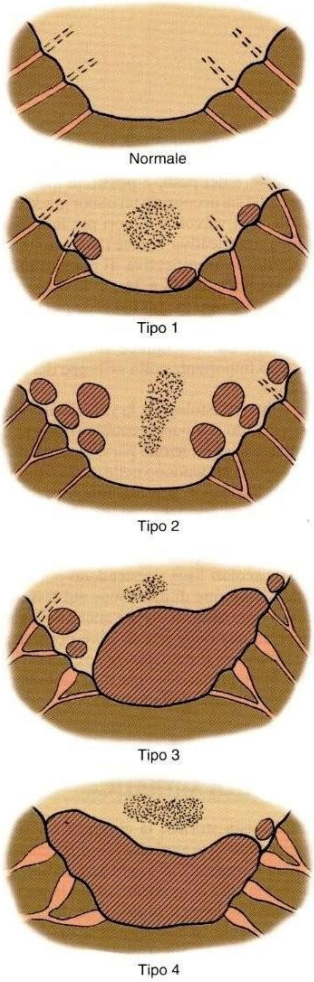

***Figure 1**- Whitney and Pomerance classification of mitral valve lesions in dogs (Manuale di cardiologia del cane e del gatto, 2018)*

The pathophysiology of the disease, on the other hand, is well described: the structural alterations of the valve of the respective chordae tendineae are responsible for a dislocation of the mitral valve flaps during atrial systole, which leads to a prolapse of the valve itself [28].
As a result of this degeneration, part of the systolic blood volume of the left ventricle returns to the left atrium [8][29].

The left ventricle compensates for the loss of the antegrade systolic volume (i.e. that volume of blood that returns to the left atrium) by increasing the end-diastolic volume, therefore the preload, while the heart rate is affected only in the advanced stages of the disease. Increasing the preload improves the cardiac contraction force due to the Frank-Starling mechanism, according to which the heart muscle regulates the force of its contraction based on the amount of blood present in the ventricle at the end of diastole [30][31].
With the progression of valve lesions and the worsening of the rate of mitral insufficiency, a series of compensatory mechanisms are activated including the sympathetic nervous system, the renin-angiotensin-aldosterone system (RAAS) and the action of the antidiuretic hormone, which contribute to maintain an adequate antegrade systolic volume [3][8].

The left atrium plays a fundamental role in the pathogenesis of mitral insufficiency, as its progressive dilation allows for normal filling pressures to be maintained even in the presence of severe mitral regurgitation [3][32]. Its chronic remodeling is often associated with atrial hypertrophy and dilatation, which over time reduce the compliance of the heart chamber and lead to an easier development of pulmonary edema [3][8].

One of the most hopeful directions appears to be focusing on the differences between valvular cells from myxomatous and unaffected regions of mitral valves from dogs of all ages [33].

2.4 Staging system for myxomatous mitral valve disease

In order to properly understand and manage a patient with MMVD, it is necessary to follow the directions outlined by the ACVIM (American College Of Veterinary Internal Medicine), which has reviewed the guidelines for the diagnosis and treatment of mitral myxomatous disease, previously drafted and published in 2009. In these new guidelines, there are several innovations regarding the management of the mitral patient, the early diagnosis of MMVD, and the recommendations for treatment (both medical, surgical, and dietary).

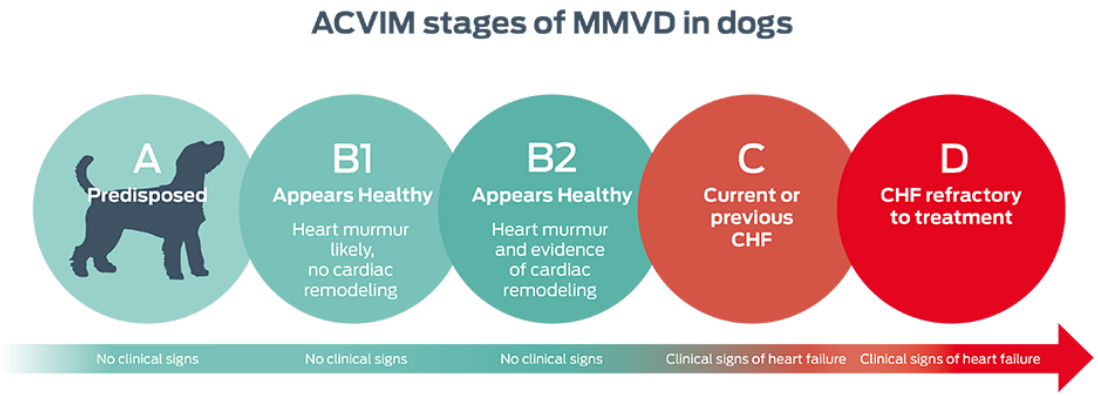

***Figure 2**- ACVIM stages of MMVD in dogs (https://www.purinainstitute.com)*

2.4.1 Stage A

Dogs classified as "Stage A" are patients at high risk of developing the disease, but who at present do not present signs and symptoms attributable to the same. Regarding the diagnosis, it is recommended to carry out periodic checks with cardiac auscultation in all small dogs, especially in predisposed breeds (for example Cavalier King Charles Spaniels, Dachshunds, Poodles, Maltese, small mixed-breed dogs) [18].

Particularly in CKCS, in which a genetic predisposition has been demonstrated - the prevalence of the disease in subjects over 10 years exceeds 90% [34]. The panelists recommend anticipating echocardiography controls already at a young age (<6-8 years). Regarding medical indications of this category of patients, it is not necessary to administer drugs or dietary treatments [18].

2.4.2 Stage B

Dogs classified as "Stage B" are patients who have structural abnormalities, but who have never shown clinical signs referable to heart failure due to cardiac alterations. Stage B is divided into two subclasses, Stage B1 and Stage B2.

- **Stage B1**: dogs classified as "B1" are asymptomatic patients who have mitral regurgitation caused by valve degeneration, but who do not yet need therapy as they do not have cardiomegaly. From a diagnostic point of view, B1 patients have a normal size of the atrium and left ventricle, maintained systolic function at the echocardiographic visit, and absence of cardiomegaly on radiography [18].

- **Stage B2**: Dogs classified as “B2” are asymptomatic patients who have mitral regurgitation so severe that it leads to cardiac remodeling. To be classified in this category, dogs must meet certain criteria:

    - Heart murmur intensity of at least 3/6
    - Echocardiographic LA:Ao (ratio between the left atrium and the aorta) in right parasternal short-axix view at the beginning of diastole ≥ 1.6
    - LVIDDN (Left ventricular internal diameter in diastole normalized for bodyweighyt) ≥ 1.7
    - Breed-adjusted VHS (vertebral heart score), which is evaluated by radiography ≥ 10.5

Theoretically, B2 patients should meet all of the above criteria. If it is not possible to perform an echocardiographic examination, the ACVIM panel recommends using cardiac auscultation (heart murmur ≥3/6) and radiography: a patient with VHS ≥ 11.5 can be classified as B2 [18].

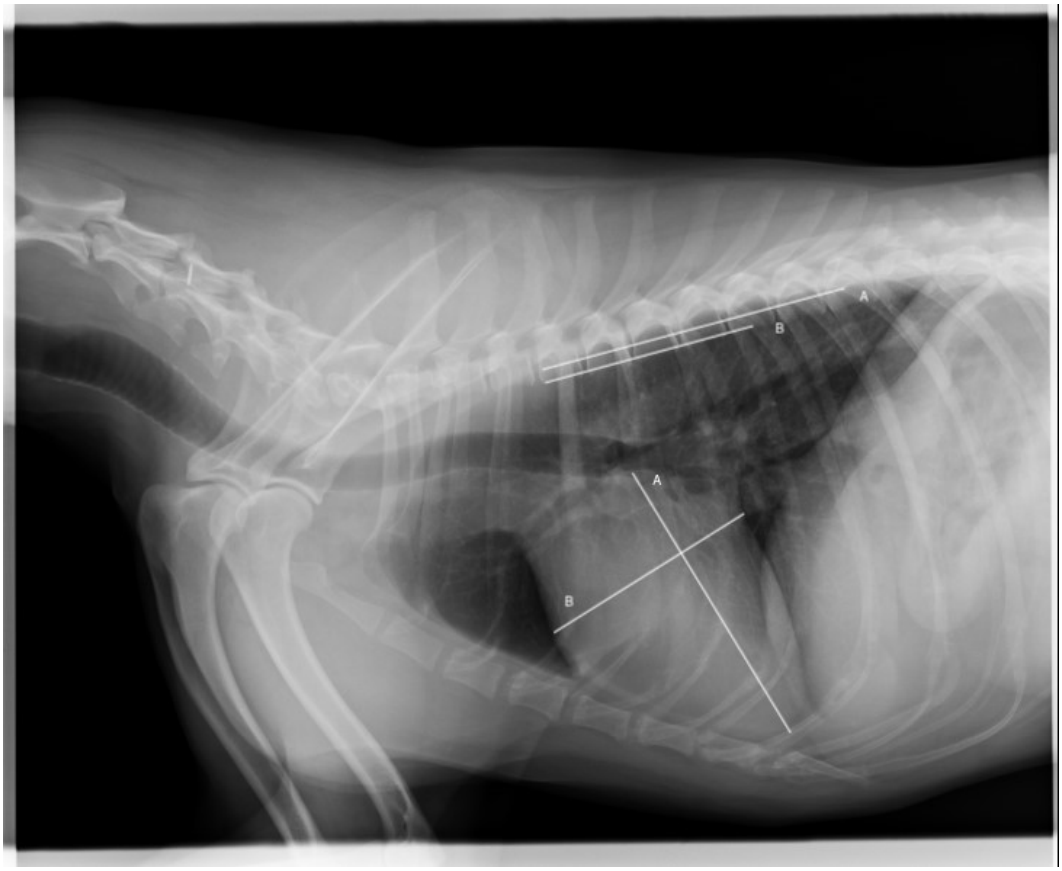

***Figure 3**- Method for determination of VHS measurement on a chest radiograph in laterolateral projection. The measurement of the major axis of the cardiac silhouette (A) corresponds to 6.2 thoracic vertebrae; that of the minor axis (B) corresponds to 4.5 vertebrae. The value of VHS is therefore a+b, hence 10.7. This result is compatible with a slightly increased heart size.*

2.4.3 Stage C

Dogs classified as “Stage C” are patients who have presented or present with clinical signs of heart failure caused by MMVD and who are not refractory to standard therapy [18]. Stage C marks the end of a chronic pathological process that began long before with the onset of valve insufficiency [8]. As regards the diagnosis, it is important to start with the medical history and the clinical visit. Patients classified as “Stage C” are often dogs who have presented or present with tachypnea, restlessness, respiratory distress, and cough. The clinical examination should include the non-invasive measurement of systemic systolic pressure and it is of fundamental importance to perform a chest X-ray, echocardiography, and laboratory tests. In the event that a subject C should undergo a successful mitral valve repair, it can return to be classified as "B2" [18].

2.4.4 Stage D

Dogs classified as “Stage D” are end-stage patients with clinical signs referable to heart failure caused by MMVD and refractory to therapy [18]. These are the patients with the highest risk of developing complications, such as arrhythmias (mainly ventricular and supraventricular arrhythmias), rupture of the chordae tendineae, pulmonary hypertension and rupture of the left atrium [3]. The rupture or fissure of the left atrium is the major complication in dogs with MMVD. This is an urgency that can manifest clinically as hemopericardium, intracardiac thrombosis, or both [35].

2.5 Diagnosis

2.5.1 Physical examination

The diagnostic suspect of mitral valve disease is generally related to the recognition of a systolic heart murmur at the left apical level during auscultation [3]. Generally, the loudness of the murmur is related to the severity of the valve insufficiency, but not to the clinical status of the patient [36][37]. In the event of more advanced disease, the heart murmur is radiated dorso-caudally and to the right hemithorax, due to the atrioventricular enlargement resulting from the progression of the disease and the possible involvement of the tricuspid valve [3]. Although the discovery of a systolic murmur is a very common finding, it is not a necessary condition in mild forms of the disease [3].

In fact, in the early stages of the disease, the only auscultation finding is a mid-systolic click, which increases in intensity in patients with a high heart rate. The mid-systolic click is caused by mild valve prolapse associated with mitral regurgitation [3] or by the tension of the thickened tendon cords and the rapid deceleration of blood against the mitral flaps [34].

On auscultation of the lung fields of mitral patients without signs of heart failure, sounds are normal [8]; on the contrary, in dogs with pulmonary edema, it is possible to hear sounds such as crackles/clicks/popping. This means that the evaluation of the respiratory system is essential to understand the progression of the disease: symptoms such as dyspnea, tachypnea, and exercise intolerance are clinical indicators of disease progression [38][39].

At the physical examination of MMVD patients in an early stage of the disease, the mucous membranes are usually normal, with a capillary refill of less than two seconds. In patients in the advanced stage of the disease, the mucous membranes are cyanotic or greyish [8]. As for the clinical signs referable to mitral disease subjects classified as ACVIM A and ACVIM B do not show pathognomonic symptoms. The only symptoms reported - not always found - are exercise intolerance and cough which are not specific signs of heart disease [1][8].

The first clinical signs of decompensation in subjects classified as ACVIM C are usually mild, but can rapidly worsen within a few days or weeks, leading to congestion and pulmonary edema with tachypnea and dyspnoea [8]. Dogs with decompensated MMVD are more restless, inactive

patients with varying degrees of loss of appetite. Cardiac cachexia may also develop, although weight loss may be associated with concomitant fluid retention (ascites) and edema [8]. Some dogs with MMVD have syncopal episodes, often associated with episodes of tachyarrhythmia, episodes of coughing, or exercise in the presence of pulmonary hypertension [8].
Having developed a deep awareness of the spread of this disease in some breeds (such as, for example, CKCS), breed screening tests often allow for the early diagnosis of MMVD [40].

2.5.2 Thoracic radiography

Performing thoracic radiography is a fundamental part of the diagnostic process for all patients with suspected MMVD [3][18]. The goals of radiographic examination are different: to evaluate the hemodynamic consequences of mitral disease, to check for the presence of pulmonary edema, and to evaluate the presence of concomitant respiratory diseases [3]. In addition, in patients who have already been diagnosed with MMVD, thoracic radiography allows to evaluate changes in the left atrium and left ventricle, the pulmonary vessels, and the main bronchi [8].

Thoracic radiography is often used to differentiate dogs classified as B1 from those classified as B2. Generally, the larger the left heart, the more severe the underlying mitral pathology [41].
To objectively express cardiac enlargement, the consensus on mitral disease drawn up by ACVIM suggests the use of two quantitative radiographic measures: the vertebral heart score (VHS) and the vertebral left atrial size (VLAS) [18].

VHS is a cardiac measurement method that compares the size of the heart silhouette with the length of the thoracic vertebral bodies [42]. The measurement is done using a lateral projection radiograph. The dimensions of the long axis and the short cardiac axis are brought to the vertebral column with the beginning of both segments on the cranial margin of T4. The number of vertebrae covered by the segments will be counted and the two values are added together [41]. Normal values depend on the breed: it is, therefore, advisable, when present, to use the numerical values related to the individual races [3].

According to the ACVIM consensus, a VHS greater than or equal to 10.5, associated with an echocardiographic picture compatible with atrial magnification, is compatible with an ACVIM B2 subject.

The VLAS, on the other hand, is a useful parameter to verify the presence of cardiomegaly of the left heart, which allows to distinguish stage B1 dogs from stage B2 dogs. In particular, it has been estimated that a cut-off of 2.5 may be useful for identifying dogs that require a control echocardiography, while a cut-off of 3.1 may be useful for distinguishing B2 from B1 patients [43].

From the most recent studies. VLAS appears to be a more specific atrial magnification parameter than VHS [44].

2.5.3 Echocardiography

The echocardiographic examination is considered the gold standard method, according to the ACVIM guidelines [18] for the diagnosis of degenerative disease of the mitral valve [3][45]. Echocardiography, therefore, allows the cardiologist to confirm or exclude a diagnosis of MMVD, identify any concomitant pathologies [3], and evaluate any thickening of the mitral valve and its protrusion in the left atrium [8].

The two-dimensional study (called B-mode) and the one-dimensional (M-mode) study are useful for evaluating the morphology and type of lesion, the possible presence of valvular prolapse or broken tendon cords [46], while the measurement of the dimensions and functional parameters of heart chambers defines both the chronicity of the pathology and the degree of remodeling.

Color Doppler identifies the possible presence of regurgitation. By evaluating the extension of the regurgitating jet in the atrial chamber and the width of the jet at the level of the regurgitating orifice and the flow convergence area, an accurate estimate of the regurgitating volume can be obtained. Continuous and pulsed wave Doppler determines the hemodynamic severity of the insufficiency [46][47].

Currently, there is no perfect method to accurately establish the severity of regurgitation, but the different measurements and assessments must be used in an integrated and smart way, taking into account the primary (organic) or secondary (functional) etiology of the regurgitation and the impact on cardiac morphology and function.

Essential two-dimensional echocardiographic measurements in dogs affected by MMVD for classification according to ACVIM guidelines

- **Measurement of the left atrium/aorta ratio**

  The left atrium/aorta ratio is an important echocardiographic parameter because it allows the clinician to analyze the progression of mitral disease. The left atrium/aorta ratio allows you to relate the atrial diameter with the aortic annulus through the right parasternal short axis scan [3]. Physiologically this ratio must be less than or equal to 1.6 [48]. As the disease progresses, the size of the left atrium increases, and in dogs that develop heart failure, the ratio may be greater than 2. Currently, several studies have shown that the value of the LA/Ao ratio can be used as a prognostic factor for heart failure, and patient outcome [34][49] However, according to a recent study, the LA/Ao relationship is a moderately reliable parameter as it is operator dependent [50].

- **Left ventricular volumes**

  The estimate of ventricular volumes is important for documenting the volume overload and any ventricular dysfunction that accompanies chronic valve regurgitation. In fact, it must be considered that the extra volume of blood that is introduced into the left atrium at each diastole increases the stress of the wall and triggers a chamber remodeling response (both atrial and ventricular) [51].

- **Body weight normalized left ventricular internal diameter in diastole (LVIDDN)**

  LVIDDN is an echocardiographic measure that is commonly used to assess the degree of left ventricular internal diameter [1]. The LVIDd measurement timing coincides with end diastole, that is the maximum left ventricular dimension. From the right parasternal short-axis high papillary muscle view, the measurement extends from the mid-interventricular septum to the mid-free wall [41]. This measurement is indexed to body size.

$$\mathrm{LVIDDN} = \frac{\mathrm{LVIDD}^{a}\ (\mathrm{cm})}{\mathrm{Weigh}\ (\mathrm{kg})^{\,0.294}}$$

  LVIDDN is considered a fundamental parameter for the classification of patients who should start therapy according to the ACVIM guidelines [18]. In particular, an LVIDDN greater than or equal to 1.7 is suggestive of cardiomegaly due to mitral insufficiency and

is associated with a worse outcome [52][53]. A recent study discovered that a scaling exponent of 0.294 does not always represent ventricular enlargement. On the contrary, a scaling exponent of 0.33, with breed-specific reference limits for different breeds, reduces the misclassification of healthy dogs as having left ventricular enlargement [54].

- **VAS**

  The left atrial volume is a fundamental echocardiographic parameter capable of evaluating left atrial enlargement. This is a different method than the LA:Ao ratio. Volumetric assessments are based on dimensions obtained from multiple planes and may detect chamber enlargement with greater sensitivity than the LA: Ao, which is determined by a single linear atrial dimension [55]. Generally, left atrial volume is calculated using the biplane area-length method from the left apical 2- and 4-chamber views at the end of ventricular systole [55]. According to the study by Franco et al (2016), the mean left atrial volume in diastole was 16mL / m2, while in systole it was 9mL / m2 in healthy dogs [55], but with a great variety among the different races. LA volume increase with increasing disease severity. In fact, a large LA volume is a reflection of volume overload due to regurgitant flow [56].

Quantification of mitral valve regurgitation by Color Doppler

- **Size of the regurgitating jet**

  Color Doppler is a quick and useful method to exclude or confirm the presence of valve regurgitation [57]. However, the ability to accurately stage the severity of the regurgitation is poor: theoretically, as the severity of the regurgitation increases, the extension of the regurgitating jet in the atria also increases. Jets extended to less than 20% of the atrium are considered mild, 20 to 40% moderate and over 50% severe. The evaluation can be performed on various scan planes. In mitral regurgitation, another system is to relate the area of the regurgitating jet to the area of the left atrium through the 4-chamber apical scan. A ratio of <30% represents mild regurgitation, 30 to 70% moderate and over 70% severe. Undoubtedly, large regurgitating jets, which extend deep into the atrium above, indicate a higher rate of regurgitation. However, the relationship between the jet area and the severity of the regurgitation is very variable since the color

Doppler signal depends not only on the severity of the regurgitation but also on technical and haemodynamic factors. For example, with the same severity of regurgitation, subjects with high atrial pressures or with greatly enlarged atria may have smaller jet areas than ones with normal size and pressure atria or with centrally directed jets. Color Doppler should only be used to detect the presence of regurgitation: a quantitative approach is required if more than one small jet extending just beyond the annular plane is detected.

- **Width of the jet at vena contracta**

  The vena contracta is the narrowest and fastest regurgitating jet segment and represents the area of the regurgitating orifice (ROA). The width of the jet at the vena contracta indicates the severity of the regurgitation. To evaluate this parameter, parasternal long-axis scans or in any case scan planes are used in which the vena contracta is perpendicular to the scan plane itself. In humans, a vena contracta <3 mm indicates a mild regurgitation > 6 mm a severe regurgitation. For intermediate values, there are many overlaps. In a study in dogs with mitral disease a 4.9 mm vena contracta (IQR 5.4-4.1) corresponded to moderate-severe regurgitation and a 2.9 mm vena contracta (IQR 3.4- 2.5) to mild to moderate regurgitation [58]. The measure of the vena contracta can also be related to the aortic diameter to minimize the effects of the size of the animal. In one study, a vena contracta/aorta ratio> 0.24 was a negative prognostic factor. The measurement of the width of the vena contracta is a simple method but is based on the assumption that the regurgitating orifice is circular and fixed, and this represents a limit of this evaluation. The effective regugitant orifice area (EROA) can be calculated from the width of the vena contracta.

- **PISA method (proximal isovelocity surface area) or flow convergence**

  The PISA method relies on the hydrodynamic principle whereby the flow directed towards a circular orifice accelerates creating concentric hemispheres with progressively higher velocity and progressively smaller area. The 4-chamber apical projection is usually used for measurement. The finding of convergence of the flow at Nyquist limit values of 50-70 cm/s corresponds to significant regurgitation volumes. Mathematical formulas allow you to calculate the regurgitating flow, the EROA, the regurgitating volume and the

regurgitating fraction. In humans, an EROA <0.2 cm2 indicates mild insufficiency, between 0.2 and 0.39 cm2 moderate insufficiency, and <0.4 cm2 severe insufficiency.

In dogs, a mitral regurgitation fraction <45% corresponds to mild insufficiency, between 45% and 75% moderate, and> 75% severe. The PISA method has several limitations: it assumes a circular regurgitating orifice and a hemispherical flow convergence, and this does not happen in many cases. Another source of error is the dynamic nature of many regurgitations, and the calculation on a frame (the one with the largest PISA) could cause errors in estimating the regurgitation [59].

Quantification of mitral valve regurgitation by Pulsed Spectral Doppler

- **Volumetric method**

  The volumetric method is based on the calculation of the regurgitating volume and the regurgitating fraction starting from the volume of the transmitral flow and the aortic output. The flow through an orifice can be calculated by multiplying the area of the orifice by the time-velocity integral (VTI) measured with pulsed Doppler. The area is calculated using the diameter of the orifice and assuming a circular shape.

  The regurgitation volume is given by the difference between the volume of the transmitral flow and the aortic output and the regurgitation fraction is the regurgitation volume divided by the volume of the transmitral flow and represents the percentage of total output that regurgitates in the left atrium. For volumetric calculations of mitral regurgitation, the aortic diameter is measured from the parasternal long axis projections to the insertion points of the valve leaflets or by tracing the aortic area on the cross sections. The VTI of the aortic flow is calculated from the subcostal or apical 5-chamber view with the sample volume at the valve level. The diameter of the mitral annulus and the VTI of the transmitral flow at the level of the annulus are calculated on the apical 4-chamber projection. Multiplying the regurgitation fraction by the VTI of mitral regurgitation (continuous Doppler) we obtain the EROA. In humans, a regurgitation fraction (RF) ≥ 50% indicates severe regurgitation, a RF <30% a mild regurgitation. These measurements have the advantage of taking into account the systolic behavior of the regurgitation: the data are not obtained on a single frame and therefore represent the real volumetric overload. Potential errors can arise from the measurement of annular

diameters which are amplified in the calculation of the areas and in the positioning of the sample volume. Furthermore, measurements are not reliable in case of concomitant aortic regurgitation. The circular shape of the mitral valve is also assumed. This measuring system can be useful in the case of eccentric or multiple jets. Alternatively, the total range can be calculated by estimating the end-diastolic and end-systolic ventricular volumes using the Simpson or area-length method: the value obtained can be inserted in the formulas for the calculation of volume and regurgitation fraction and in this way the scrutiny calculation is avoided. of transmitral flow. Volumetric calculations are difficult to apply to the study of tricuspid regurgitation due to the inevitable errors in the measurement of annular diameters, the asymmetrical shape of the annulus and the difficulty in aligning correctly with the transtricuspid flow.

Other indirect signs of regurgitation severity

- **Continuous Doppler of regurgitant flow**

  A fully enveloped high-density signal is indicative of significant regurgitation. Conversely, a weak, low-density signal with an incomplete envelope suggests mild regurgitation.

- **Transmitral flow**

  In the event of significant regurgitation, there is an increase in the peak velocity of the E wave of the transmitral flow at pulsed Doppler due to the volumetric overload. However, it is not a specific marker and must be interpreted in the light of other clinical findings and evaluations. In particular, the severity of regurgitation can be deduced from the indirect estimate of the left atrial pressures on the basis of size of the atrium, pulmonary venous flow (dominant diastolic, even with retrograde flow in the veins during systole), ratio between wave velocity E and isovolumetric relaxation time (E/IVRT) and ratio between E wave velocity and E 'velocity at mitral annulus tissue Doppler (E/E').

- **Mitral /Aortic VTI ratio**

  The VTI ratio of transmitral flow to aortic flow estimated with pulsed Doppler is a further proposed index to quantify isolated organic valve regurgitation, independent of jet geometry.

Theoretically, in the absence of a left-sided valvular regurgitation, the mitral inflow must be equal to the aortic outflow. In Tribouilloy's (1994) study, the mitral to aortic VTI ratio was significantly elevated, and there was a close relationship between the mitral to aortic ratio and angiographic grade of mitral regurgitation and regurgitant fraction [60].

This is a particularly significant finding, as it is deduced that the Mitral/Aortic VTI is a reliable echocardiographic parameter that is not affected by the geometry of the regurgitating jet. This non-geometric index may be particularly useful in screening tests to assess the severity of mitral regurgitation in patients with highly eccentric regurgitant jet [60][61].

- **Pulmonary arterial pressure estimation**

  Pulmonary arterial hypertension can develop as a consequence of the increase in pulmonary venous pressures (pulmonary hypertension type II) in subjects with severe mitral insufficiency and can be estimated according to guidelines [62].

2.6 Therapy

Currently, there are no therapies capable of preventing myxomatous degeneration of the mitral valve. This means that medical treatment for patients suffering from this degenerative disease aims to control symptoms, improve quality of life and, therefore, increase survival. Since the importance of medical therapy is ascertained, most recent studies focus their trials on understanding the most suitable time to start therapy.

Regarding **class A** according to ACVIM staging, no medical therapy or dietary treatment is required [18].

As regards **class B1**, according to ACVIM guidelines, treatment is not recommended in these dogs, as, at this early stage of the disease, its progression is not certain. In these patients, echocardiographic re-evaluation every 6-12 months is recommended to monitor disease progression [18]. At the same time, new studies have emerged, such as *"The effect of treatment with Pimobendan in dogs with preclinical mitral valve disease - a placebo-controlled double-blinded crossover study*", which has shown promising results as it emerges that pimobendan has a reducing effect on biomarkers (NT-proBNP) at rest and with exertion and that reduces the size of the left ventricular volume [63]. Also, on the same line of the aforementioned article, *"Effect of Pimobendan on physical fitness, lactate and echocardiographic parameters in dogs with preclinical mitral valve disease without cardiomegaly*" underlines how some subjects classified as ACVIM B1 can benefit from therapy with Pimobendan [64].

As regards **class B2**, the ACVIM guidelines now recommend starting the administration of pimobendan at a dosage of 0.25-0.3 mg/kg PO (per os) every 12 hours. All stage B2 diagnosis criteria must be met, as pimobendan represents a lifetime therapy for the mitral patient [18]. The choice to recommend administration of this drug in the guidelines is based on as the paper "*Effect of Pimobendan in Dogs with Preclinical Myxomatous Mitral Valve Disease and Cardiomegaly: The EPIC Study — A Randomized Clinical Trial"*, which demonstrated that the administration of pimobendan in dogs with cardiomegaly secondary to MMVD in a pre-clinical stage, in the absence of other cardiovascular drugs, results in a prolongation of the preclinical period of about 15 months and is well tolerated by the animal [53].

A part of the ACVIM panelists (5 out of 10) recommends treatment with ACEI (angiotensin-converting enzyme inhibitors) in dogs with a significant increase in left atrial volume. However, the studies currently available in the literature have contradictory results. In one of the most recent studies, *"Efficacy of Enalapril for Prevention of Congestive Heart Failure in Dogs with Myxomatous Valve Disease and Asymptomatic Mitral Regurgitation*", it was shown that long-term ACE inhibitor treatment in asymptomatic dogs with MMVD does not delay the onset of CHF regardless of whether cardiomegaly is present at the start of therapy [65].

The administration of spironolactone is not recommended at this stage. A study evaluating the efficacy of the association of benazepril and spironolactone in delaying the onset of clinical signs in stage B dogs failed to show any benefit in prolonging the asymptomatic phase of this disease [66]. However, the same study points out that the treatment, which is safe and well tolerated by mitral patients, can reduce or even reverse the cardiac remodeling process [66].

At the same time, dietary treatment is recommended. The guidelines recommend starting a diet low in sodium, palatable and with a correct percentage of proteins and calories, in order to maintain an excellent BCS, or body condition score [18].

Some panelists find the administration of cough suppressants useful, especially when there is an indication that the cough is the result of increased pressure resulting from the enlargement of the heart [18].

The ACVIM guidelines also describe surgical options. Surgery is the gold standard therapy for the management of MMVD in humans. In veterinary medicine, several specialist centers have tried to develop a technique for replacing the mitral valve with a bovine or porcine valve, but the post-operative risks (e.g. thrombosis phenomena) were frequent [67].
Mitral valve repair has been shown to be superior to valve replacement in multiple studies in both the medium and long-term [67][68].

As concerns surgery in the advanced stages of B2 subjects, it is possible to perform valvuloplasty with excellent long-term results that have few side effects. At present, they are very expensive interventions that run few reference centers [18][41].

For patients classified as **Stage C**, medical treatment plays a fundamental role.
The objectives of the therapy are many: in particular, to reduce venous pressure to improve the patient's edema and effusion, to maintain cardiac output sufficient not to cause kidney damage and signs of weakness and lethargy, to protect the heart from the effects due to neurohormonal influence and finally the reduction of cardiac overload and mitral regurgitation [8].

The guidelines recommend different treatment for **acute** patients, who need hospitalization, and for chronic patients, who can be managed at home. In acute treatment, panelists recommend the following protocol [18]:

- Administration of oxygen, or through a flow by administration, or through the use of an oxygen cage
- Paracentesis or thoracocentesis is recommended to improve patient ventilation and respiratory distress
- 2 mg / kg bolus furosemide administered IV or IM every hour until the respiratory rate has stabilized. If the patient presents with pulmonary edema, continuous infusion furosemide (CRI) can be administered at a dosage of 0.66-1 mg/kg/h
- Pimobendan 0.25-0.3 mg / kg administered po q12h
- Butorphanol at a dosage of 0.2-0.25 mg /, administered IM or IV, for the management of anxiety and stress associated with dyspnoea
- ACEIs, such as enalapril or benazepril, at a dose of 0.5 mg/kg po every 12 hours (panelists do not all agree on acute ACEi administration)
- Dobutamine (2.5-10µg/kg/min as a CRI, starting at 2.5µg/kg/min and increasing the dosage incrementally) may be used to improve the left ventricular function in patients that fail to respond to pimobendan, furosemide and sedation. During the administration of dobutamine, constant ECG monitoring is recommended
- Nitroglycerin ointment, half an inch paste/10 kg BW, applied to an unhaired or shaved area of skin, can be used for the first 24 to 36 hours of hospitalization
- Constant infusion of sodium nitroprusside, which is a vasodilator, at dosages ranging from 1 to 15 µg/kg/min for up to 48 hours

In **chronic** treatment, panelists recommend the following therapeutic protocol [18]:

- Furosemide: commonly 2 mg / kg given every 12 hours by mouth orally

- Torasemide: some panelists suggest to substitute torsemide for furosemide at approximately 5% to 10% of the furosemide dosage, or approximately 0.1-0.3 mg/kg q24h for homecare in animals in which hospitalized CHF management using furosemide was difficult
- Pimobendan at 0.25-0.3 mg / kg po every 12 hours
- ACE Inhibitor (enalapril, benazepril) at 0.5 mg / kg po every 12 hours. The evidence of the efficacy and safety in the use of ACEI is demonstrated by several studies. In particular, in the study "*The effect of benazepril on survival times and clinical signs of dogs with congestive heart failure: Results of a multicenter, prospective, randomized, double-blinded, placebo-controlled, long-term clinical trial",* addition of ACEI to standard therapy is not only able to improve the quality of life of patients, but is associated with a prolongation of the same [69].
- Spironolactone (2 mg / kg po every 12-14 hours). The study "Efficacy of Spironolactone on Survival in Dogs with Naturally Occurring Mitral Regurgitation Caused by Myxomatous Mitral Valve Disease" highlights how treatment with spironolactone, in addition to pimobendan and diuretic therapy, has shown a beneficial effect when added to conventional therapy [70]. Notably, the risk of cardiac death or euthanasia was reduced by 69% compared with conventional therapy alone (i.e., ACEI plus furosemide or digoxin, if needed).
- Beta-blockers, if the dog was already using this therapy before the onset of heart failure
- Cough suppressants and bronchodilators
- Administration of a diet that allows to maintain the patient's RER (about 60 kcal / kg) to minimize weight loss. The diet must have a standard percentage of protein (low-protein diets are to be avoided) and reduce the intake of foods rich in sodium. In case, in laboratory tests, hypokalaemia is present, it is recommended to integrate it through natural or commercial sources. For individuals with decreased appetite, the omega-3 fatty acid supplement is recommended.

As for patients classified as "**Stage D**", these are dogs who are refractory to medical therapy. The therapy, also in this case, is divided into acute therapy (for those subjects who need hospitalization) and chronic therapy (ie patients that can be managed by the owners at home). Regarding the subject in the **acute** phase, the panel described the following guidelines [18].

- Initial bolus furosemide of 2 mg / kg IV for dysponoic patients, then switched to furosemide at a dose of 0.66-1 mg / kg / h in CRI for up to 4 hours or until respiratory distress did not decrease. Being a particularly high dosage, it is important to monitor the renal function of these patients.
- In case the patient no longer responds correctly to furosemide, torsemide, a long-acting diuretic at a dosage of 0.1-0.2 mg / kg every 12-24 hours can be administered
- Abdominal or thoracic centesis to improve the respiratory distress situation.
- Supplementation of flow-by oxygen or, alternatively, assisted mechanical ventilation.
- Hydralazine 0.5-2 mg / kg po or amlodipine (0.05-0.1 mg / kg po) for the management of heart failure
- Pimobendan at a dosage of 0.3 mg / kg / day by mouth
- ACEI as indicated for the patient in Stage C
- Bronchodilators to manage cardiogenic pulmonary edema
- If the patient has CHF complicated by clinically relevant pulmonary hypertension, the combination of Sildenafil is recommended (starting with a dosage of 1-2 mg / kg po every 8 hours).

As regards the therapy of D subjects in the **chronic** phase, therefore as home therapy, the following are used:

- Furosemide used in patients with pulmonary edema or fluid accumulation in the abdomen and chest. The correct dosage is a source of debate among panelists. It is important to monitor the renal function of these patients.
- Torasemide if the patient no longer responds to furosemide treatment (0.1-0.2 mg / kg / PO). Comparing dogs receiving a twice daily dose of furosemide to patients receiving torasemide alone has seen similar success when used as a first-line treatment. If instead it is used as a substitute for furosemide, it has been associated with a decrease in the risk of reaching the cardiac endpoint (decompensation, death from cardiac causes or euthanasia). Dogs treated with this diuretic must constantly monitor kidney function and electrolyte disturbances [71].
- Spironolactone if the patient has not already started therapy at stage C

- Hydrochlorothiazide in combination with furosemide or torasemide. The risk of this drug is that of developing acute renal failure associated with electrolyte disturbances. It is possible to administer hydrochlorothiazide even intermittently every 2-4 days.
- Pimobendan at a dosage of 0.3 mg / kg as a daily dose. It is possible to increase the dosage or add a third daily dose.
- Amplodipine or hydralazine, to reduce cardiac afterload
- Digoxin and other anti-arrhytmic drugs as needed for the management of atrial fibrillation. The recommended dosage is the same for patients classified as stage C.
- Sildenafil at 1-2 mg / kg po every 8 hours, for the management of patients with ascites referable to severe pulmonary hypertension
- Cough relievers and bronchodilators for the management of chronic cough
- The recommended diet is the same as for patients classified as stage C. In subjects with refractory fluid accumulations in the thoracic and abdominal area, it is recommended to further reduce sodium intake with the diet.

2.7 Follow-up

The ACVIM guidelines report as expert opinion a recommended yearly follow-up in stage A dogs, and a 6-12 months follow-up in stage B1 dogs. No recommendations are given for stage B2 to D dogs [18]. Currently, there are no accurate indications regarding echocardiographic monitoring for ACVIM B2 patients.

2.7 Pimobendan

Pimobendan, a benzimidazole-pyridazinone derivative, is known as an inodilator which possesses the unique combination of a positive inotropic and a vasodilator [72].
The effects that Pimobendan has on the cardiovascular system have been known since 1987, the year from which studies began to define, in detail, the clinical implications of this drug [73].

2.7.1 Mechanism of action

Pimobendan has two different mechanisms of action: it reversibly inhibits phosphosiesterase 3 (PDE-3) at the level of myocytes and vascular smooth muscle cells and sensitizes Ca2 + at the myocardial level [74]. Pimobendan has two main effects on the cardiovascular system. First, Pimobendan increases the intracellular content of cAMP in both myocytes and vascular smooth muscle cells, resulting in increased cardiac contraction and promoting vascular relaxation, respectively. Second, pimobendan increases the affinity of troponin C with intracellular calcium, resulting in a positive inotropic effect [75].

This means that Pimobendan improves cardiac output without increasing energy needs at the same time as with digoxin or catecholamines. This unique combination of properties makes pimobendan suitable for the treatment of congestive heart failure (CHF) secondary to myxomatous mitral valve disease or dilated cardiomyopathy in dogs [72].

Furthermore, Pimobendan has other relevant pharmacological properties, such as antithrombotic activity, repression of sympathetic nerve activity, improvement of left ventricular (LV) relaxation, depression of nitric oxide (NO) production, such as effects anti-cytokines that reduce tumor necrosis factor-α [76].

2.7.2 Pharmacokinetics and pharmacodynamics

Pimobendan is absorbed by oral administration and reaches maximum plasma peaks after two to three hours. As for the oral route of administration, bioavailability is between 65% and 70%,

but is reduced in the presence of food [74]. Pimobendan is metabolised in the liver to its active metabolite UD-CG 212 via an oxidative demethylation reaction. The metabolite is in turn biotransformed into O- and N-glucuronides. Elimination occurs through the faeces and biliary excretion [74]. The clearance of Pimobendan is approximately 90 mL / min / kg, its elimination half-life is 0.5 hours and the active metabolite 2 hours [77].

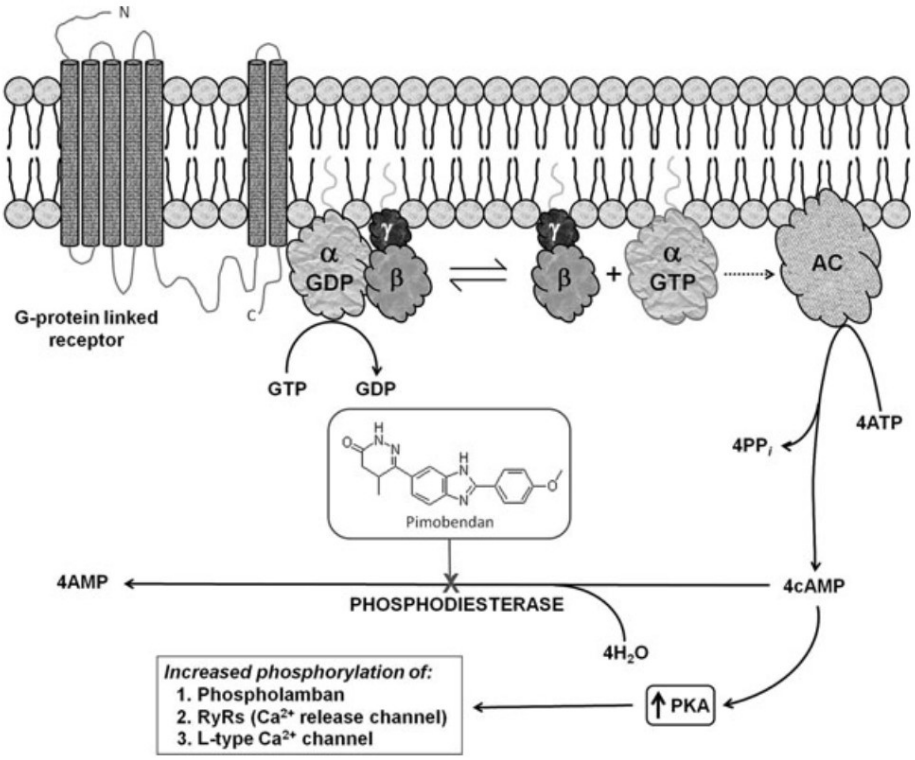

***Figure 4**- Signal trasduction, G-Protein, cAMP, PKA, and PDEs (Boyle, 2012)*

On studies carried out in human medicine, it has been seen that, although Pimobendan has a short half-life, the pharmacodynamic effects last longer than 8 hours [78].

Although the oral route of administration is the most frequently used, in emergency situations it is important to have different options, as dogs with CHF from MMVD often come to the emergency room with respiratory problems. For these scenarios, injectable Pimobendan was developed, as it provides a rapid inotropic effect and decreases left ventricular diastolic pressure. Currently, the oral administration of Pimobendan is available in the form of chewable tablets and is widespread in a limited number of states [72].

2.7.3 Therapeutic indications

Pimobendan has the same therapeutic applications as other PDE3 inhibitors, so it is used for the treatment of congestive heart failure (CHF) due to dilated cardiomyopathy or mitral valve insufficiency, in conjunction with systolic / diastolic dysfunction [74].

Although the potential of this drug had been known since the 1980s, large-scale studies have only been carried out in the last twenty years to study its effects and clinical applications.

Study population	Type of study	Key findings	Reference
Asymptomatic CVHD			
24 client-owned dogs ISACHC Ib CVHD	Prospective Blinded Controlled	Pimobendan group: • No decrease in RF • Significant increase in the EF at 30 days • Nonsustained decrease in end systolic LVID	Ouellet et al. (2009)
12 nonclient-owned beagles NYHA class I CVHD	Prospective Double blinded Randomized Parallel group	Pimobendan group: • Decreased systolic LVID • Increased RF • Histologic grades of mitral valve lesions were more severe	Chetboul et al. (2007)
4 beagles Induced mild MR	Experimental	Pimobendan effects: • Increased cardiac contractility • Vasodilation • Reduced volumetric load of the LV and LA	Kanno et al. (2007)
2 Dogs Without echocardiographic exams On long-term pimobendan	Case report	Dog 1 (10-month treatment with pimobendan) and Dog 2 (5-month treatment with pimobendan) both with evidence of increased MR and myocardial hypertrophy, which resolved with cessation of pimobendan	Tissier et al. (2005)
Symptomatic CVHD			
252 client-owned dogs In CHF caused by CVHD Class not defined 124 treated w/pimobendan 128 treated w/benazepril	Prospective Single blinded Randomized	Compared with benazepril, pimobendan prolongs Time to: • Sudden death • Time to euthanasia for cardiac reasons • Treatment failure	Haggstrom et al. (2008)
76 client-owned dogs ISACHC Class II or III CVHD	Prospective Double blinded Randomized Controlled Multicentre	Pimobendan • Improvement in ISACHC class in 84% of dogs • Long-term median survival 415 days Benazepril • Improvement in ISACHC class in 56% of dogs • Long-term median survival 128 days	Lombard et al. (2006)
43 client-owned dogs NYHA class II or III CVHD	Prospective Single blinded Randomized Parallel group	Pimobendan group: • Well tolerated compared with ramipril • 25% as likely as ramipril dogs to have an adverse heart failure outcome	Smith et al. (2005)

***Figure 5**- Studies evaluating Pimobendan in dogs (Boyle, 2012)*

Most of the studies described improved clinical conditions and survival times in dogs with dilated cardiomyopathy and mitral insufficiency treated with Pimobendan. An example is the study “The effect of treatment with Pimobendan in dogs with preclinical mitral valve disease - a placebo-controlled double-blinded crossover study”, in which the effect of Pimobendan on ACVIM B1 subjects was studied: that Pimobendan has reducing effects on pre- and post-exercise cardiac biomarker concentrations and left ventricular size, as well as beneficial effects on the quality of life and activity of dogs under therapy [63].

In 2006, a large multicenter randomized case-control study showed that Pimobendan in addition to diuretic therapy increases the survival of mitral subjects compared to an ACE-I [3]. Dogs with heart failure resulting from chronic mitral valve disease demonstrated improved quality of life associated with prolonged survival times when treated with Pimobendan with or without Furosemide, compared to those treated with Benazepril hydrochloride with or without Furosemide. Based on the data obtained, it follows that Pimobendan should be considered as a first-line drug when AV disease progresses to symptomatic heart failure [79]. The fact that Pimobendan improves short-term heart function more effectively than Benazepril was confirmed by the study "Short-term hemodynamic and neuroendocrine effects of Pimobendan and benazapril in dogs with myxomatous mitral valve disease and congestive heart failure study"[80].

However, the studies that have most revolutionized the way of using Pimobendan and that have allowed its use increasingly on a large scale are mainly two: the QUEST study ("*Effect of Pimobendan or Benazepril Hydrochloride on Survival Times in Dogs with Congestive Heart Failure Caused by Naturally Occurring Myxomatous Mitral Valve Disease: The QUEST Study"*) and the EPIC study (*Effect of Pimobendan in Dogs with Preclinical Myxomatous Mitral Valve Disease and Cardiomegaly: The EPIC Study – A Randomized Clinical Trial*).

- In the **QUEST** study, it has been found that pimobendan combined with furosemide was more beneficial than benazepril and furosemide for survival in a group of dogs with symptomatic CVD. In particular, the study was carried out on small and medium-sized dogs classified as ACVIM B2, which were administered Pimobendan at a dosage of 0.4-0.6 mg/kg/day [81] However, this study does not allow to clarify whether the combination of an ACE inhibitor and Pimobendan has therapeutic advantages over therapy with single Pimobendan and furosemide [3].

- In the **EPIC** study, in which 360 owned dogs classified as ACVIM B2 were recruited, the primary outcome variable was the onset of heart failure, death from cardiac causes, or euthanasia. The median time to primary endpoint was 1228 days (95% CI: 856 – NA) in the Pimobendan group and 766 days (95% CI: 667–875) in the placebo group, which means that the median time to onset linked heart failure is prolonged by about 15 months. Chronic oral administration of Pimobendan to dogs with echocardiographic and

radiographic evidence of cardiomegaly secondary to MMVD, in the absence of concomitant cardiovascular therapy, results in prolongation of the preclinical period and is safe and well tolerated [53].

	QUEST STUDY	EPIC STUDY
REFERENCES	Häggström et al., 2008	Boswood et al., 2016
STUDY POPULATION	Two hundred and sixty client-owned dogs in CHF caused by MMVD were recruited from 28 centers in Europe, Canada, and Australia	360 client-owned dogs with MMVD with left atrial-to-aortic ratio ≥1.6, normalized left ventricular internal diameter in diastole ≥1.7, and vertebral heart sum>10.5.
TYPE OF STUDY	Prospective, single-blinded, randomized study	Prospective, randomized, placebo-controlled, blinded, multicenter study
CONCLUSIONS	Pimobendan plus conventional therapy prolongs time to sudden death, euthanasia for cardiac reasons, or treatment failure in dogs with CHF caused by MMVD compared with benazepril plus conventional therapy.	Administration of pimobendan to dogs with MMVD and echocardiographic and radiographic evidence of cardiomegaly results in prolongation of preclinical period and is safe and well tolerated. Prolongation of preclinical period by approximately 15 months represents substantial clinical benefit

***Table 1**- Comparison between the QUEST study and the EPIC study [53][81]*

Another 2018 study, "Longitudinal Analysis of Quality of Life, Clinical, Radiographic, Echocardiographic, and Laboratory Variables in Dogs with Preclinical Myxomatous Mitral Valve Disease Receiving Pimobendan or Placebo: The EPIC Study" prospective, blinded with dogs classified as ACVIM B2 randomized to Pimobendan or placebo, set out to investigate the effect of Pimobendan on clinical variables and the relationship between change in heart size and the time to onset of congestive heart failure (CHF) or cardiac death (CRD) in dogs with MMVD and cardiomegaly. In dogs with preclinical MMVD, treatment with Pimobendan results in a smaller heart size in both the short and long term [82]. The degree to which the size of the heart shrinks in the short term is predictive of a successful or bad outcome. The smaller the size of the heart (in particular, the end-systolic size of the left ventricle), the greater the prolongation of the time to onset of heart failure. If from an echocardiographic point of view, significant differences can be noted between the group that received the drug and the placebo group, the quality of life of the two study groups were comparable at the primary endpoint [82].

In conclusion, it can be said that thanks to the studies carried out to date, the ACVIM panelists strongly agree on recommending the initiation of therapy with Pimobendan in B2 staged subjects [18].

RETROSPECTIVE STUDY

3 Purpose of the research

Several studies showed that left atrial and ventricular enlargement as well as the entity and rate of progressive increase of left heart chambers dimensions are important prognostic factors for heart failure development and predicting outcome. In ACVIM B2 canine patients, published guidelines do not give recommendations about follow-up intervals.

The aim of this study was to evaluate the effect of Pimobendan administration on selected echocardiographic parameters of left cardiac chambers volume overload and mitral regurgitation severity in dogs affected by ACVIM B2 MMVD in a mean period of 6 months from the start of Pimobendan.

The hypothesis was that left heart chambers do not enlarge significantly in dogs administered with Pimobendan and therefore a follow-up interval of less than 6 months is not recommended in this population of dogs.

4 Materials And Methods

4.1 Study design

This is an observational, retrospective, multicenter study. Because of the retrospective study design, no informed consent was given to patient owners. The databases of the Cardiology Unit of the Veterinary Teaching Hospital of Parma University (OVUD), the Centro Veterinario Imperiese and the Veterinary Teaching Hospital of Turin University were retrospectively searched for dogs diagnosed with ACVIM stage B2 myxomatous mitral valve disease referred from January 2019 to July 2022. This range period was selected according to the date of publication of the updated ACVIM Consensus guidelines for the diagnosis and treatment of canine myxomatous mitral valve disease.

Dogs who met the following inclusion criteria were included in the study:

1. Diagnosis of MMVD ACVIM B2
2. No cardiological treatment was administered at the time of diagnosis

3. Dogs having a follow-up complete cardiological check-up performed over a period between 4 and 8 months
4. Absence of concomitant systemic pathologies that might affect the patient's cardiovascular status (systemic hypertension, endocrinopathies, chronic kidney disease, gastrointestinal disorders, neoplasia)

All patients not complying with the aforementioned inclusion criteria were excluded from the study.

4.2 Methods

For each patient included in the study, data regarding signalment, history, and physical examination were collected. Particularly, for signalment: body weight (in kg), age (in months), breed, sex, and the reason for the visit.

After the anamnesis was collected, a complete clinical examination was carried out. Each dog included should have a complete cardiological examination and echocardiography. The presence and characters of heart murmurs were assessed through auscultation.

Echocardiographic examinations were carried out by the cardiology team of the Veterinary Teaching Hospital of Parma University and Turin University and by the cardiology team of the Centro Veterinario Imperiese.

The ultrasound systems used were Philips Epiq CVX and Mindray 9 and under continuous ECG monitoring. Echocardiographic examinations were performed on dogs restrained in lateral recumbency on a table with an opening that allowed transducer manipulation and examination from beneath the animal, and according to standards from right parasternal, subcostal, left cranial parasternal, and left apical parasternal windows. Off-line measurements were made on high-quality video clips of standard echocardiographic views and high-quality M-mode, PW- and CW-Doppler images of at least 6 cardiac cycles previously acquired and stored.

For the purpose of this study, the following echocardiographic parameters were taken into account at Time 0 (inclusion and start of Pimobendan administration) and Time 1 (follow-up after 4-8 months) and included in the statistical analysis:

- Left ventricular internal diameter in diastole normalized for body weight (LVIDDn ($cm/kg^{0,294}$))
- Left ventricular internal diameter in systole normalized for body weight (LVIDSn ($cm/kg^{0,315}$))
- Fractional shortening (FS (%))
- Left atrium/aortic ratio (LA/Ao)
- Aortic Velocity Time Integral (Aortic VTI (cm))
- Mitral E Wave Peak Velocity (E Vel (m/s))
- Mitral Velocity Time Integral (Mitral VTI (cm))
- Left Atrial Volume (LAV (ml))
- Mitral to Aortic Velocity Time Integral Ratio (Mitral to Aortic VTI Ratio)
- Left ventricular early inflow-outflow (LVEIO)

Symptoms eventually developed, the presence or not of radiographic signs of congestive heart failure and variation of therapy at the follow-up visit were also considered.

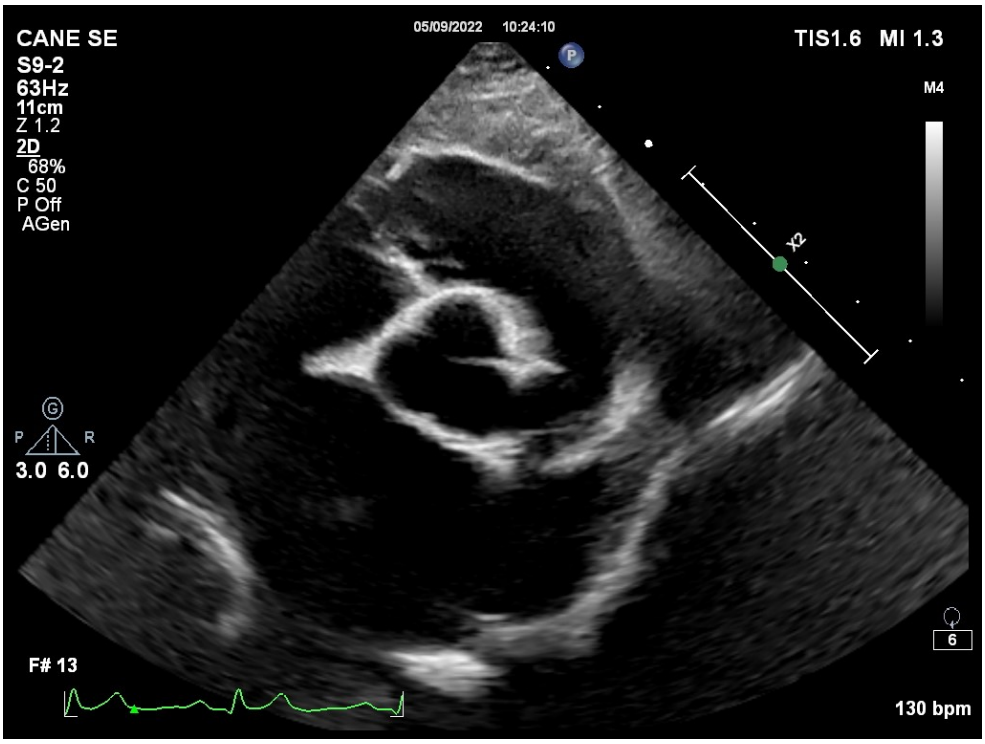

***Figure 6**- Right parasternal short axis view at the base of the heart: visualization of choice for LA/Ao assessment*

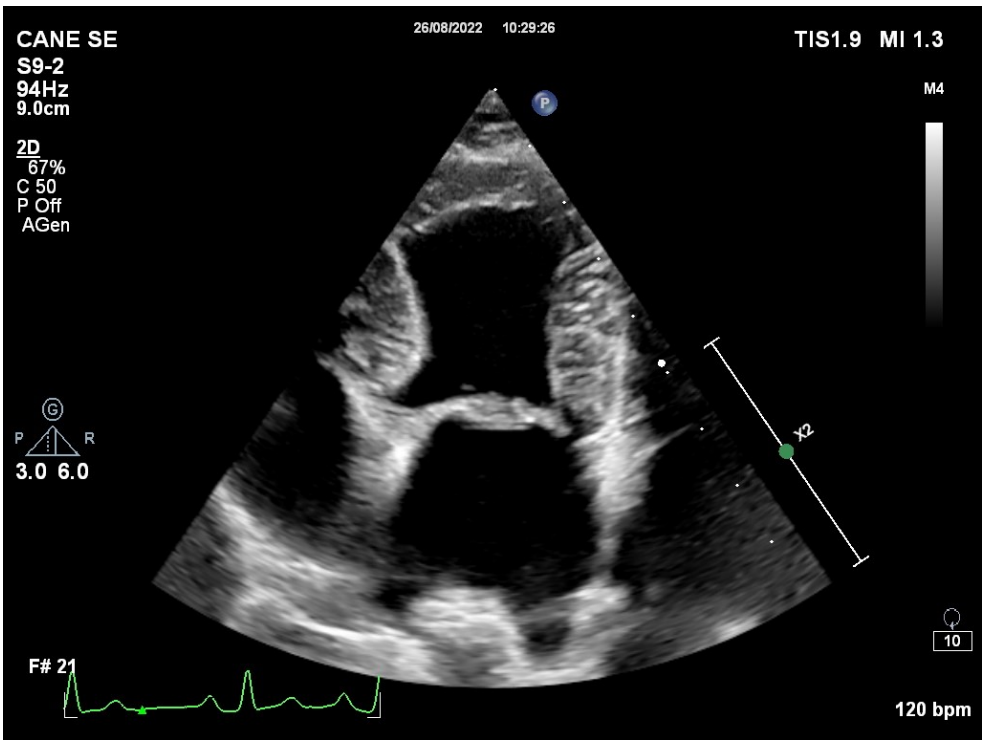

***Figure 7**- Left apical four-chamber view: visualization of choice for visualization of LAV*

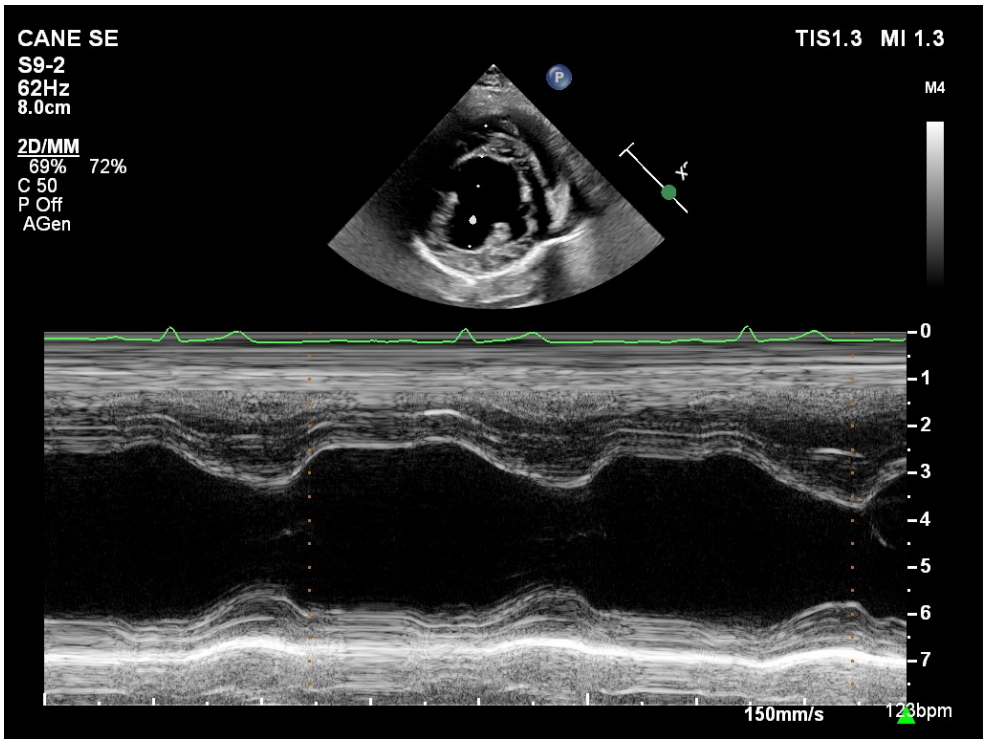

***Figure 8**- M-mode scan acquired from the right parasternal short axis view of the left ventricle: display of IVSd, LVIDd, LVIDs, FS%*

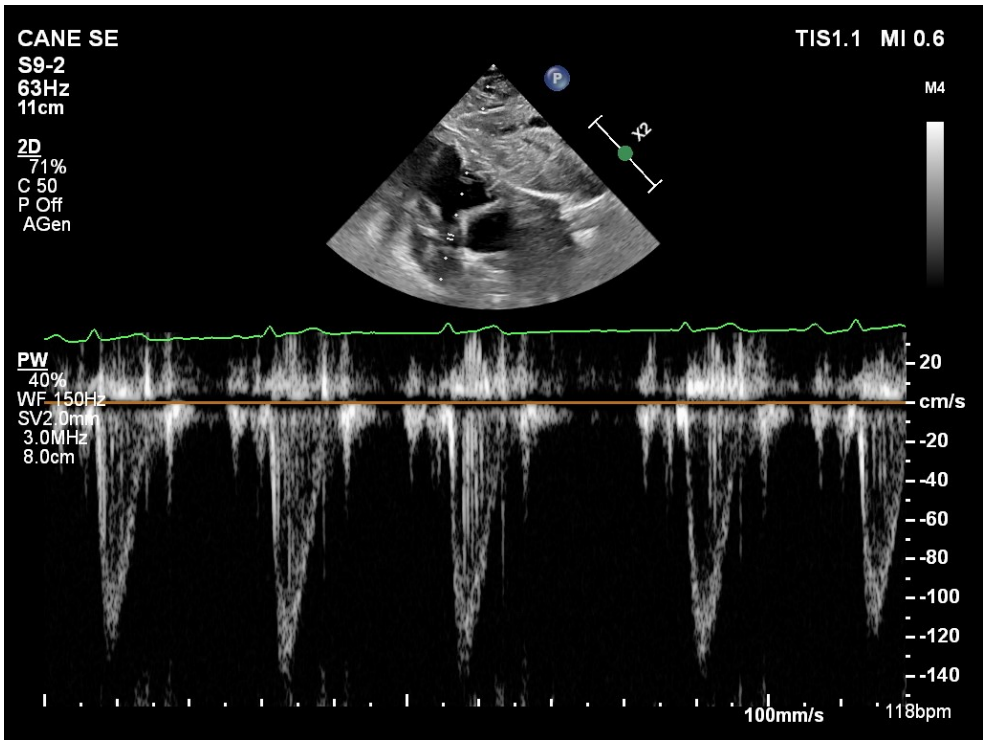

***Figure 9**- Transaortic flow profile assessed using pulsed wave Doppler acquired from the subcostal view: evaluation of Aortic VTI*

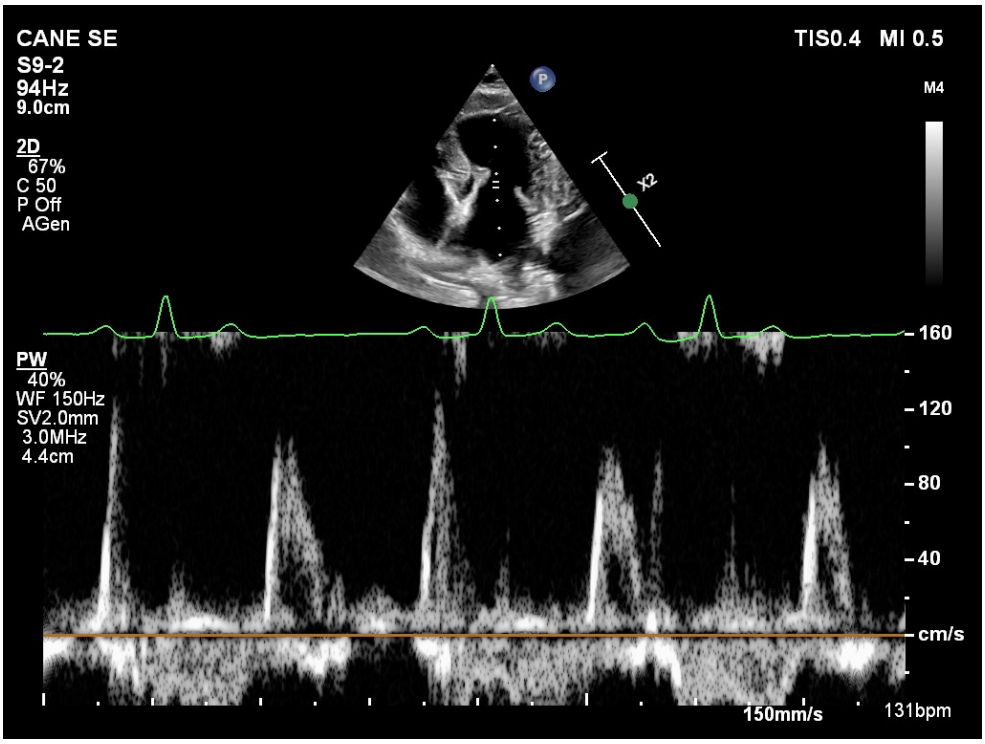

***Figure 10**- Pulsed Doppler of transmitral flow acquired from the left apical four-chamber view: evaluation of E Vel, Mitral VTI*

4.3 Statistical Analysis

All data were collected into electronic spreadsheets (*Microsoft Excel, Microsoft Corporation Redmond, USA*) and then imported into a statistical software (*MedCalc ® Version 20.110, Ostend, Belgium).* Data distribution was assessed using the Shapiro-Wilk test. Data were expressed by standard descriptive statistics and presented as mean +/- standard deviation or median and range (minimum-maximum) based on normal or non-normal data distribution. To compare the values between the different variables, the Wilcoxon test was performed and continuous values were reported as median with 95% confidence intervals (95% CI). A P-value <0,05 was considered significant.

5 Results

5.1 Animals

In the period of time between January 1st 2019 and 31st July 2022, 57 dogs met the inclusion criteria and were included in this study. Among these 57 dogs, 25 (43.86%) were females of which 17 were neutered (29,8%) and 32 were males (56.14%) of which 8 were neutered (14%).

Several breeds were represented: Cavalier King Charles Spaniel (CKCS) (n=11, 19%), Chihuahua (n=5, 9%), Mixed breed (N=21, 37%), Maltese (n=2, 3%) Dachshund (n=5, 9%), Poodle (n=1; 2%), Miniature Schnauzer (n=2, 3%), Pinscher (n=3, 5%), German Sheperd (n=1; 2%), Yorkshire Terrier (n=2, 3%), Tibetan Terrier (n=1; 2%), Setter (n=1; 2%), Border Collie (n=1; 2%), Breton (n=1, 2%) . Most of the dogs included in the study were mixed breed (n=21, 37%), followed by CKCS (n=11, 19%).

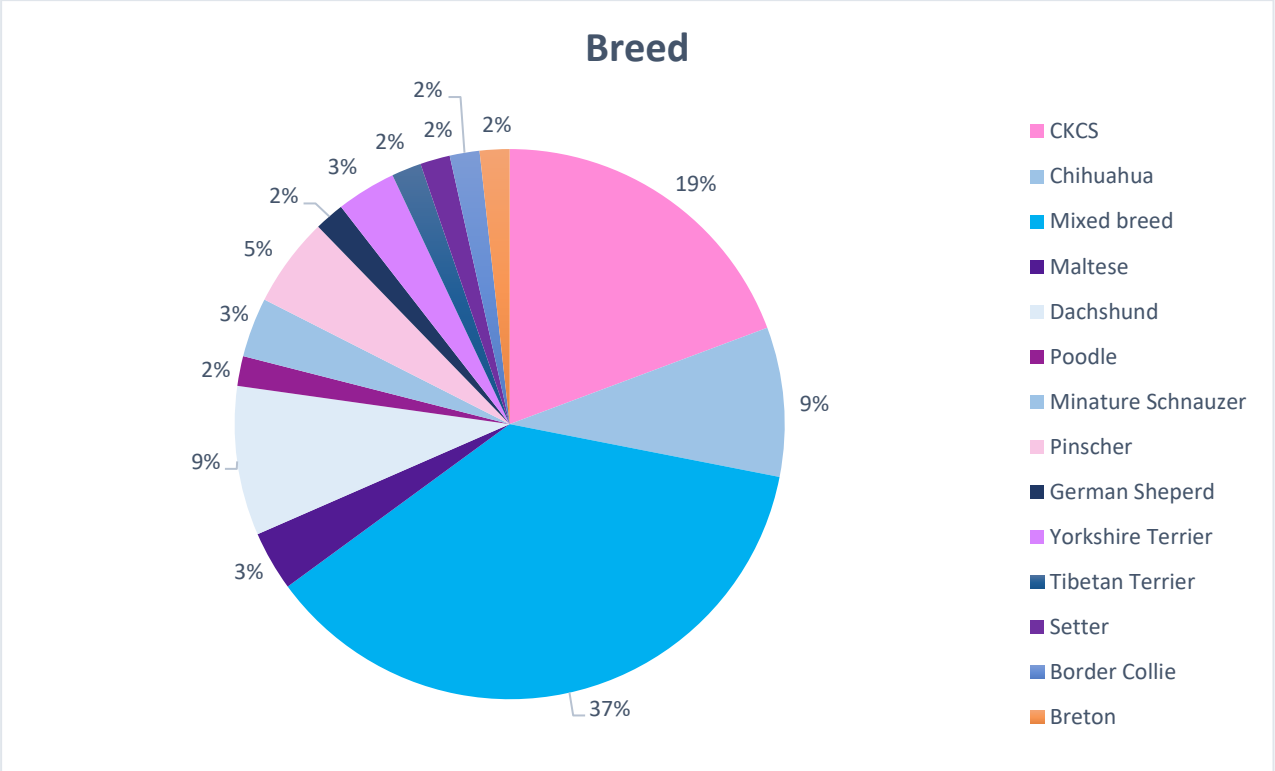

***Figure 11**- Distribution of breed included in the study*

The median weight of the dogs was 8,8 kg (range 1,95 kg-30 kg). The 95% CI for the median is 7,5 to 10,54.

The mean age of the dogs was 134 ± 32 months.

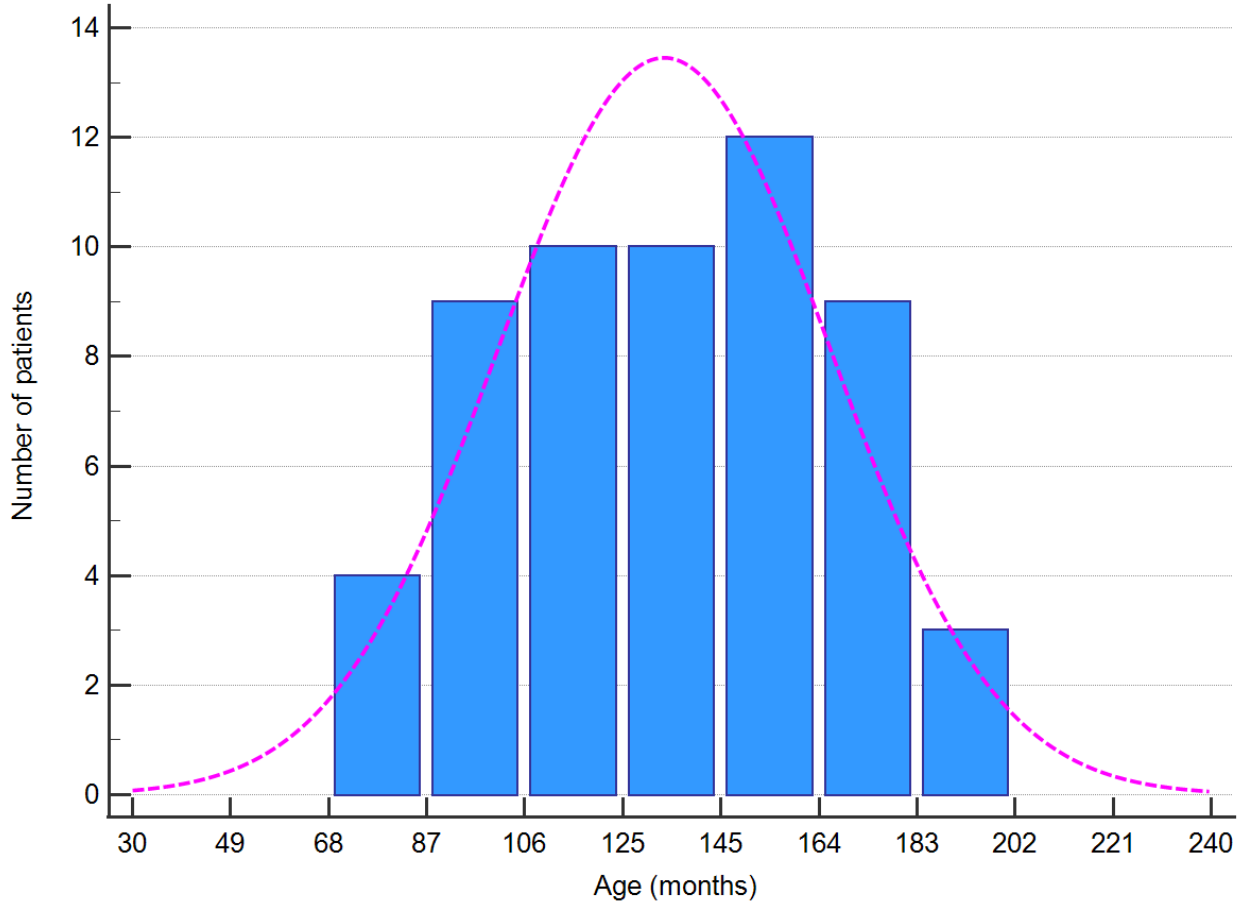

***Figure 12**- The figure shows the number of patients analyzed, as their age varies in months. It is noted that the age of the patients follows an almost normal distribution, even if the real distribution appears slightly shifted to the right - that is, towards older ages - with respect to the Gauss curve.*

5.2 Echocardiography

Several echocardiographic parameters were considered in the study.

For **LVEIO** (left ventricular early inflow-outflow) 48 dogs were evaluated. At baseline, the median was 9.2 (range 4.2-17.5; 95% CI 7.6 to 10.27), while at control the median was 9.15 (range 3.6-27.1; 95% CI 8.3 to 10.57). P-value was 0,66.

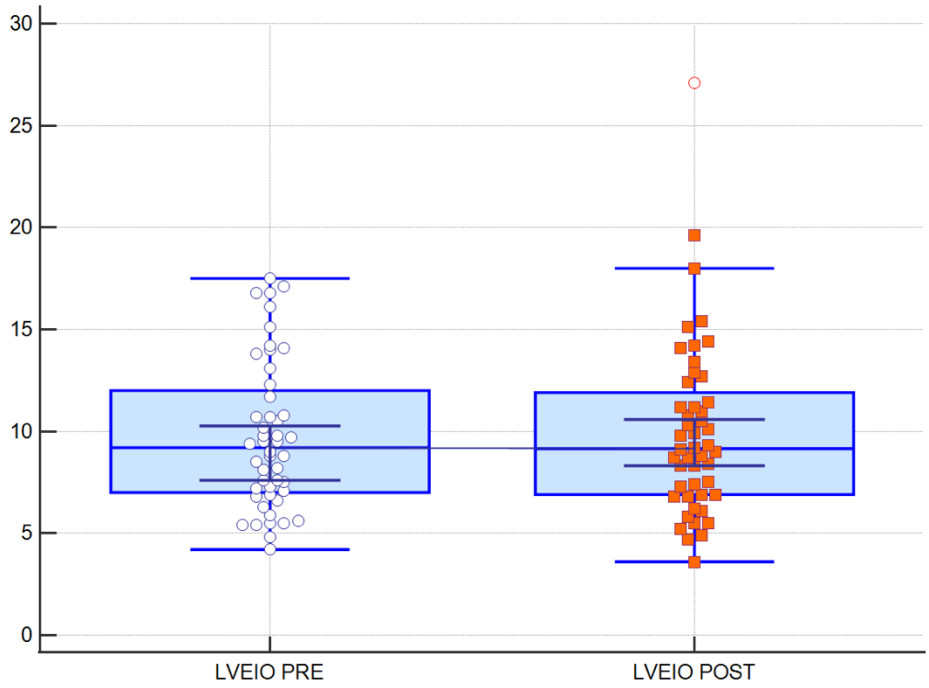

***Figure 13**- Box plots of LVEIO, at the time of diagnosis and 6 months after the introduction of Pimobendan. The central box represents the values from the lower to upper quartile (25 to 75 percentile). The middle line represents the median. The vertical line extends from the minimum to the maximum value, excluding outliers. At 6 months, LVEIO remains almost steady on control. At baseline, the median was 9.2 (range 4.2-17.5), while at control the median was 9.15 (range 3.6-27.1)*

.

Fifty-one canines were examined in relation to the **Aortic VTI** (Aortic Velocity Time Integral). At control, the median was 10.9 cm (range 5.8-18, 95% CI 10 to 11.9), while at baseline, it was 11.5 cm (range 6.2-21, 95% CI 10 to 12). P-value was 0,6.

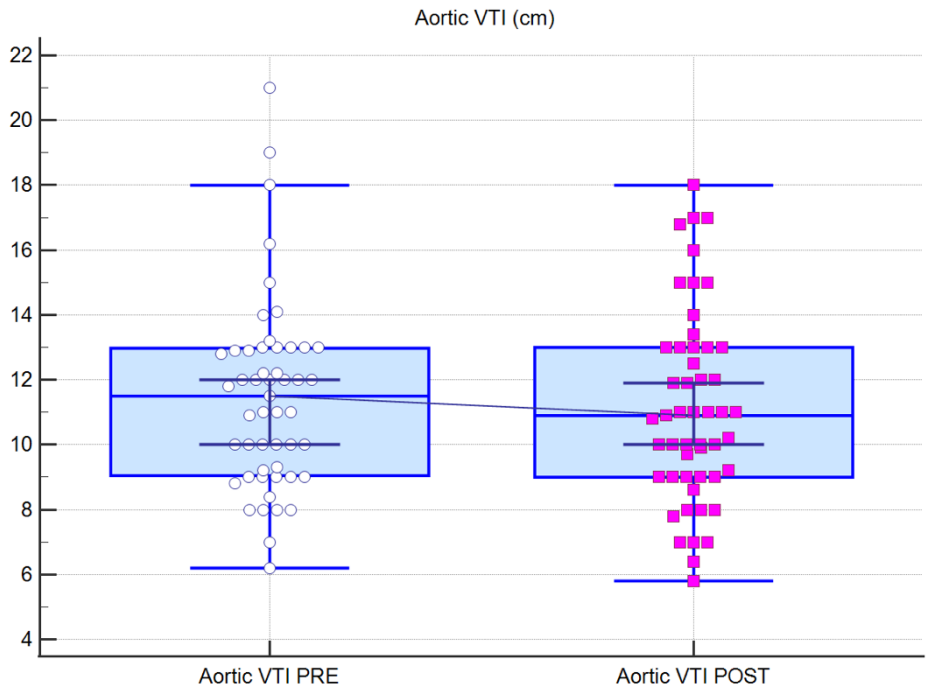

***Figure 14**- Box plots of Aortic VTI, at the time of diagnosis and 6 months after the introduction of Pimobendan. The central box represents the values from the lower to upper quartile (25 to 75 percentile). The middle line represents the median. The vertical line extends from the minimum to the maximum value, excluding outliers. Aortic VTI decreases slightly on control. At control (POST), the median was 10.9 (range 5.8-18)), while at baseline (PRE), it was 11.5 (range 6.2-21).*

As for the **E Vel** (Mitral E Wave Peak Velocity), 54 dogs were evaluated. At baseline, the median was found to be 1.065 m/s (range 0.65-1.85; 95% CI 0.94 to 1.14); at control, the median is 1.015 m/s (range 0.61-2.17; 95% CI 0.91 to 1.14). P-value was 0,89.

Fifty dogs were evaluated for **LAV** (ml) (Left Atrial Volume). The median at baseline was 20.65 ml (range 2.25-81.5, 95% CI 15.8 to 25.28), while the median at control was 20.6 ml (4.17-90.6, 95% CI 16.88 to 26.15). P-value was 0,3.

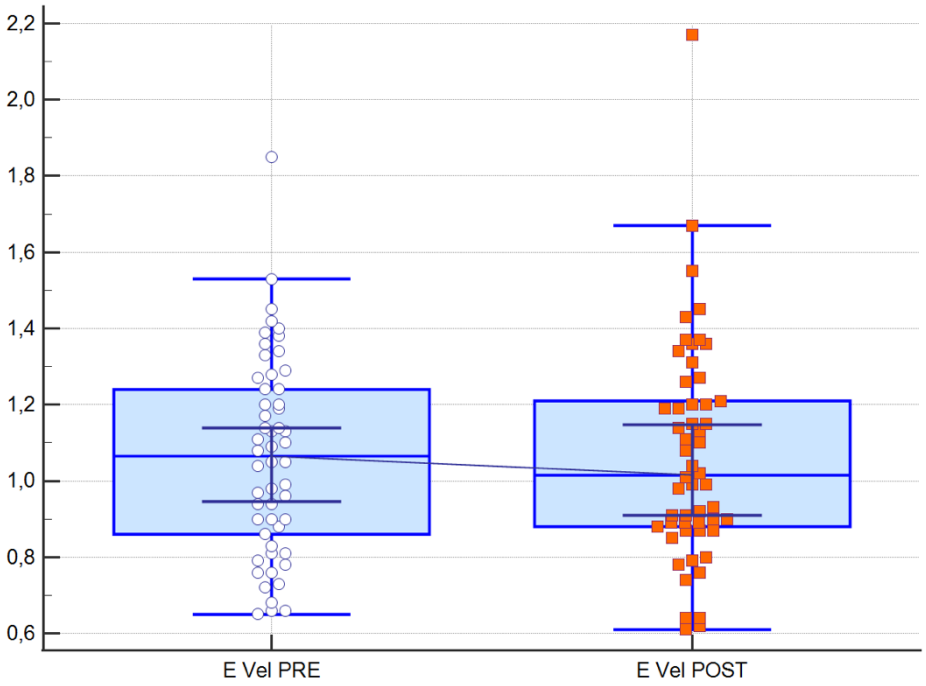

***Figure 15**- Box plots of E Vel, at the time of diagnosis and 6 months after the introduction of Pimobendan. The central box represents the values from the lower to upper quartile (25 to 75 percentile). The middle line represents the median. The vertical line extends from the minimum to the maximum value, excluding outliers. E Vel is not significantly different at follow-up. At baseline, the median was found to be 1.065 m/s (range 0.65-1.85); at control, the median is 1.015 m/s (range 0.61-2.17).*

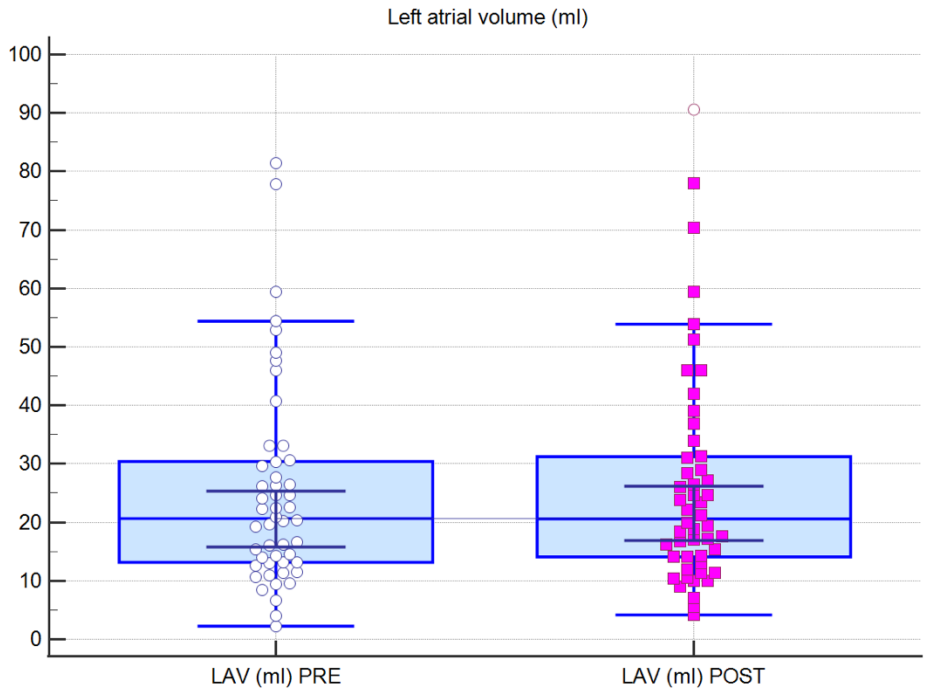

***Figure 16**-Box plots of LAV (ml), at the time of diagnosis and 6 months after the introduction of Pimobendan. The central box represents the values from the lower to upper quartile (25 to 75 percentile). The middle line represents the median. The vertical line extends from the minimum to the maximum value, excluding outliers. LAV (ml) doesn't change on control. The median at baseline (PRE) was 20.65 (range 2.25-81.5), while the median at control (POST) was 20.6 (4.17-90.6).*

Fifty-sever dogs were assessed to determine the **SF%** (Fractional shortening). The median at the control was found to be 45% (range 25–64; 95% CI 43–48), while the median at the baseline was found to be 44% (range 22–63; 95% CI 42–47). P-value was 0,05.

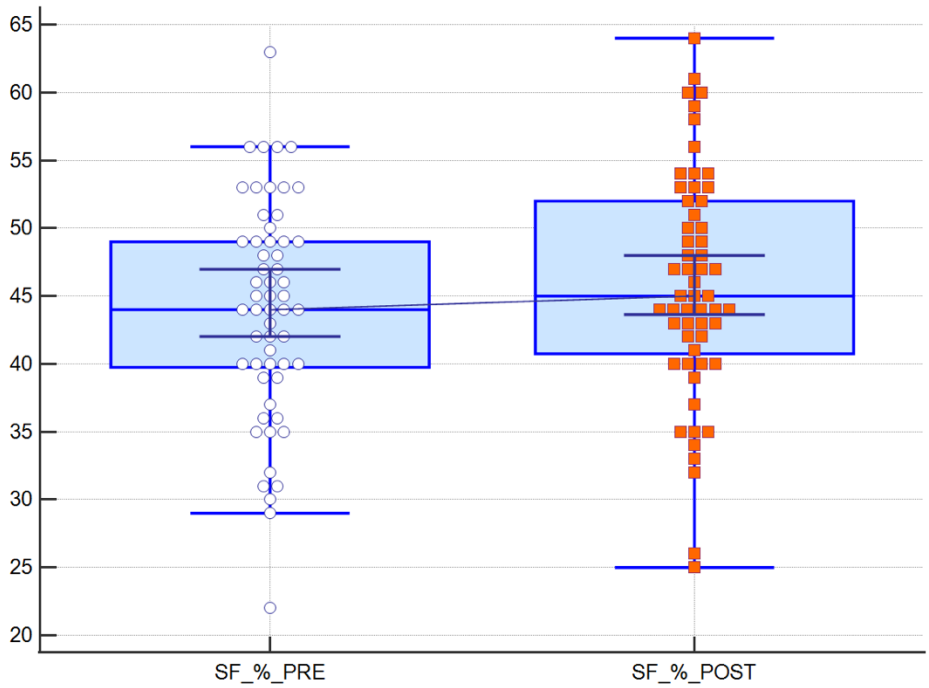

***Figure 17**- Box plots of SF%, at the time of diagnosis and 6 months after the introduction of Pimobendan. The central box represents the values from the lower to upper quartile (25 to 75 percentile). The middle line represents the median. The vertical line extends from the minimum to the maximum value, excluding outliers. SF% is conserved at control. The median at the control was found to be 45% (range 25–64), while the median at the baseline was found to be 44% (range 22–63).*

Fifty-one dogs were assessed in terms of **LA/Ao** (Left atrium/aortic ratio). The median at baseline was 1.78 (range 1.08-2.75; 95% CI 1.71 to 1.83), and the median at control was 1.79 (range 1.05-2.8, 95% CI 1.75 to 1.91). P-value was 0,93.

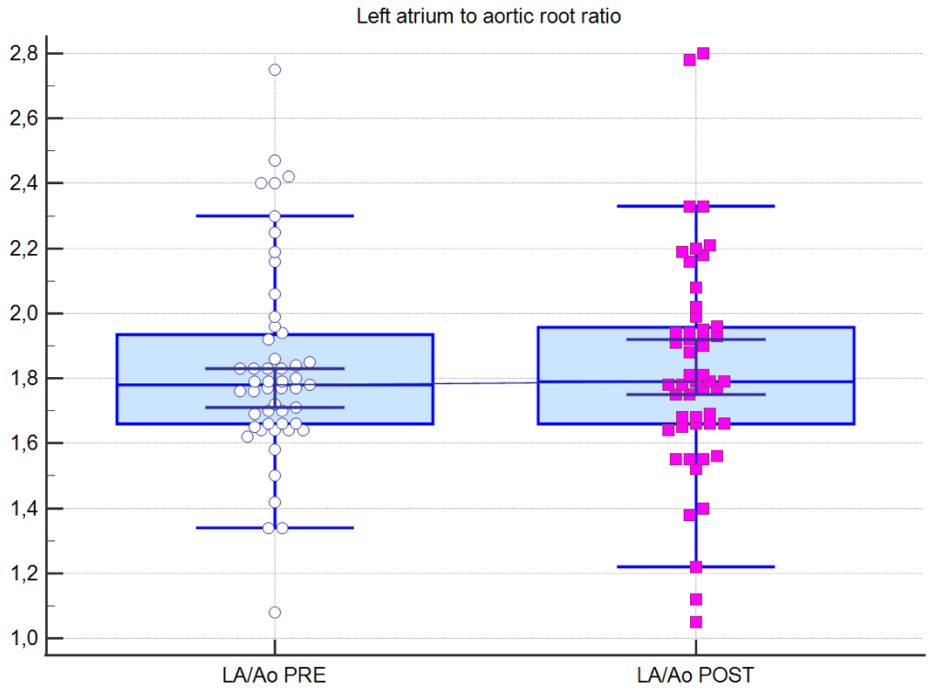

***Figure 18**- Box plots of LA/Ao, at the time of diagnosis and 6 months after the introduction of Pimobendan. The central box represents the values from the lower to upper quartile (25 to 75 percentile). The middle line represents the median. The vertical line extends from the minimum to the maximum value, excluding outliers. LA/Ao has minimal change: in fact, the median at baseline was 1.78 (range 1.08-2.75), and the median at control was 1.79 (range 1.05-2.8).*

For the evaluation of the **LVIDSn** (Left ventricular internal diameter in systole normalized for bodyweight), 57 dogs were evaluated. At baseline, the median was 1.01 (range 0.67-1.34, 95% CI 0.95 to 1.07), at control the median was 0.99 (range 0.54-1.4, 95% CI 0.92 to 1.07). P-value was 0,17.

LVIDDn (Left ventricular internal diameter in diastole adjusted for body weight) was measured in 57 dogs. The median at baseline was 1.89 (range 1.55-2.29, 95% CI 1.84 to 1.98), while the median at control was 1.89 (range 1.45-2.66, 95% CI 1.84 to 1.99). P-value was 0,91.

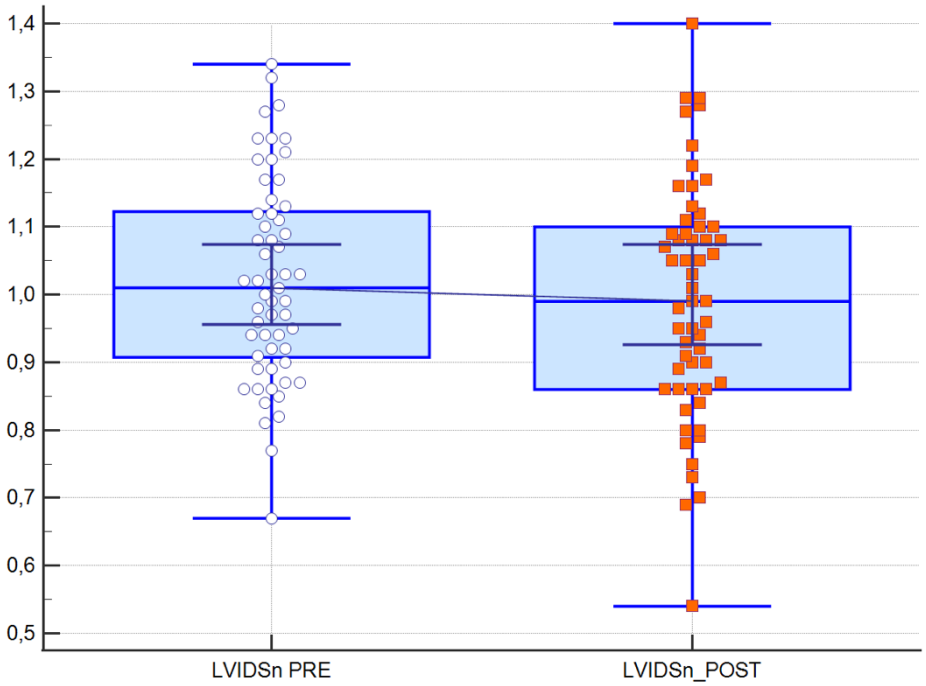

***Figure 19**-Box plots of LVIDSn, at the time of diagnosis and 6 months after the introduction of Pimobendan. The central box represents the values from the lower to upper quartile (25 to 75 percentile). The middle line represents the median. The vertical line extends from the minimum to the maximum value, excluding outliers. At baseline, the median was 1.01 (range 0.67-1.34), at control the median was 0.99 (range 0.54-1.4). LVIDSn does not change at control.*

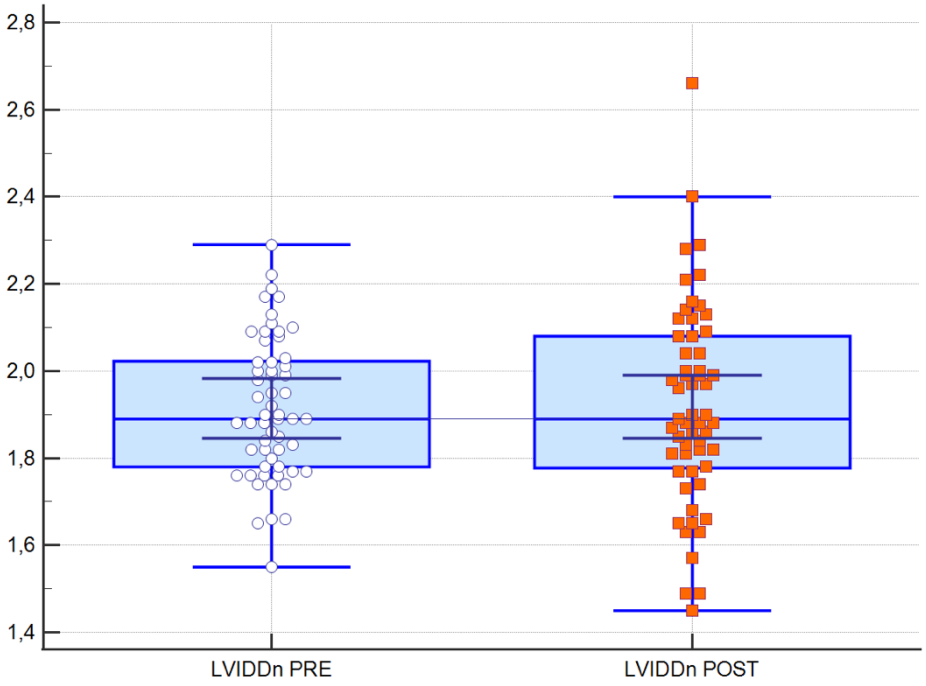

***Figure 20**-Box plots of LVIDDn, at the time of diagnosis and 6 months after the introduction of Pimobendan. The central box represents the values from the lower to upper quartile (25 to 75 percentile). The middle line represents the median. The vertical line extends from the minimum to the maximum value, excluding outliers. The median at baseline was 1.89 (range 1.55-2.29), while the median at control was 1.89 (range 1.45-2.66). LVIDDn does not change at control.*

As for the only clinical parameter considered in the statistical analysis, which is **HR (heart rate)**, 45 dogs were evaluated. At baseline, the median was 135 (range 80-183), at the control the median was 130 (range 80-190). P-value was 0,14.

Only two echocardiographic parameters, **Mitral VTI** (Mitral Velocity Time Integral) and **Mitral to Aortic VTI Ratio** (Mitral to Aortic Velocity Time Integral Ratio) resulted statistically significantly different.

A total of 50 dogs were examined for **Mitral VTI**. The median at baseline was 12.15 cm (range 7.3-22.8), while the median at control was 13.45 cm (range 8-19.9), with a p-value of **0,008**.

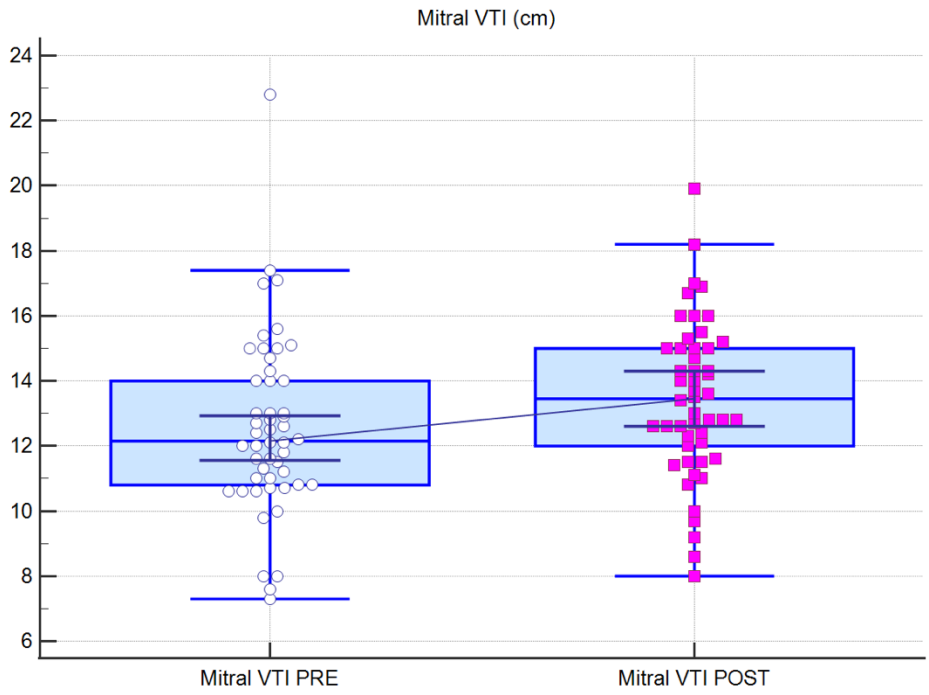

***Figure 21**- Box plots of Mitral VTI, at the time of diagnosis and 6 months after the introduction of Pimobendan. The central box represents the values from the lower to upper quartile (25 to 75 percentile). The middle line represents the median. The vertical line extends from the minimum to the maximum value, excluding outliers. The graph shows a worsening of Mitral VTI: the median at baseline was 12.15 (range 7.3-22.8), while the median at control was 13.45 (range 8-19.9).*

Forty-five dogs were evaluated for the assessment of the **Mitral to Aortic VTI Ratio**. At baseline, the median was 1.1 (range 0.44-2.28), while at control the median was 1.26 (range 0.56-2.28), with a p-value of **0,03.**

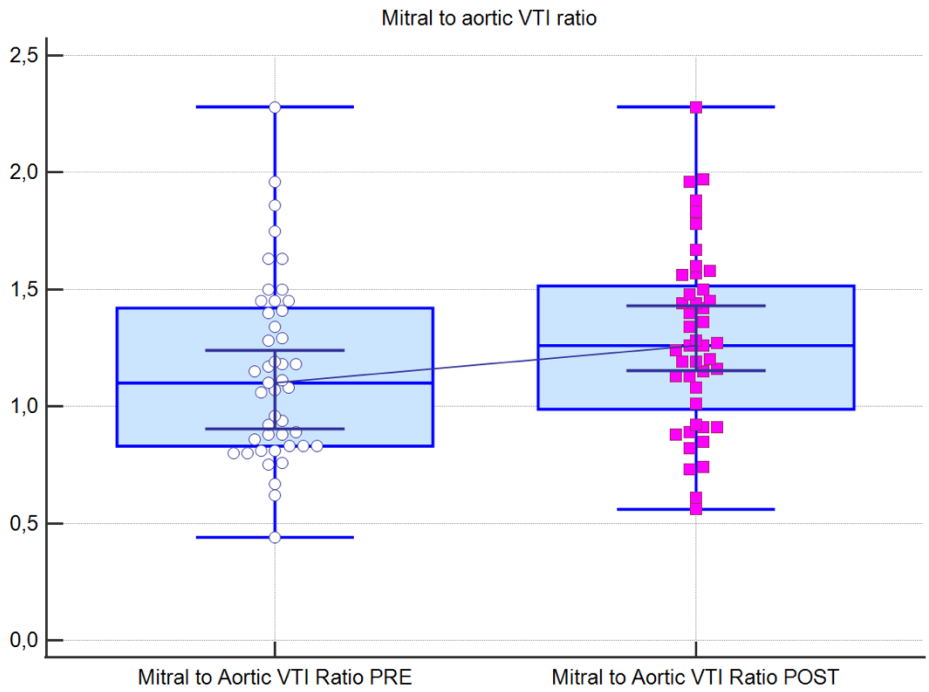

***Figure 22**- Box plots of Mitral to Aortic VTI, at the time of diagnosis and 6 months after the introduction of Pimobendan. The central box represents the values from the lower to upper quartile (25 to 75 percentile). The middle line represents the median. The vertical line extends from the minimum to the maximum value, excluding outliers. The graph shows a worsening of Mitral to Aortic VTI Ratio. At baseline, the median was 1.1 (range 0.44-2.28), while at control the median was 1.26 (range 0.56-2.28).*

Results are reported in the following synoptic table for easly consultation:

Ecocardiographic indices	Median PRE (Time 0)	95% CI for the median PRE (Time 0)	Median POST (Time 1)	95% CI for the median POST (Time 1)	p-value
LVIDDn	1,89	1,84 to 1,98	1,89	1,84 to 1,99	0,916
LVIDSn	1,01	0,95 to 1,07	0,99	0,92 to 1,07	0,177
FS (%)	44	42 to 47	45	43,63 to 48	0,058
LA/Ao	1,78	1,71 to 1,83	1,79	1,75 to 1,91	0,935
Aortic VTI (cm)	11,5	10 to 12	10,9	10 to 11,9	0,604
E Vel (m/s)	1,06	0,94 to 1,14	1,01	0,91 to 1,14	0,891
Mitral VTI (cm)	12,15	11,56 to 12,93	13,45	12,6 to 14,3	**0,008**
LAV (ml)	20,65	15,8 to 25,28	20,6	16,88 to 26,15	0,303
Mitral to Aortic VTI Ratio	1,1	0,9038 to 1,2385	1,26	1,1546 to 1,4308	**0,03**

LVEIO	9,2	7,6 to 10,27	9,15	8,3 to 10,57	0,664
Heart Rate (bpm)	135	125 to 150	130	120 to 140	0,14

***Table 2**- Echocardiographic data*

5.3 Outcome At Follow-Up (POST/Time 1)

The median time interval from starting Pimobendan (PRE/Time 0) and follow-up (POST/ Time 1) was 6 months. At time 0 (PRE), all 57 included dogs were classified as ACVIM B2. At time 1 (POST, follow-up examination), 14 switched to ACVIM C with the introduction of diuretic therapy (n = 14, 24,56%). Of these 14 who started diuretic therapy, in 7 dogs the cardiologist decided to introduce furosemide because of worsening of the echocardiographic parameters, even if the dogs were still asymptomatic (n = 7; 50%); 4 dogs had a worsening cough in recent weeks as reported by owners (n = 4; 28.57%), 2 dogs had increased resting respiratory rate as reported by owners (n = 2; 14.28%), only one dog presented, according to the history, both a worsening of the cough and an increase in the respiratory rate at rest (n = 1; 7.14%).

6 Discussion

This retrospective study is based on a population of 57 dogs admitted to three different veterinary hospital (Veterinary Teaching Hospital of Parma University (OVUD), the Centro Veterinario Imperiese and the Veterinary Teaching Hospital of Turin University) with a diagnosis of MMVD ACVIM B2 in a period between January 1st 2019 and 31st July 2022.

The demographic parameters of the population included in this study match those documented in the literature for dogs with chronic mitral valve disease [2][83].

In fact, our MMVD ACVIM B2 dog population included a higher proportion of males (56.4% out of a total of 57 dogs) and small-medium sized dogs as mixed breed dogs (37%), Cavalier King Charles Spaniel (19%), Chihuahua (9%), and Dachshunds (9%).

According to the data currently available in the literature, the median age of diagnosis of ACVIM B2 mitral disease was 132 months or 11 years of age [1], [2]. This study supports the tight relationship between age and the disease's gradual and degenerative course [84]. The results obtained in our study agree with the literature.

The median weight of the dogs in the study was 8.8 kg, which is in keeping with the literature, which shows that most dogs with mitral valve disease are small to medium-sized and weigh less than 9 kg [12][85].

Regarding the evaluation of the heart rate, it has been noticed that the median value at time 0 was 135 beats per minute (with a maximum value of 183 beats per minute), whereas the median at time 1 was 130 beats per minute (with a maximum value of 190 beats per minute). This mild reduction, even if not significant, is in accordance with existing evidence in the literature, which shows that heart rate decreases in patients treated with Pimobendan, improving their outcome [80][81]. More noteworthy, however, is that heart rate is not significantly increased at follow-up: in fact, increasing heart rate is one of the major compensatory mechanisms that the cardiovascular system adopts to preserve cardiac output, by activation of the sympathetic system. Therefore, we can assume that sympathetic activation is not important at mean 6-month follow-up after initiation of Pimobendan therapy in ACVIM B2 MMVD dogs.

Regarding what has been published in the literature, there are confirmations as well as novelties in the evaluation of echocardiographic parameters.

The left ventricular early inflow-outflow (**LVEIO**) was unchanged at follow-up. LVEIO is an easy-to-use echocardiographic parameter that reliably detects severe mitral regurgitation, so it may be supposed that there was no severe worsening of mitral regurgitation in the group under study [86][87].

The **aortic velocity time integral (VTI)** is an echocardiographic parameter used to estimate stroke volume (SV). It can be used as a left-ventricular (LV) output parameter. It basically indicates the distance travelled by blood during ventricular systole. The median Aortic VTI has decreased slightly, but not significantly, at follow-up. This finding might imply that there is a decline in the stroke volume 6 months after starting treatment, but not hemodynamically significant, as also demonstrated also by the failure to increase heart rate.

Mitral E Wave Peak Velocity (E Vel) was not significantly different at follow-up. The researchers have linked an increase in Mitral E Wave Peak Velocity values to a worse prognosis as a sign of worsening pressure gradient between the LA and LV with rising regurgitation volumes [49]. Since this echocardiographic parameter is a proxy for the severity of mitral regurgitation, it may be concluded that there was no severe worsening of the mitral regurgitation in the study group.

Left atrial size is one of the most important predictive factors of worst outcome in dogs affected by MMVD. The volume of LA increases as disease severity increases. A large LA volume reflects volume overload caused by regurgitant flow [56], [88].
LAV (Left Atrial Volume) remained unchanged at follow-up, showing no sign of deterioration. According to published research, dogs with MMVD had larger LA volumes and worse LA function. More specifically, LA dimensions and function worsened with increasing disease severity, as shown by a declining reservoir and active contractile activity [56], [88] These findings can be interpreted in a variety of ways based on what has been written in the literature. To start, a stable LAV may be caused by the presence of cardiac compensation mechanisms or by a stable level of disease severity. Another hypothesis is that Pimobendan slowed the disease's course by helping the heart compensate, maintaining a sufficient ventricular filling volume. However, it can be inferred that there is no evidence of atrial enlargement six months following the start of medication.

One of the main measures used to assess left atrial (LA) size in dogs is the **left atrial/aortic ratio (LA/Ao).** This ratio provides an indication of LA size that is unaffected by body size [90]. The size of the left atrium gets larger as the disease advances, and in dogs who develop heart failure, the ratio may be greater than 2: in this case, the median at baseline was 1.78, and the median at control was 1.79. The population under study shows no increase in left atrial enlargement at follow-up. In conclusion, both parameters of left atrial enlargement, LAV and LA/Ao, were not significantly changed at follow-up and these data have prognostic relevance.

Left ventricular diameters, measured as **LVIDDn** (left ventricular internal diameter in diastole normalized for body weight) and **LVIDSn** (left ventricular internal diameter in systole normalized for body weight), were unchanged at follow-up. Left ventricular diameters, especially diastolic, increase as the disease progresses, because of volume overload. In this instance, it may be claimed that the study's target population didn't exhibit any increase in left ventricle enlargement after 6 months of therapy with Pimodendan.

In conclusion, dogs included in this study did not show an increase in left heart chambers remodelling at follow-up.

Fractional shortening, as expected is conserved at Time 1 (POST), suggesting no deterioration of circumferential systolic function at follow-up. Fractional shortening is generally increased in the hyperdynamic ventricle of affected dogs and tends to reduce only in the more advanced stage of the disease [89]. However, parameters of longitudinal systolic function were not evaluated in this retrospective study.

Mitral velocity time integral (Mitral VTI) is one of the two data points that were statistically different in this statistical analysis. It was significantly increase at follow-up. In this scenario, a rise in mitral VTI indicates a worsening of the rate of regurgitation in the population under investigation. In the absence of left-sided valve regurgitation, the mitral inflow should be equivalent to the aortic outflow [60]. In this scenario, the mitral VTI is greater than the aortic VTI.

The **mitral to aortic velocity time integral ratio** (Mitral to Aortic VTI Ratio) investigation provides similar results, showing a significant increase at follow-up. This measure assesses the severity of

mitral regurgitation without regard to jet geometry. Current literature demonstrates a tight association between the mitral to aortic ratio, angiographic grade of MR, and regurgitant fraction [60][61]. As a result, based on the data presented in the study, it is possible to argue that mitral regurgitation worsens at 6-month follow-up.

However, as other parameters linked to severity of mitral regurgitation are not significantly different at follow-up (i.e. LVEIO, E Wave Peak velocity, Aortic VTI) and parameters of left heart chambers volume overload are unchanged, it can be concluded that this retrospective study shows that at six month follow-up after initiation of Pimobendan therapy, mitral regurgitation increase is not severe enough to determine significant hemodynamic consequences and remodelling. When considering the outcome at follow-up, no dogs developed congestive heart failure documented by radiography. It can be concluded that after initiation of Pimobendan therapy a follow-up interval of less than 6 months is not recommended.

The study has several limitations that must be considered when interpreting the results. First of all, for ethical reasons it was not possible to build a control group that does not receive Pimobendan. However, the EPIC study has already demonstrated that Pimobendan administration in dogs affected by MMVD ACVIM B2 delays the onset of clinical signs of about 15 months as compared with untreated dog. The same study showed a reduction of left atrium dimension after one month of therapy with Pimobendan, but no information is available about later follow-ups. As a consequence, the results of this study are in line with those of the EPIC study and can be considered reliable even in the absence of an untreated control group. Another study limitation was the small sample size. The echocardiographic indices with low measurement variability, might have achieved statistical significance with larger samples and higher statistical power. Furthermore, because of the retrospective nature of the study, there are limitations related to this type of the study. For example, the echocardiographic images used for the statistical analysis were acquired by different operators. For this reason, inter-operator variability must be taken into account. A prospective multicenter study involving a larger number of dogs would be necessary to confirm the results of this study.

7 Conclusion

Despite an increase in regurgitation fraction, this retrospective study shows that volume overload of the left heart chambers and development of congestive heart failure is unlikely after an average of 6 months of Pimobendan administration.

At the moment, it is safe to presume that MMVD B2 patients starting Pimobendan therapy do not need to be re-examined for 6 months.

These data must be considered a preliminary analysis to lead a larger prospective study with more cases of MMVD B2 dogs, according to the considered criteria.

8 References

[1] Franchini, A., Borgarelli, M., Abbott, J. A., Menciotti, G., Crosara, S., Häggström, J., Lahmers, S., Rosenthal, S., & Tyrrell, W. (2021). The Longitudinal Outcome Of Canine (K9) myxomatous mitral valve disease (LOOK-Mitral registry): Baseline characteristics, *Journal of veterinary cardiology,* vol.36, pp. 32–47.

[2] Fox P. R. (2012). Pathology of myxomatous mitral valve disease in the dog. *Journal of veterinary cardiology, vol. 14*(1), pp. 103–126.

[3] Santilli R.A., Bussadori C., Borgarelli M. (2018) *Manuale di cardiologia del cane e del gatto.*

[4] O'Brien, M. J., Beijerink, N. J., & Wade, C. M. (2021). Genetics of canine myxomatous mitral valve disease. *Animal genetics*, vol. 52(4), pp. 409–421.

[5] Häggström, J., Höglund, K., & Borgarelli, M. (2009). An update on treatment and prognostic indicators in canine myxomatous mitral valve disease. *The Journal of small animal practice*, vol. 50, pp. 25–33.

[6] Oyama, M. A., & Chittur, S. V. (2006). Genomic expression patterns of mitral valve tissues from dogs with degenerative mitral valve disease. *American journal of veterinary research*, vol. 67(8), pp. 1307–1318.

[7] Stern, J. A., Hsue, W., Song, K. H., Ontiveros, E. S., Luis Fuentes, V., & Stepien, R. L. (2015). Severity of Mitral Valve Degeneration Is Associated with Chromosome 15 Loci in Whippet Dogs. *PloS one*, vol.10.

[8] Ettinger, S., Feldman, E., & Cotè, E. (2017). *Textbook of Veterinary Internal Medicine*

[9] Fuentes, V.L., Johnson, L. (2010). BSAVA Manual Of Canine and Feline Cardiorespiratory Medicine, pp. 186–194.

[10] Connell, P. S., Han, R. I., Grande-Allen, K. J. (2012). Differentiating the aging of the mitral valve from human and canine myxomatous degeneration. *Journal of veterinary cardiology,* vol. 14, pp. 31–45.

[11] Serfass, P., Chetboul, V., Sampedrano, C. C., Nicolle, A., Benalloul, T., Laforge, H., Gau, C., Hébert, C., Pouchelon, J. L., Tissier, R. (2006). Retrospective study of 942 small-sized dogs: Prevalence of left apical systolic heart murmur and left-sided heart failure, critical effects of breed and sex. *Journal of veterinary cardiology,* vol. 8(1), pp. 11–18.

[12] Parker, H. G., Kilroy-Glynn, P. (2012). Myxomatous mitral valve disease in dogs: does size matter?. *Journal of veterinary cardiology*, vol. 14, pp. 19–29.

[13] Mattin, M. J., Boswood, A., Church, D. B., López-Alvarez, J., McGreevy, P. D., O'Neill, D. G., Thomson, P. C., Brodbelt, D. C. (2015). Prevalence of and risk factors for degenerative

mitral valve disease in dogs attending primary-care veterinary practices in England. *Journal of veterinary internal medicine*, vol. 29(3), pp. 847–854.

[14] Lu, C. C., Liu, M. M., Culshaw, G., French, A., Corcoran, B. (2016). Comparison of cellular changes in Cavalier King Charles spaniel and mixed breed dogs with myxomatous mitral valve disease. *Journal of veterinary cardiology,* vol. 18, pp. 100–109.

[15] Bionda, A., Cortellari, M., Bagardi, M., Frattini, S., Negro, A., Locatelli, C., Brambilla, P. G., Crepaldi, P. (2020). A Genomic Study of Myxomatous Mitral Valve Disease in Cavalier King Charles Spaniels. *Animals: an open access journal from MDPI*, vol. 10, p. 1895.

[16] Madsen, M. B., Olsen, L. H., Häggström, J., Höglund, K., Ljungvall, I., Falk, T., Wess, G., Stephenson, H., Dukes-McEwan, J., Chetboul, V., Gouni, V., Proschowsky, H. F., Cirera, S., Karlskov-Mortensen, P., & Fredholm, M. (2011). Identification of 2 loci associated with development of myxomatous mitral valve disease in Cavalier King Charles Spaniels. *The Journal of heredity*, vol. 102, pp. 62–67.

[17] Swenson, L., Häggström, J., Kvart, C., & Juneja, R. K. (1996). Relationship between parental cardiac status in Cavalier King Charles spaniels and prevalence and severity of chronic valvular disease in offspring. *Journal of the American Veterinary Medical Association*, vol. 208, pp. 2009–2012.

[18] Keene, B. W., Atkins, C. E., Bonagura, J. D., Fox, P. R., Häggström, J., Fuentes, V. L., Oyama, M. A., Rush, J. E., Stepien, R., & Uechi, M. (2019). ACVIM consensus guidelines for the

diagnosis and treatment of myxomatous mitral valve disease in dogs. *Journal of veterinary internal medicine*, vol.33, pp. 1127–1140.

[19] Olsen, L. H., Fredholm, M., & Pedersen, H. D. (1999). Epidemiology and inheritance of mitral valve prolapse in Dachshunds. *Journal of veterinary internal medicine*, vol.13, pp. 448–456.

[20] French, A. T., Ogden, R., Eland, C., Hemani, G., Pong-Wong, R., Corcoran, B., & Summers, K. M. (2012). Genome-wide analysis of mitral valve disease in Cavalier King Charles Spaniels. *Veterinary Journal,* vol. 193(1), pp. 283–286.

[21] Meurs, K. M., Friedenberg, S. G., Williams, B., Keene, B. W., Atkins, C. E., Adin, D., Aona, B., DeFrancesco, T., Tou, S., & Mackay, T. (2018). Evaluation of genes associated with human myxomatous mitral valve disease in dogs with familial myxomatous mitral valve degeneration. *Veterinary Journal)*, vol. 232, pp. 16–19.

[22] Cremer, S. E., Singletary, G. E., Olsen, L. H., Wallace, K., Häggström, J., Ljungvall, I., Höglund, K., Reynolds, C. A., Pizzinat, N., & Oyama, M. A. (2014). Serotonin concentrations in platelets, plasma, mitral valve leaflet, and left ventricular myocardial tissue in dogs with myxomatous mitral valve disease. *Journal of veterinary internal medicine*, vol. 28, pp. 1534–1540.

[23] Oyama, M. A., & Levy, R. J. (2010). Insights into serotonin signaling mechanisms associated with canine degenerative mitral valve disease. *Journal of veterinary internal medicine*, vol. 24, pp. 27–36.

[24] Hadian, M., Corcoran, B. M., & Bradshaw, J. P. (2010). Molecular changes in fibrillar collagen in myxomatous mitral valve disease. *Cardiovascular pathology: the official journal of the Society for Cardiovascular Pathology*, vol. *19*, pp. e141–e148.

[25] Han, R.I., Black, A., Culshaw, G.K., French, A.T (2010) Structural and Cellular Changes in Canine Myxomatous Mitral Valve Disease: An Image Analysis Study Proteomics of calcific aortic valve disease View project Thoracic veterinary Diagnostic Imaging View project. *The Journal of heart valve disease*, vol. 19, pp. 60-70.

[26] Marcato, P. S. (2015). *Patologia Sistematica Veterinaria*, vol. 1.

[27] Pomerance, A., & Whitney, J. C. (1970). Heart valve changes common to man and dog: a comparative study. *Cardiovascular research*, vol. 4, pp. 61–66.

[28] Beardow, A. W., & Buchanan, J. W. (1993). Chronic mitral valve disease in cavalier King Charles spaniels: 95 cases (1987-1991). *Journal of the American Veterinary Medical Association*, vol. 203, pp. 1023–1029.

[29] Tilley, L.P., Smith, F.W., Oyama, M., Sleeper, M.M. (2008). *Manual of Canine and Feline Cardiology*.

[30] Komamura, K., Shannon, R. P., Ihara, T., Shen, Y. T., Mirsky, I., Bishop, S. P., & Vatner, S. F. (1993). Exhaustion of Frank-Starling mechanism in conscious dogs with heart failure. *The American journal of physiology*, vol. 265, pp. H1119–H1131.

[31] Sjaastad, Ø.V., Sand, O., and Hove, K. (2019). *Fisiologia degli animali domestici*, vol. 1.

[32] Nakamura, K., Kawamoto, S., Osuga, T., Morita, T., Sasaki, N., Morishita, K., Ohta, H., & Takiguchi, M. (2017). Left Atrial Strain at Different Stages of Myxomatous Mitral Valve Disease in Dogs. *Journal of veterinary internal medicine*, vol. 31, pp. 316–325.

[33] Connell, P. S., Han, R. I., & Grande-Allen, K. J. (2012). Differentiating the aging of the mitral valve from human and canine myxomatous degeneration. *Journal of veterinary cardiology: the official journal of the European Society of Veterinary Cardiology*, vol. 14(1), pp. 31–45.

[34] Borgarelli, M., & Haggstrom, J. (2010). Canine degenerative myxomatous mitral valve disease: natural history, clinical presentation and therapy. *The Veterinary clinics of North America. Small animal practice*, vol. 40(4), pp. 651–663.

[35] Caivano, D., Marchesi, M. C., Birettoni, F., Lepri, E., Porciello, F. (2021). Left Atrial Mural Thrombosis and Hemopericardium in a Dog with Myxomatous Mitral Valve Disease. *Veterinary sciences*, vol. 8(6), p. 112.

[36] Ljungvall, I., Rishniw, M., Porciello, F., Ferasin, L., Ohad, D. G. (2014). Murmur intensity in small-breed dogs with myxomatous mitral valve disease reflects disease severity. *The Journal of small animal practice*, vol. 55(11), pp. 545–550.

[37] Ljungvall, I., Ahlstrom, C., Höglund, K., Hult, P., Kvart, C., Borgarelli, M., Ask, P., Häggström, J. (2009). Use of signal analysis of heart sounds and murmurs to assess severity of mitral valve regurgitation attributable to myxomatous mitral valve disease in dogs. *American journal of veterinary research*, vol. 70(5), pp. 604–613.

[38] Garncarz, M., Parzeniecka-Jaworska, M., Jank, M., Łój, M. (2013). A retrospective study of clinical signs and epidemiology of chronic valve disease in a group of 207 Dachshunds in Poland. *Acta veterinaria Scandinavica*, vol. 55(1), p. 52.

[39] Kim, H. T., Han, S. M., Song, W. J., Kim, B., Choi, M., Yoon, J., Youn, H. Y. (2017). Retrospective study of degenerative mitral valve disease in small-breed dogs: survival and prognostic variables. *Journal of veterinary science*, vol. 18(3), pp. 369–376.

[40] Häggström, J., Duelund Pedersen, H., Kvart, C. (2004). New insights into degenerative mitral valve disease in dogs. *The Veterinary clinics of North America. Small animal practice*, vol. 34(5), pp. 1209–viii.

[41] Visser, L.C. (2022) *Atti Congressuali Congresso Internazionale SCIVAC*

[42] Lamb, C.R., Boswood, A. (2003). Il ruolo dell'esame radiografico nella diagnosi delle cardiopatie del cane, Veterinaria, n°3, pp.21-31

[43] Mikawa, S., Nagakawa, M., Ogi, H., Akabane, R., Koyama, Y., Sakatani, A., Ogawa, M., Miyakawa, H., Shigemoto, J., Tokuriki, T., Toda, N., Miyagawa, Y., Takemura, N. (2020). Use of vertebral left atrial size for staging of dogs with myxomatous valve disease. *Journal of veterinary cardiology,* vol. 30, pp. 92–99.

[44] Duler, L., Visser, L. C., Jackson, K. N., Phillips, K. L., Pollard, R. E., Wanamaker, M. W. (2021). Evaluation of radiographic predictors of left heart enlargement in dogs with known or suspected cardiovascular disease. *Veterinary radiology & ultrasound: the official journal of the American College of Veterinary Radiology and the International Veterinary Radiology Association*, vol. 62(3), pp. 271–281.

[45] Rabozzi, R., Oricco, S. (2021). *Ecografia Point-of-Care nel cane e nel gatto*, vol. 1.

[46] Boon, J.A. (2010) *Veterinary Echocardiography*, vol. 1

[47] Terzo, E., Di Marcello, M., McAllister, H., Glazier, B., Lo Coco, D., Locatelli, C., Palermo, V., Brambilla, P. G. (2009). Echocardiographic assessment of 537 dogs with mitral valve prolapse and leaflet involvement. *Veterinary radiology & ultrasound: the official journal of the American College of Veterinary Radiology and the International Veterinary Radiology Association*, vol. 50(4), pp. 416–422.

[48] Rishniw, M., Erb, H. N. (2000). Evaluation of four 2-dimensional echocardiographic methods of assessing left atrial size in dogs. *Journal of veterinary internal medicine*, vol. 14(4), pp. 429–435.

[49] Baron Toaldo, M., Romito, G., Guglielmini, C., Diana, A., Pelle, N. G., Contiero, B., Cipone, M. (2018). Prognostic value of echocardiographic indices of left atrial morphology and function in dogs with myxomatous mitral valve disease. *Journal of veterinary internal medicine*, vol. 32(3), pp. 914–921.

[50] Hsue, W., Visser, L. C. (2020). Reproducibility of echocardiographic indices of left atrial size in dogs with subclinical myxomatous mitral valve disease. *Journal of veterinary internal medicine*, vol. 34(5), pp. 1779–1786.

[51] Dillon, A. R., Dell'Italia, L. J., Tillson, M., Killingsworth, C., Denney, T., Hathcock, J., Botzman, L. (2012). Left ventricular remodeling in preclinical experimental mitral regurgitation of dogs. *Journal of veterinary cardiology*, vol. 14(1), pp. 73–92.

[52] Gordon, S. G., Saunders, A. B., Wesselowski, S. R. (2017). Asymptomatic Canine Degenerative Valve Disease: Current and Future Therapies. *The Veterinary clinics of North America. Small animal practice*, vol. 47(5), pp. 955–975.

[53] Boswood, A., Häggström, J., Gordon, S. G., Wess, G., Stepien, R. L., Oyama, M. A., Keene, B. W., Bonagura, J., MacDonald, K. A., Patteson, M., Smith, S., Fox, P. R., Sanderson, K., Woolley, R., Szatmári, V., Menaut, P., Church, W. M., O'Sullivan, M. L., Jaudon, J. P.,

Kresken, J. G., … Watson, P. (2016). Effect of Pimobendan in Dogs with Preclinical Myxomatous Mitral Valve Disease and Cardiomegaly: The EPIC Study-A Randomized Clinical Trial. *Journal of veterinary internal medicine*, vol. 30(6), pp.1765–1779.

[54] Rishniw, M., Brown, D. (2022). The ACVIM consensus statement definition of left ventricular enlargement in myxomatous mitral valve disease does not always represent left ventricular enlargement. *Journal of veterinary cardiology*, vol. 42, pp. 92–102.

[55] Wesselowski, S., Borgarelli, M., Bello, N. M., Abbott, J. (2014). Discrepancies in identification of left atrial enlargement using left atrial volume versus left atrial-to-aortic root ratio in dogs. *Journal of veterinary internal medicine*, vol. 28(5), pp. 1527–1533.

[56] Höllmer, M., Willesen, J. L., Tolver, A., Koch, J. (2017). Left atrial volume and function in dogs with naturally occurring myxomatous mitral valve disease. *Journal of veterinary cardiology*, vol. 19(1), pp. 24–34.

[57] Zoghbi, W. A., Enriquez-Sarano, M., Foster, E., Grayburn, P. A., Kraft, C. D., Levine, R. A., Nihoyannopoulos, P., Otto, C. M., Quinones, M. A., Rakowski, H., Stewart, W. J., Waggoner, A., Weissman, N. J., American Society of Echocardiography (2003). Recommendations for evaluation of the severity of native valvular regurgitation with two-dimensional and Doppler echocardiography. *Journal of the American Society of Echocardiography: official publication of the American Society of Echocardiography*, vol. 16(7), pp. 777–802.

[58] Di Marcello, M., Terzo, E., Locatelli, C., Palermo, V., Sala, E., Dall'Aglio, E., Bussadori, C. M., Spalla, I., Brambilla, P. G. (2014). Assessment of mitral regurgitation severity by Doppler color flow mapping of the vena contracta in dogs. *Journal of veterinary internal medicine*, vol. 28(4), pp. 1206–1213.

[59] Gouni, V., Serres, F. J., Pouchelon, J. L., Tissier, R., Lefebvre, H. P., Nicolle, A. P., Sampedrano, C. C., Chetboul, V. (2007). Quantification of mitral valve regurgitation in dogs with degenerative mitral valve disease by use of the proximal isovelocity surface area method. *Journal of the American Veterinary Medical Association*, vol.231(3), pp. 399–406.

[60] Tribouilloy, C., Shen, W. F., Rey, J. L., Adam, M. C., Lesbre, J. P. (1994). Mitral to aortic velocity-time integral ratio. A non-geometric pulsed-Doppler regurgitant index in isolated pure mitral regurgitation. *European heart journal*, vol.15(10), pp.1335–1339.

[61] Ascione, L., De Michele, M., Accadia, M., Tartaglia, P. F., Guarini, P., Sacra, C., Tuccillo, B. (2003). Usefulness of the mitral/aortic flow velocity integral ratio as a screening method to identify patients with hemodynamically significant mitral regurgitation. *Journal of the American Society of Echocardiography: official publication of the American Society of Echocardiography*, vol. 16(5), pp. 437–441.

[62] Reinero, C., Visser, L. C., Kellihan, H. B., Masseau, I., Rozanski, E., Clercx, C., Williams, K., Abbott, J., Borgarelli, M., Scansen, B. A. (2020). ACVIM consensus statement guidelines for the diagnosis, classification, treatment, and monitoring of pulmonary hypertension in dogs. *Journal of veterinary internal medicine*, vol.34(2), pp. 549–573.

[63] Klein, S., Nolte, I., Rumstedt, K., Sehn, M., Raue, J. F., Weiner, F., Treese, J. S., Beyerbach, M., Bach, J. P. (2021). The effect of treatment with pimobendan in dogs with preclinical mitral valve disease - a placebo-controlled double-blinded crossover study. *BMC veterinary research*, vol. 17(1), p. 310.

[64] Iwanuk, N., Wall, L., Nolte, I., Raue, J., Rumstedt, K., Pilgram, A., Sehn, M., Rohn, K., Bach, J. P. (2019). Effect of pimobendan on physical fitness, lactate and echocardiographic parameters in dogs with preclinical mitral valve disease without cardiomegaly. *PloS one*, vol.14(10), pp. 1-12.

[65] Kvart, C., Häggström, J., Pedersen, H. D., Hansson, K., Eriksson, A., Järvinen, A. K., Tidholm, A., Bsenko, K., Ahlgren, E., Ilves, M., Ablad, B., Falk, T., Bjerkfås, E., Gundler, S., Lord, P., Wegeland, G., Adolfsson, E., Corfitzen, J. (2002). Efficacy of enalapril for prevention of congestive heart failure in dogs with myxomatous valve disease and asymptomatic mitral regurgitation. *Journal of veterinary internal medicine*, vol. 16(1), pp.80–88.

[66] Borgarelli, M., Ferasin, L., Lamb, K., Bussadori, C., Chiavegato, D., D'Agnolo, G., Migliorini, F., Poggi, M., Santilli, R. A., Guillot, E., Garelli-Paar, C., Toschi Corneliani, R., Farina, F., Zani, A., Dirven, M., Smets, P., Guglielmini, C., Oliveira, P., Di Marcello, M., Porciello, F., … Baron Toaldo, M. (2020). DELay of Appearance of sYmptoms of Canine Degenerative Mitral Valve Disease Treated with Spironolactone and Benazepril: the DELAY Study. *Journal of veterinary cardiology*, vol. 27, pp.34–53.

[67] Bristow, P. (2020). Mitral valve repair surgery in dogs. *Veterinary practice today*, vol.8, pp.10-13

[68] Uechi M. (2012). Mitral valve repair in dogs. *Journal of veterinary cardiology,* vol.14(1), pp.185–192.

[69] BENCH (BENazepril in Canine Heart disease) Study Group (1999). The effect of benazepril on survival times and clinical signs of dogs with congestive heart failure: Results of a multicenter, prospective, randomized, double-blinded, placebo-controlled, long-term clinical trial. *Journal of veterinary cardiology,* vol. 1(1), pp. 7–18.

[70] Bernay, F., Bland, J. M., Häggström, J., Baduel, L., Combes, B., Lopez, A., Kaltsatos, V. (2010). Efficacy of spironolactone on survival in dogs with naturally occurring mitral regurgitation caused by myxomatous mitral valve disease. *Journal of veterinary internal medicine*, vol.24(2), pp. 331–341.

[71] Chetboul, V., Pouchelon, J. L., Menard, J., Blanc, J., Desquilbet, L., Petit, A., Rougier, S., Lucats, L., Woehrle, F., TEST study investigators (2017). Short-Term Efficacy and Safety of Torasemide and Furosemide in 366 Dogs with Degenerative Mitral Valve Disease: The TEST Study. *Journal of veterinary internal medicine*, vol. 31(6), pp. 1629–1642.

[72] Her, J., Kuo, K. W., Winter, R. L., Cruz-Espindola, C., Bacek, L. M., Boothe, D. M. (2020). Pharmacokinetics of Pimobendan and Its Metabolite O-Desmethyl-Pimobendan Following Rectal Administration to Healthy Dogs. *Frontiers in veterinary science*, vol. 7, p. 423.

[73] Bagardi, M., Palermo, V., Locatelli, C., Colombo, F. M., Pazzagli, L., Brambilla, P. G. (2022). Database development and survival analysis in a clinical and historical cohort of dogs affected by myxomatous mitral valve disease treated or not with pimobendan using causal inference techniques. *Veterinary research communications*, vol. 46(2), pp. 481–486.

[74] Carli, S., Ormas, P., Re, G., Soldani, G. (2009) *Farmacologia Veterinaria*, vol. 1.

[75] Pichayapaiboon, P., Tantisuwat, L., Boonpala, P., Saengklub, N., Boonyarattanasoonthorn, T., Khemawoot, P., Kijtawornrat, A. (2021). Pharmacodynamics and Pharmacokinetics of Injectable Pimobendan and Its Metabolite, O-Desmethyl-Pimobendan, in Healthy Dogs. *Frontiers in veterinary science*, vol. 8, pp. 1-11.

[76] Kanno, N., Kuse, H., Kawasaki, M., Hara, A., Kano, R., Sasaki, Y. (2007). Effects of pimobendan for mitral valve regurgitation in dogs. *The Journal of veterinary medical science*, vol. 69(4), pp. 373–377.

[77] Plumb, D.C. (2008). *Plumb's Veterinary Drug Handbook*.

[78] Boyle, K. L., Leech, E. (2012). A review of the pharmacology and clinical uses of pimobendan. *Journal of veterinary emergency and critical care,* vol.22(4), pp. 398–408.

[79] Lombard, C. W., Jöns, O., Bussadori, C. M. (2006). Clinical efficacy of pimobendan versus benazepril for the treatment of acquired atrioventricular valvular disease in dogs. *Journal of the American Animal Hospital Association*, vol. 42(4), pp. 249–261.

[80] Häggström, J., Lord, P. F., Höglund, K., Ljungvall, I., Jöns, O., Kvart, C., Hansson, K. (2013). Short-term hemodynamic and neuroendocrine effects of pimobendan and benazepril in dogs with myxomatous mitral valve disease and congestive heart failure. *Journal of veterinary internal medicine*, vol.27(6), pp. 1452–1462.

[81] Häggström, J., Boswood, A., O'Grady, M., Jöns, O., Smith, S., Swift, S., Borgarelli, M., Gavaghan, B., Kresken, J. G., Patteson, M., Ablad, B., Bussadori, C. M., Glaus, T., Kovacević, A., Rapp, M., Santilli, R. A., Tidholm, A., Eriksson, A., Belanger, M. C., Deinert, M., … DiFruscia, R. (2008). Effect of pimobendan or benazepril hydrochloride on survival times in dogs with congestive heart failure caused by naturally occurring myxomatous mitral valve disease: the QUEST study. *Journal of veterinary internal medicine*, vol. 22(5), pp. 1124–1135.

[82] Boswood, A., Gordon, S. G., Häggström, J., Wess, G., Stepien, R. L., Oyama, M. A., Keene, B. W., Bonagura, J., MacDonald, K. A., Patteson, M., Smith, S., Fox, P. R., Sanderson, K., Woolley, R., Szatmári, V., Menaut, P., Church, W. M., O'Sullivan, M. L., Jaudon, J. P., Kresken, J. G., … Watson, P. (2018). Longitudinal Analysis of Quality of Life, Clinical, Radiographic, Echocardiographic, and Laboratory Variables in Dogs with Preclinical Myxomatous Mitral Valve Disease Receiving Pimobendan or Placebo: The EPIC Study. *Journal of veterinary internal medicine*, vol. 32(1), pp. 72–85.

[83] Borgarelli, M., Buchanan, J. W. (2012). Historical review, epidemiology and natural history of degenerative mitral valve disease. *Journal of veterinary cardiology*, vol. 14(1), pp. 93–101.

[84] Borgarelli, M., Savarino, P., Crosara, S., Santilli, R. A., Chiavegato, D., Poggi, M., Bellino, C., La Rosa, G., Zanatta, R., Haggstrom, J., Tarducci, A. (2008). Survival characteristics and prognostic variables of dogs with mitral regurgitation attributable to myxomatous valve disease. *Journal of veterinary internal medicine*, vol. 22(1), pp. 120–128.

[85] Thrusfield, M.V., Aitken, C.G.G., Darke, P.G.G. (1985). Observations on breed and sex in relation to canine heart valve incompetence. Journal of small animal practice, vol. 26, pp. 709-717.

[86] Morgan, K., Monteith, G., Raheb, S., Colpitts, M., Fonfara, S. (2020). Echocardiographic parameters for the assessment of congestive heart failure in dogs with myxomatous mitral valve disease and moderate to severe mitral regurgitation. *Veterinary journal*, vol. *263*, p. 105518.

[87] Lee, M. M., Salahuddin, A., Garcia, M. J., Spevack, D. M. (2015). Left ventricular early inflow-outflow index: a novel echocardiographic indicator of mitral regurgitation severity. *Journal of the American Heart Association*, vol. 4(6), p. e000781.

[88] Franco, R. P., Zacche, E., Camacho, R. R., Sousa, M. G., Camacho, A. A. (2016). Determination of left atrial volume in healthy dogs and dogs with myxomatous mitral valve disease, *Pesquisa Veterinaria Brasileira*, vol. 36, pp. 743–748.

[89] Menciotti, G., Borgarelli, M. (2017). Review of Diagnostic and Therapeutic Approach to Canine Myxomatous Mitral Valve Disease. *Veterinary sciences*, vol. 4(4), p. 47.

[90] Marchesotti, F., Vezzosi, T., Tognetti, R., Marchetti, F., Patata, V., Contiero, B., Zini, E., Domenech, O. (2019). Left atrial anteroposterior diameter in dogs: reference interval, allometric scaling, and agreement with the left atrial-to-aortic root ratio. *The Journal of veterinary medical science*, vol. 81(11), pp. 1655–1662.