ESCOLA UNIVERSITÁRIA VASCO DA GAMA

MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

CANINE MYXOMATOUS MITRAL VALVE DISEASE: A FOCUSED UPDATE

Mariana de Sousa Pinheiro

Coimbra, julho 2021

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List of Abbreviations and Acronyms

5-HT – Serotonin

ACEI – Angiotensin-converting enzyme inhibitor

ADAMTSL4 – ADAMTS Like 4

aVIC – Activated myofibroblast

BW – Body weight

CASQ2 – Calsequestrin – 2

CFA – Canine Chromosome

CHF – Congestive heart failure

CKCS – Cavalier King Charles Spaniel

CPB – Cardiopulmonary bypass

Ea – Effective arterial elastance

ECG - Electrocardiography

Ees – Left ventricular end-systolic elastance

ePTFE – Expanded-polytetrafluoroethylene

FAH – Fumarylacetoacetate Hydrolase

FBN1 – Fibrilin – 1

FGFR1 – Fibroblast growth factor receptor 1

FSV – Forward stroke volume

GAG - Glycosaminoglycan

GWAS – Genome-wide association study

HF – Heart Failure

LA:Ao – Left atrial to aortic root ratio

LA:Ao – Left atrium to aorta ratio

LA:LV – Left atrium to left ventricle ratio

LV:Ao – Left ventricle to aorta ratio

LAP – Left atrial pressure

LVIDD – Left ventricular internal diameter at end-diastole

LVIDS – Left ventricular internal diameter at end-systole

MMVD – Myxomatous mitral valve disease

M-VLAS – Modified-VLAS

MVR – Mitral valve replacement

NT-proBNP – N-terminal pro-B-type natriuretic peptide

PH – Pulmonary Hypertension

qVIC – Valvular interstitial cells before the phenotypical change

RAA – Renin-Angiotensin-Aldosterone cascade

RHF – Right heart failure

RLAD – Radiographic left atrium dimension

RSV – Regurgitation stroke volume

RYR2 – Ryanodine receptor 2

TGF-β1 – Transforming growth factor beta 1

TGF-β – Transforming growth factor beta

TNNI3 – Troponin I3

TPH1 – Tryptophan hydroxylase

TSV – Total stroke volume

VAC – Left ventricular-arterial coupling

VHS – Vertebral Heart Size

VICs – Valvular interstitial cells

VLAS – Vertebral left atrial size

Canine Myxomatous Mitral Valve Disease: A Focused Update

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Abstract

The myxomatous mitral valve disease (MMVD) is the most common cardiac condition in dogs, being the leading cause of congestive heart failure that precede the cardiac related death or euthanasia. It is a disease mostly frequent in small to median breed dogs (under 20kg), with a symptomatic phase most common in older patients. The Cavalier King Charles Spaniel is the most predisposed breed, with almost all the individuals presenting the disease and clinical signs in early ages when comparing to other breeds. This condition is a polygenic disease, and the mechanisms that lead to the mitral valve degeneration are not yet completely known. A phenotypic modification of the valvular interstitial cells seems to be a major feature in the development of the changes observed in the mitral valve. The changes in connective tissue, collagen organisation and glycosaminoglycans deposition leads to the formation of nodules on the valve. These nodules are what give the disease its name. The changes result in an inability of complete closure of the mitral valve, which leads to mitral regurgitation, the primary cause for cardiac remodelling and all the following clinical signs observed in the course of the disease. The medical treatment aims to delay the onset of clinical signs and congestive heart failure in earlier stages of the disease. Nonetheless, in the last stage of the disease, the medical treatment is no longer effective. The surgical treatment is possible but not frequent, due to its high cost and the need for specialized teams. Being such a common disease, studies in the area are still need, to increase the efficacy of the medical treatment and extend the life expectancy of the affected dogs.

Keywords: Dog, congestive heart failure, mitral cardiac regurgitation, mitral valve repair, polygenic disease, serotonin

Resumo

A Doença Valvular Mitral Mixomatosa (MMVD) é a doença cardíaca mais frequente em cães, sendo a primeira causa para o desenvolvimento de falha cardíaca congestiva, morte ou eutanásia por causas cardíacas. É mais comum em cães com menos de 20kg, e a fase sintomática ocorre geralmente em idades mais avançadas. A raça de cães mais predisposta é a Cavalier King Charles Spaniel, com a maioria dos animais como portadores da doença. Nestes, o início dos sinais clínicos ocorre mais cedo, comparativamente a outras raças. A MMVD é uma doença poligénica, mas os mecanismos que levam à degeneração da válvula mitral ainda não são completamente conhecidos. Uma mudança fenotípica acontece nas células intersticiais da válvula mitral, e este acontecimento parece ser uma das principais causas para as alterações visualizadas na válvula. Há alterações no tecido conjuntivo, organização do colagénio e deposição de glicosaminoglicanos, que levam à formação de nódulos na válvula. Estes nódulos são responsáveis por dar à doença a expressão "mixomatosa". Todas estas alterações levam à incapacidade de encerramento completo valvular, levando a regurgitação mitral, que parece ser a causa principal para todas as alterações hemodinâmicas que acontecem com o avançar da doença. O tratamento médico é importante nas fases iniciais da doença para retardar o aparecimento dos sinais clínicos e da falha cardíaca congestiva. No entanto, em cães com esta condição em fase muito

avançada, o tratamento pode deixar de ser eficaz. O tratamento cirúrgico é também possível, mas não muito frequente, devido ao elevado custo monetário e à necessidade de uma equipa médico-veterinária especializada para efetuar o procedimento. Sendo uma doença com elevada prevalência, é necessário continuar a efetuar estudos, de modo a aumentar a eficácia do tratamento médico e aumentar a esperança de vida dos cães afetados.

Palavras-Chave: *Cão, doença poligénica, falha cardíaca congestiva, regurgitação cardíaca mitral, restauração cirúrgica válvula mitral, serotonina*

1. Background

The myxomatous mitral valve disease (MMVD) also commonly known as degenerative mitral valve disease, chronic valvular disease or endocardiosis (Hezzell, 2018) is the most frequently diagnosed cardiac disease in the companion animal practice (Keene *et al.*, 2019). MMVD is associated with development of congestive heart failure (CHF), being one of the major causes for its occurrence (Borgarelli & Buchanan, 2012).

Delabere Blaine made the first mention of the disease more than 200 years ago, in 1817. The English veterinary surgeon described it as “a kind of thrill, very different from usual sensation produced by the beating of the heart of a healthy dog” when a hand was placed in the thorax of an affected dog (Borgarelli & Buchanan, 2012).

The course of the disease is equivalent to the mitral valve prolapse described in humans (Madsen *et al.*, 2011). For that reason, both species have been studied in a comparative perspective in the last years.

The age of the animal is a major factor (Franchini *et al*., 2021), although some variations occur, especially in predisposed breeds. The disease is more common in animals with a body weight under 20kg (Keene *et al*., 2019), and the arising of the clinical signs seems to be earlier in breeds such as the Cavalier King Charles Spaniel (CKCS) (Keene *et al*., 2019).

Despite the various hypotheses described, the real cause of the onset of MMVD is still unknown. The possibilities are related to genetic mechanisms (Bionda *et al.*, 2020; Birkegård *et al.*, 2016; Madsen *et al*., 2011; Markby *et al.*, 2020), that influence a series of events that lead to the development of changes observed in affected dogs. One of these mechanisms is related with the influence of serotonin (Disatian & Orton, 2009; Ljungvall *et al*., 2013; Menciotti & Borgarelli, 2017).

For being the most common cardiac disease in the veterinary clinical practice, the number of published studies, research, and review paper, regarding MMVD increased in the last years, as depicted in Figure 1, reflecting a simple search in PubMed with the keywords “MMVD” and “Dog” in the previous 18 years. For that reason, the aim of this study was to update and summarize the most recent knowledge on MMVD focused on the aetiology, pathogenesis and natural course of the disease, diagnosis, as well as recent advances on medical and surgical treatments.

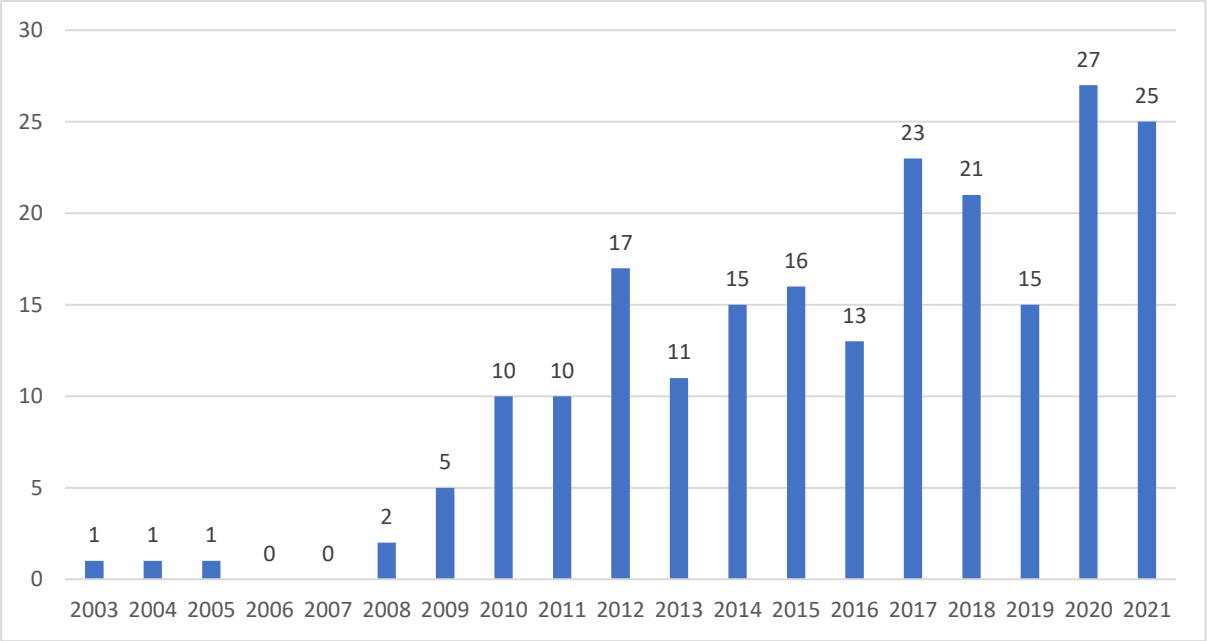

Figure 1: The evolution of published reports on MMVD in the last 18 years from PubMed (accessed on May 26, 2021)

2. Predisposition

The prevalence of MMVD seems to be related to some factors, such as sex, body size and breed (Keene *et al*., 2019), being further considered an age-dependent disease (Franchini *et al*., 2021). Recent studies have revealed that predisposition is higher in males (1.5 times), smaller dogs (average body weight less than 20kg) and in some breeds, such as the CKCS (Keene *et al*., 2019)**.** In this particular breed it is estimated that half of the individuals are affected by the age of five, and almost all the dogs have the disease by the age of ten (Hezzell, 2018). Moreover, in the CKCS the disease seems to be detected at younger ages than in other breeds. Nonetheless its progression appears to be similar to other affected animals (Franchini *et al.*, 2021; Keene *et al*., 2019).

Other breeds in which the prevalence seems to be higher are the Dachshund, the Poodle, the Chihuahua and the Cocker Spaniel (Hezzell, 2018). Some larger breeds can also be affected, but the prevalence seems to be less pronounced. However, when affected, these larger size breeds tend to have a more severe myocardial dysfunction, thus leading to a faster progression and, consequently, a worst prognosis (Keene *et al*., 2019)**.**

3. Aetiology

In MMVD, the changes in the myocardium affect the cellular components and the *valvus apparatus*, comprised by the valve leaflets, *chordae tendineae* and the valve annulus (Hezzell, 2018), which transforms its intercellular matrix. As a result, the collagen content changes and, a new alignment of the collagen fibrils in the valve occurs (Keene *et al*., 2019).

As mention before, the real cause for this disease to develop is still unknown, underlying genetic mechanisms in the CKCS, and in other predisposed breeds, are suggested by several studies (Bionda *et al*., 2020; Madsen *et al.*, 2011; Markby *et al*., 2020).

Madsen *et al.,* (2011) conducted a genome-wide association study (GWAS) to identify the *loci* in the CKCS genome that had an influence in the development of MMVD. This was only possible because the genome within a breed is sufficiently homogenous, due to the small pool of individuals selected for breeding. In the 139 individuals on the study group, they identified markers on canine chromosome (CFA) 13 and CFA 14, in which 99% of the dogs had at least one haplotype D13 or D14, and only one individual was homozygous with d13 and d14. Conversely, in the control group (n=102), only four individuals were homozygous for D13 and D14. The authors further concluded that the haplotype D14 seems to have a pathogenic effect, whilst the d13 may have a protective effect. Since some of the genes located in these regions play a role in the composition of the connective tissue, collagen formation and deposition of proteoglycans, this can be a possible explanation for the development of the disease (Madsen *et al*., 2011).

Parker and Kilroy-Glynn (2012) theorised about the morphologic and historical factors that could possibly be associated with the development of MMVD. Morphologically, the most affected breeds have usually less than 20 kg of body weight (Keene *et al*., 2019). Historically, there has been a selection for the best traits. This selection has led to some degree of inbreeding and the use of the same individuals to become sires. Over time, this practice resulted in a decrease in heterozygosity, leading to an increase in the prevalence of some mutations, and, therefore, an increase in the development of certain diseases that later become characteristic of that group of animals. Another theory is that all small breeds have a common ancestor, and that the MMVD gene was introduced very early, and became more frequent in the population due to the small pool of individuals that were able to breed (Parker & Kilroy-Glynn, 2012).

In the work of Birkegård *et al.* (2016), a breeding restriction program and the effects of the choices of breeders over the litters of CKCS were studied, for a period of 10 years. The animals were examined by means of auscultation and echocardiography and approved, or not, for breeding, depending on the results. After this period of time, the authors concluded that with a breeding scheme based on auscultation and echocardiography, it was possible to decrease the prevalence of MMVD, with significantly lower cases of mitral regurgitation (Birkegård *et al.*, 2016).

The serotonin (5-hydroxytryptamine, 5-HT) appears to be also implicated in the establishment of the disease, namely because of interactions with transforming growth factor beta 1 (TGF-β1), in which a

transformation of the phenotype of the fibroblast-like valvular interstitial cells (qVIC) in a more active myofibroblast phenotype (aVIC) may occur (Ljungvall *et al.,* 2013; Rajamannan *et al.*, 2001).

Ljungvall *et al.* (2013), conducted a study about the influence of the serum serotonin concentration in the severity of MMVD in dogs. They reach two important conclusions, first that the serum 5-HT concentration decreased with the increasing severity of disease and secondly, the CKCS individuals on the study presented a higher serum 5-HT concentration than individuals from other breeds (non-CKCS). The decreased concentration suggests that if the 5-HT has an important role in the establishment of the disease, it is restricted to the earlier stages of valvular degeneration. With all these findings, it seems that despite other factors, some modifications in the 5-HT signalling system may contribute to the process that leads to valvular degeneration (Ljungvall *et al.*, 2013).

The tryptophan hydroxylase 1 (TPH1) is the major serotonin-synthesizing enzyme. A local increase in the expression of TPH1 by the activation of tension mechanosensors may increase the autocrine production of 5-HT in the leaflets (Aupperle & Disatian, 2012; Menciotti & Borgarelli, 2017)**.**

Bionda *et al*. (2020), performed a study to identify the genomic regions associated with early-stage MMVD, in CKCS. They found that the fumarylacetoacetate hydrolase (FAH) interacts with ADAMTS like 4 (ADAMTSL4), with the latter facilitating the fibrillin-1 (FBN1) microfibril biogenesis, which regulates the signalling of the transforming growth factor beta (TGFβ). It was also found that the HEPACAM2 which interacts with the fibroblast growth factor receptor 1 (FGFR1), associated with abnormal heart development, and the HEPACAM2 may be localized on the region CFA 14q1.3, previously identified (Bionda *et al.*, 2020; Madsen *et al.*, 2011).

A comparative study about the mitral valve transcriptome profile between CKCS and non-CKCS dogs was recently reported (Markby *et al.*, 2020). The enrolled dogs presented MMVD in severe and very severe stages. The results showed differences between the valves of CKCS and non-CKCS with severe MMVD, in particular, some differences in the expression of genes associated with cardiomyocytes, namely Calsequestrin-2 (CASQ2), Troponin I3 (TNNI3) and Ryanodine Receptor 2 (RYR2) (Markby *et al*., 2020)**.**

4. Pathogenesis

The normal mitral valve is composed of two leaflets, one anterior or septal, and another posterior or mitral (Markby *et al.*, 2017). They are connected to the heart by the *annulus fibrosus*, and the *chordae tendineae* connect them to the papillary muscles (Markby *et al*., 2017). The surface of the underside of the leaflets is rough and irregular because of the insertions of the *chordae tendineae*, while the surface on the mitral side is smooth. Three layers, the *atrialis*, the *spongiosa* and the *fibrosa* constitute the middle of the leaflet. All these three layers are composed by varying amounts of collagen I, III, IV and VI, laminin, fibronectin and heparan sulphate (Markby *et al*., 2017).

The *atrialis,* is a thin layer that also contains large amounts of elastin, scattered collagen I and III (Oyama & Levy, 2010). In the *spongiosa* it can be found lose collagen fibres, scattered collagen bundles and small numbers of thin elastin fibres embedded in glycosaminoglycan (GAG) with scattered valvular interstitial cells (VICs). The *fibrosa* is dense with collagen bundles and scattered VICs (Markby *et al*., 2017). It is not always possibly to make the complete distinction between the *spongiosa* layer and the *fibrosa* layer, which is not considered pathologic (Markby *et al.*, 2017).

The most affected valve in a situation of myxomatous degeneration is the mitral valve, but it can also damage the tricuspid valve, and less frequently the pulmonary and aortic valves (Hezzell, 2018). The valves affected by the disease have an increase in cellularity and matrix deposition (Menciotti & Borgarelli, 2017; Oyama & Levy, 2010; Oyama *et al*., 2008)

The most important feature in MMVD is a phenotypic modification of the valvular interstitial cell to an activated myofibroblast type of cell (Han *et al.*, 2008; Markby *et al.*, 2017). These activated myofibroblasts localised primarily close to the endothelium have a tropism for the *atrialis* layer (Han *et al*., 2008; Markby *et al*., 2017), and may be responsible for the destruction and disorganisation of the extracellular matrix, one of the major features in MMVD (*Han et al*., 2008; Rabkin *et al.*, 2001; Walker *et al.*, 2004). With the progression of disease there is an increase in the number of activated myofibroblasts, which correlates with the condition's severity (Blake *et al*., 2020; Han *et al*., 2008).

The aVICs increase proteolytic enzymes that degrade collagen and elastin faster than they are produced by the valvular interstitial cells before the phenotypical change (Markby *et al.*, 2017; Markby *et al.,* 2020; Yang *et al.,* 2017).

There is an overexpression of collagenolytic enzymes and matrix metalloproteinases (Menciotti & Borgarelli, 2017). The *spongiosa* layer is infiltrated by GAGs, which alters the collagen fibre orientation (Menciotti & Borgarelli, 2017).

Because of the increase aVICs during the course of the disease, their survival may be a contribution for the development and progression of MMVD, along with other possible mechanisms previously reported. The reason for the increase in the number of these cells may possibly be a heightened senescence or a reduced apoptosis (Blake *et al*., 2020; Surachetpong *et al.*, 2013), because if there is no destruction of these cells, they promote a continuous tissue remodelling, leading to tissue damage (Blake *et al*., 2020).

The importance of the survival of aVICs lead Black *et al.* (2020) to hypothesise that this increase in aVICs may be explained by a defect in the activation and progression of apoptotic pathways. The survival of the aVICs was confirmed by their examination of the spatial and temporal distribution and then it was made an examination of the expression of the apoptotic genes both in aVIC and qVIC, before and after stimulation with doxorubicin, that was used as an inductor of apoptosis (Blake *et al*., 2020). In the first step it was clearly identified a modification both in density and distribution of aVICs, with an increase in the number of the alpha smooth muscle actin positive cells as the disease progresses, with

a partition of cells along all the leaflet. It is noteworthy that in a normal leaflet these cells only occupy a single layer in the basal area (Blake *et al*., 2020). In addition, when the doxorubicin was introduced, the cells continued increasing their number, maintaining the morphology. While this cannot be absolutely conclusive, their findings suggest that cell survival is due to heightened cell senescence and/or reduced apoptosis (Blake *et al*., 2020).

Progressive weakening of the connective tissue because of an abnormal collagen organisation and GAG accumulation affects the valve apparatus zone. This process is what gives the disease the name of myxomatous degeneration (Hezzell, 2018). The modifications lead to the formation of nodules, especially in the valve apposition area, that progressively coalesces to plaque-like lesions.

The areas where the nodules develop are called myxomatous areas, where a decrease in connective tissue density and cell numbers with an increase of GAG content occurs (Han *et al.*, 2010; Markby *et al*., 2017)**.** In the *chordae tendineae* the collagen also suffers a considerable reduction, an increase GAG content and a modification in the GAG composition (Markby *et al*., 2017), possibly leading to their rupture (Hezzell, 2018). The increase in GAG content leads to the accumulation of them on the valve, which is a major factor for the thickening and further mechanical failure (Markby *et al.*, 2017).

On the histopathologic view, the most severe changes are the expansion of the *spongiosa,* and the partially or complete destruction of the *fibrosa* (Markby *et al.,* 2017). There are modifications on the laminin, elastin and collagens IV and VI in the subendothelium, and deeper in the leaflet are seen changes in elastin, fibronectin and collagens I and II. Nonetheless these changes are dependent of the severity of the disease (Markby *et al.*, 2017). The number of cells increase in the proximity of subendothelium zones, and in areas where a endothelium cell loss occurred (Markby *et al.*, 2017).

The affected valve become thicker and nodular, with nodules in large areas of the leaflets and eventually affecting the *chordae tendineae* (Menciotti & Borgarelli, 2017).

Because of the instability provoked by all the modifications described previously, in a mechanical point of view, the final result is an inability to complete apposition of the leaflets that make up the valve, developing mitral regurgitation (Menciotti & Borgarelli, 2017). This inability is most seen during the ventricular systole, which leads to a regurgitation of the blood from the ventricle to the atrium. The resulting high velocity jets lead to trauma in the atrial endocardium (Hezzell, 2018).

The severity of the mitral regurgitation is what determines the left atrium and ventricle remodelling, and consequential haemodynamic changes (Menciotti & Borgarelli, 2017).

In a normal functioning heart, the total stroke volume, and the forward stroke volume (FSV) present equal numbers. In a dog with MMVD, a dysfunctional mitral valve allows a certain degree of mitral regurgitation. As a result, some of the blood that should progress to the aorta returns to the left atrium (Bonagura & Twedt, 2009; Hezzell, 2018; Mama, 2016).

In the following diastole, this blood returns to the left ventricle, increasing the tension in the diastolic cardiac wall. In result, there are remodelling reactions in both the left atrium and ventricle (Lord *et al*., 2010; Menciotti & Borgarelli, 2017).

Despite this cardiac remodelling, the cardiac output can be maintained normal for a limited period. This happens because of renal and neurohormonal mechanisms, and vascular compensation (Ljungvall *et al.*, 2011).

The aorta has baroreceptors that detect a decrease in the cardiac output. This leads to an increase of the sympathetic drive to the sinus node, increasing the heart rate in an attempt to normalize the cardiac output (Hezzell, 2018; Mama, 2016).

Nonetheless, as the FSV decrease, the systemic blood pressure lowers, reducing the concentration of sodium chloride in the distal tubules in the kidney. This decrease is a stimulus for the delivery of renin, triggering the renin-angiotensin-aldosterone cascade (RAA). The RAA will provide vasoconstriction and retention of salt and water, normalizing blood volume and restoring de FSV (Hezzell, 2018; Mama, 2016).

The restored FSV increases the total stroke volume (TSV), since the regurgitation is still present, which leads to ventricular eccentric hypertrophy with addition of cardiomyocytes in series, making the ventricle appear more globoid. Furthermore, with the regurgitation, the atrium dilates. This enlargement of the left heart leads to normal chamber pressures, decreasing the risk of secondary pulmonary hypertension that could further result in pulmonary oedema (Hezzell, 2018; Ljungvall *et al.*, 2011; Mama, 2016). At this stage, although the blood pressure remains normal, the dog can present some clinical signs, as cough due to the left bronchial compression (Bonagura & Twedt, 2009).

Although the compensatory mechanisms described above seem to be effective for a while, the progressive characteristics of this disease, lead to an aggravation of mitral deterioration, increasing the mitral regurgitation (Hezzell, 2018; Mama, 2016)**.** The problem with the eccentric hypertrophy is that besides the wall of the heart, there is also an enlargement of the valve *annulus*, which increases the difficulty of the total valve closure. These means that all the mechanisms initially beneficial, become latter a worsening factor for the progression of MMVD. The left atrium is no longer able to respond to the high-pressure jet from the left ventricle, increasing the pressure in the atrium and hence the pulmonary vein pressure. Because of the increased pressure, the fluid from the pulmonary interstitium it is not totally reabsorbed, forming interstitial oedemas and leading the animal to the final stage of the disease, the congestive heart failure (Hezzell, 2018; Mama, 2016)**.**

5. Clinical Manifestation

The term ‘heart failure’ (HF) is used to describe an onset of clinical signs originated by heart dysfunction. The HF, namely the congestive heart failure (CHF), results from a heart disease that has

an impact in heart function, resulting in an abnormally increased venous pressure, so that fluids begin to accumulate in the lungs or in body cavities (Keene *et al*., 2019).

The development of clinical signs varies from animal to animal. It can take a gradual course or have an acute onset. Sudden aggravation of MMVD can happen due to rupture of a *chordae tendineae,* the beginning of an arrythmia or because the dog was subjected to some kind of stress (Petrič, 2015).

The first sign of the disease is a characteristic left apical systolic murmur (Hezzell, 2018) that is often increased by exercise or excitement (Nelson & Couto, 2014), but the animal can have a long asymptomatic period (Petrič, 2015). This heart murmur can be detected in a regular check-up that precedes a vaccination appointment (Hezzell, 2018).

When the clinical signs begin, usually the left heart failure also starts. The owner can complain about tachypnoea or dyspnoea, cough or even syncope (Petrič, 2015), but also exercise intolerance, increased respiratory rate (Nelson & Couto, 2014; Ware, 2011) and weight loss (Boswood *et al.*, 2018). If the animal has CHF, it can also show signs of right-sided heart failure such as ascites or hepatomegaly secondary to venous congestion (Nelson & Couto, 2014).

The cough is usually harsh and dry (Petrič, 2015), usually more frequently at night or morning (Nelson & Couto, 2014). The reason for the appearance of this clinical sign is the presence of pulmonary oedema or some degree of compression of the main stem bronchus by an enlarged left atrium, without other pulmonary concomitant conditions (Franchini *et al.*, 2021; Petrič, 2015). As small dogs are predisposed to MMVD but also to chronic tracheobronchial disease, the reason for their cough must be well investigated (Keene *et al.*, 2019).

When right heart failure (RHF) is present, the MMVD is in an advance state, which means pulmonary hypertension and tricuspid degeneration or insufficiency can be present, as can dyspnoea resulting of ascites or pleural effusions (Petrič, 2015).

6. Staging

The macroscopic and histological changes observed on the valve can be classified using the Whitney Classification, graded from 0 to 4 (Table 1) (Borgarelli & Buchanan, 2012; Markby *et al.*, 2017). The grades 0 and 1 are the hardest to distinguish (Borgarelli & Buchanan, 2012; Markby *et al*., 2017), and it is possible to establish a close correspondence between the grade, the age and the intensity of the cardiac murmur (Markby *et al.*, 2017).

Table 1: Whitney Classification (adapted from Markby *et al*., 2017)

Grade	Description
0	Normal valve
1	Small nodules become apparent in the free edge of the leaflet, in small numbers, and all the valve is majorly translucent
2	Less translucent valve with bigger nodules
3	Coalescent nodules become visible
4	Nodules in the *chordae tendinae* and markedly ticker, making possible the ruptures of the *chordae.* The leaflet becomes distorted and ballooning

On the other hand, it is also possible to categorise the disease in four stages according to the ACVIM Consensus Guidelines from 2019: A, B, C and D, in increasing order of severity (Keene *et al*., 2019), as presented in Table 2.

Table 2: Staging of MMVD

Stage	Description
A	Includes all the dogs in high risk of development of this condition, but with no sign of structural change when clinical examined by the assistant veterinarian. The CKCS, a breed with high prevalence of MMVD, fits in this as an example (Keene *et al*., 2019)
B	Includes dogs with a heart disorder consistent with MMVD but without any suggestion of CHF (Keene *et al*., 2019). This group can be further divided in B1 and B2.
B1	No indication to start medical treatment, having mitral valve regurgitation but without any symptoms (Boswood *et al*., 2016, 2018; Keene *et al.*, 2019)
B2	Have indication to start medical treatment, with signs of left chamber enlargement enough to start therapy as an attempt to delay the onset of clinical signs of CHF (Boswood *et al.*, 2016, 2018; Keene *et al*., 2019). To be classified as a stage B2, there are some particularities on the medical exams that have to be present to classify the heart enlargement, such as a grade 3 or higher heart murmur (Keene *et al*., 2019), a left atrial to aortic root ratio (LA:Ao) >1.6, accessed during echocardiography in a right-sided short axis during early diastole (Hansson *et al*., 2002), left ventricular internal diameter in end-diastole (LVIDD) (normalise for body weight) ≥ 1.7 (Cornell *et al.*, 2004; Keene *et al*., 2019) and a Vertebral Heart Size (VHS), adjusted for the body weight, of >10.5 (Keene *et al.*, 2019).
C	Dogs with CHF, or previously CHF-diagnosed but responsive to the standard treatment for heart failure. A dog that as had an episode of cardiac failure and has recovered, is still in stage C. The only way to go back to stage B, is with a mitral valve repair surgery, and even then is just in very special cases (Keene *et al*., 2019).
D	Dogs with CHF and clinical signs non-responsive to treatment (Keene *et al.*, 2019)

7. Diagnosis

The clinical history of the animal is an important feature, where every relevant information should be assessed. In some dogs, a heart murmur can be discovered in a normal primary health care appointment (Hezzell, 2018), but in others, for example geriatric dogs, the sound of a left apical holosystolic murmur can be almost pathognomonic of MMVD (Côté *et al.*, 2015; Menciotti & Borgarelli, 2017).

A very important part of the diagnostic process is the estimation of the severity of the disease. For such propose it is essential to evaluate the animal´s history, physical examination and imaging diagnostic methods (i.e. thoracic radiographs and echocardiography) (Menciotti & Borgarelli, 2017).

7.1 Thoracic Radiography

A thoracic radiography should be made on the first suspicion of the presence of MMVD, even in an asymptomatic animal. This exam has numerous benefits, including or excluding other causes (Keene *et al.*, 2019). Furthermore, as mentioned above, small breeds also have predisposition for tracheal and bronchial diseases, so in a case of an animal that presents cough, these conditions should be excluded. Another key factor for using the thoracic radiography is to have a first impression of how the disease is affecting the animal and so be able to evaluate the progression. The thoracic radiography is the election method to evaluate heart enlargement by means of VHS and vertebral left atrium size (LVAS) when echocardiography is not available (Keene *et al*., 2019), and can also be used to measure the radiographic left atrium dimension (RLAD) (Lam *et al.*, 2021).

The capacity to assess the heart dimensions was described by Buchanan and Bücheler (1995). The authors proposed a method of measuring the VHS, that compares the dimensions of the heart with the length of midthoracic vertebrae and sternebrae (Buchanan & Bücheler, 1995). The conclusion was a good correlation between these two parameters in thoracic radiographs on lateral views, permitting the follow-up of cardiac disease that include cardiomegaly (Buchanan & Bücheler, 1995). The proposed upper limit for a normal dimensioned heart was <10.5 vertebras, but the authors also assumed some differences between breeds. For example, in dogs with a short thorax the upper limit is probably higher (11 vertebras) and in long thorax dogs this limit will be decreased (9.5 vertebras) (Buchanan & Bücheler, 1995). The assessment of heart size is extremely important in the decision to when start the medical treatment (Poad *et al*., 2020).

Table 3: Normal VHS for different dog breeds. (Adapted from Lamb *et al.*, 2001)

Breed	Normal Vertebral Heart Size (VHS) (v)
Boxer	10.3 - 12.6
Labrador Retriever	9.7 – 11.7
CKCS	9.9 – 11.7
German Shepherd	1.7 – 11.2
Dobermann	9.0 – 10.8
Yorkshire Terrier	9.0 – 10.5

(v, vertebrae)

More recently, Poad *et al.* (2020) evaluated the utility of VHS and VLAS to the prognosis of the echocardiographic criteria for left heart augmentation. The comparisons were made using Standard 2-D, M-mode and Doppler on the echocardiographic examination, besides thorax radiographs in three different views (Poad *et al.*, 2020). On the echocardiography different measures were performed: the LVIDD, left ventricular internal diameter at end-systole (LVIDS) and LA:Ao. Thoracic radiographs determined both VHS and VLAS. All those measures were compared, and the authors concluded that the VHS was clinically advantageous to ascertain left heart enlargement at echocardiography in dogs with MMVD in an asymptomatic phase. They found that dogs with VHS <10.8 were unexpected to have left heart enlargement and, on the other hand, 91% of the dogs with VHS >11.7 had left heart enlargement (Poad *et al*., 2020). Nonetheless is important to mention that interbreeds variations may alter some of these results, and further studies are needed (Poad *et al*., 2020).

Lam *et al.* (2021) reported a new method of measuring left atrium on radiographs to qualify this chamber enlargement in dogs with MMVD (through a LA:Ao ≥1.6). The study included healthy dogs, as well as animals in the stages B1, B2 and C of MMVD. The new measure was called Modified-VLAS (M-VLAS) and is quantified firstly from the centre of the most ventral feature of the carina and lengthened to the joining of the most caudal aspect of the left atrium and the dorsal border of the caudal vena cava. The second measure is made from the most distal border of the left atrium and stretched to intersected with the first line, in a perpendicularly manner. These two lines are then converted to number of vertebras from the T4 and caudally (Lam *et al*., 2021). Besides the M-VLAS measured, the authors also include VLAS, VHS, RLAD and LA:Ao. At the end of the study the conclusion was that the M-VLAS cut-off of ≥3.4 vertebra presented a sensitivity and specificity of 93% in the prediction of left atrium enlargement assessed by the quantification of the LA:Ao ≥1.6, being superior than the VHS measurement (Lam *et al*., 2021).

7.2 Systemic blood pressure

It is important to determine the baseline typical values of systemic blood pressure of an animal, and in this way track the progression of this parameter during the course of the disease (Keene *et al.*, 2019).

7.3 Cardiac biomarkers

The serum concentration of N-terminal pro-B-type natriuretic peptide (NT-proBNP) is also of great importance. The B-type natriuretic represents a potential cardiac disease biomarker (Oyama *et al.*, 2008). Oyama *et al.* (2008) conducted a study evaluating the serum concentration of NT-proBNP and its impact in cardiac disease and severity (Oyama *et al.*, 2008). The results were very different between healthy and cardiac dogs. A serum concentration higher than 445pmol/L was observed in dogs with MMVD. Values higher than 680pmol/L were predictors of heart enlargement whereas higher than 1725pmol/L were associated with CHF (Oyama *et al*., 2008).

7.4 Echocardiography

Among the diagnostic techniques available in the clinical practice, echocardiography is suggested to clarify the cause of the murmur (Keene *et al*., 2019). It is also important to confirm the mitral regurgitation (Menciotti & Borgarelli, 2017), and to evaluate the correct size of the left atrial and ventricular chambers (Poad *et al.*, 2020). With an experienced echocardiographer it may be possible to discover pulmonary hypertension and high left atrial pressure, which are haemodynamic irregularities common in MMVD. Nonetheless, it is essential to consider the breed specificity of these parameters (Keene *et al.*, 2019).

In the presence of mitral regurgitation, assessed by doppler, it is visible a regurgitant jet at a great speed, in the systolic part of the cardiac cycle. It has its origin in the left ventricle, and gains velocity in the mitral valve area, going in direction of the left atrium (Menciotti & Borgarelli, 2017).

Strohm *et al.* (2018), identified three variables to assess left ventricular and atrial chambers enlargement with a two-dimensional, long axis echocardiography (Strohm *et al*., 2018). The ratios used were Left Ventricle:Aorta (LV:Ao), Left Atrium:Aorta (LA:Ao) and Left Atrium:Left Ventricle (LA:LV) (Keene *et al.,* 2019; Strohm *et al.,* 2018).

Recently, Osuga *et al.* (2021) reported an echocardiographic estimation of left ventricular-arterial coupling (VAC) in dogs with MMVD (Osuga *et al.*, 2021). The VAC represents the relation between the left ventricle and the systemic arterial system, being associated with the injection efficacy of the left ventricle. The goal of the study was to find out if an inadequate VAC, estimated by parameters on the echocardiography, was associated with a severe stage of MMVD. Thus, dogs in stages B1, B2 and C were enrolled in this study, besides healthy dogs, as controls (Osuga *et al*., 2021). The echocardiography parameter was, besides others, the ratio effective arterial elastance (Ea):left

ventricular end-systolic elastance (Ees). In stage B2 dogs, the ratio Ea:Ees was increased mainly by a decrease in Ee, which suggests a ventricular systolic dysfunction. In stage C dogs, the ratio Ea:Ees is increased because of an increase in Ea and a decrease in Ee, suggesting both a dysfunctional left ventricle systolic function and an increase in the total amount on arterial blood in the left ventricle. The results in both stages suggest that a deterioration of the systolic ventricular function occurs as the disease progresses. Also the initial assumption of a correlation of the VAC with the severity of the diseases, was confirmed (Osuga *et al*., 2021).

7.5 Electrocardiography

The trace of the electrocardiography (ECG) can be suggestive of left heart enlargement. The P-*mitrale* wave may be present, in animals with left atrial enlargement, and ST segment that show depressions or slurring are suggestive of left ventricle enlargement (Bonagura & Twedt, 2009).

Atrial and supraventricular arrythmias can be present, the most common being premature atrial depolarizations. Ventricular arrythmias are uncommon. Atrial fibrillation, sinus tachycardia, supraventricular and ventricular premature complexes and sustained supraventricular tachycardias are often present in the last stages of the disease (Bonagura & Twedt, 2009; Nelson & Couto, 2014; Ware, 2011).

Although the variations previously mentioned can be present, in a high number of animals the trace is completely normal (Nelson & Couto, 2014; Ware, 2011).

8. Treatment

8.1 Medical Treatment

According to the ACVIM Guidelines Stage, the treatment varies according to the stage of the disease (Table 4).

Table 4: Clinical Management of MMVD

MMVD Stage	Clinical Management	Diet
B1	• Dogs should be clinically examined on a regular basis (6-12 months). The frequency will depend on the changes found on the last exam performed (Hezzell *et al.*, 2012; Keene *et al.*, 2019).	
B2	• The drug treatment should only begin in this stage (Keene *et al*., 2019)e, as demonstrated by a variety of studies (Borgarelli *et al*., 2020; Boswood *et al.*, 2016, 2018). • Pimobendan contributes with a positive inotropic effect and as a vasodilator, acting as an improver of damaged cardiac function (van Meel & Diederen, 1989). It should be use *per os* at a dosage of 0.25-0.3 mg/kg/day (Boswood *et al.*, 2016, 2018; Keene *et al*., 2019). • Spironolactone with Benazepril can be used in asymptomatic dogs to decrease the cardiac rearrangement (Borgarelli *et al.*, 2020).	• The diet is also of great importance. • At this stage, it should be provided a high palatable diet, to prevent any decrease in intake, and contribute to the maintenance of the body condition (Freeman *et al*., 2006; Keene *et al*., 2019). • Studies have also showed that a reduction of sodium in the diet, associated with supplements such as antioxidants, n-3 fatty acids, carnitine, taurine and arginine have a positive effect, such as a reduction in left ventricle and atrium size, and in an increase circulation concentration of a number of essential compounds (Freeman *et al*., 2006)

Table 4: Clinical Management MMVD (continued)

MMVD Stage	Clinical Management	Diet
C	• Dogs in a controlled phase of CHF, should be treated at home (Keene *et al.*, 2019). It should be administered Furosemide, a diuretic, *per os,* in a dosage of 2mg/kg/q12h, knowing that if needed, this should be adapted according to the animal (Keene *et al*., 2019). It is important to underline, that a biochemical control should be made, with measurements of serum concentrations of creatinine, blood urea nitrogen and electrolytes, 3-14 days after starting this medication (Keene *et al*., 2019) • It is also important to start an angiotensin-converting enzyme inhibitor (ACEI). Recommendations are for Enalapril or Benazepril at a dosage of 0.5mg/kg/q12, *per os*. It is essential to undertake a kidney function control regularly, with serum concentrations of creatine and electrolytes, because of the risks of development of acute kidney injury (Keene *et al.*, 2019). • Spironolactone has, also, an important part at this stage. In a previous study, it was reported that the use of this drug, along with the rest of the therapy, has a role in decreasing the risk of cardiac-related death and euthanasia for cardiac reasons (Bernay *et al*., 2010). It has been hypothesized that it is not only the diuretic potential of Spironolactone that is important in this case, but the capacity for its counteractive effects on arterial changes and replacement fibrosis (Bernay *et al*., 2010). • It some cases, a cough suppressor and a bronchodilator can also be used, and in the presence of atrial fibrillation a combination of Digoxin with Diltiazem can be prescribed, and continue as the dog enters a Stage D (Keene *et al.*, 2019). Pimobendan should continue to be administered (Keene *et al*., 2019).	• On a CHF situation, it is frequent to find a loss of body mass, and is common for these dogs to develop cardiac cachexia (Bonagura & Twedt, 2009). The calories intake should be strictly controlled to avoid weight loss (60 kcal/kg of body weight (BW)) (Keene *et al.*, 2019), and make a reduction in sodium intake (Rush *et al*., 2000), giving also a sufficient amount of protein (Bonagura & Twedt, 2009).

Table 4: Clinical Management MMVD (continued)

MMVD Stage	Clinical Management	Diet
D	• When the animal reach the this stage, the Furosemide dosage should be increased to provide comfort and decrease any accumulation of fluids (Keene *et al*., 2019). If Furosemide is no longer effective, another diuretic can be considered, such as the Torsemide (Oyama *et al*., 2011). • Spironolactone should continue. If the animal is not being medicated with this drug, it should begin (Keene *et al*., 2019). • Pimobendan can be increased for three times a day, at a 0.3 mg/kg dosage (Keene *et al*., 2019). • As a cough suppressant, with beneficial haemodynamic effects, Amlodipine or Hydralazine can be prescribed (Keene *et al.*, 2019). • Although not demonstrating a significant decrease in pulmonary hypertension (PH), Sildenafil can be used when PH is present, as it demonstrated an improvement of the clinical condition and the quality of life (Kellum & Stepien, 2007). The recommended dosage is 1-2 mg/kg/q8h, *per os* (Keene *et al*., 2019).	• The diet should be equal to that recommended to a Stage C dog. Nonetheless in animals with fluid accumulations, the decrease of sodium should be more intensive (Keene *et al*., 2019).

The importance of using Pimobendan in dogs with cardiomegaly, but without evidences of CHF, was first demonstrated by the EPIC Study – A Randomized Clinical Trial (2016), in a prospective, randomized, placebo-controlled, blinded and multicentre clinical trial (Boswood *et al*., 2016). The results showed a gain of 15 months until the establishment of CHF or cardiac-related death, when compared to dogs in the same initial conditions, but treated with placebo, expanding the preclinical phase of the disease (Boswood *et al*., 2016).

The Epic Study (2018) was aimed to understand the clinical and imaging variables in dogs in Stage B2 treated with Pimobendan in opposition to dogs treated with placebo. It was also evaluated the progression from Stage B2 to Stage C, and possible differences between dogs treated with Pimobendan compared to non-treated dogs. The results showed a decrease in heart size among the Pimobendan group, giving these dogs more time until the onset of CHF or cardiac related death. Nonetheless, once the CHF was installed, both animals with Pimobendan or placebo showed no difference in quality of life, radiographic and echocardiographic variables as well as laboratory findings. With the onset of CHF, both groups were identical, with no differences between them (Boswood *et al*., 2018).

The Delay Study (2020) evaluated the effects of using Spironolactone with Benazepril, an ACEI, in Stage B2 asymptomatic dogs, and the effects this drugs had in delaying the onset HF (Borgarelli *et al.*, 2020). Despite the negative result in the delaying of the clinical sings, this study showed an apparent efficacy in reducing the cardiac rearrangement, showed by imaging variables and the NT-proBNP (Borgarelli *et al.*, 2020).

8.2 Surgical Treatment

Although the medical treatment plays an important role in controlling the clinical signs and in the delay of death for cardiac reasons or euthanasia, it plays no role in the process to CHF, which is the MR due to valvular dysfunction (Borgarelli *et al.*, 2017; Menciotti & Borgarelli, 2017; Uechi, 2012). The final goal of a surgical approach is to decrease or eliminate the MR, by means of a restored valve coaptation and replacement of damaged *chordae tendinae* (Borgarelli *et al*., 2017; Menciotti & Borgarelli, 2017).

The main problem of the mitral valve surgery is the high monetary cost and the need of an specialized team, not available in common primary care veterinary centres (Borgarelli *et al*., 2017).

The mitral valve can be replaced or repaired (Uechi, 2012), with some techniques requiring cardiopulmonary bypass (CPB) (Takashima *et al.*, 2007; Uechi, 2012) and others where it can be made on a beating heart (Uechi, 2012).

The CPB is performed with an extracorporeal circuit, connected to a heart and lung machine, that provides oxygenation and changes in body temperature. Decrease of the body temperature of the animal is important to reduce the demand of oxygen and preserve the peripheral tissues (Uechi, 2012).

During CPB the anaesthesia can be made by a continuous rate of fentanyl and propofol, instead of an inhalant agent (Uechi, 2012)

The mitral valve replacement (MVR) can be carried out by use of mechanical or bioprosthetic valves (Takashima *et al.*, 2007). The big disadvantage of using a mechanical valve is the need of a chronic anticoagulation therapy due to the high thrombogenicity potential associated, contrary to the bioprosthetic valves (Takashima *et al*., 2007). Although, these valves have a higher durability time, due to the high probability of development of thrombosis, the life expectancy in dogs that are submitted to this surgery is reduced (Takashima *et al*., 2007).

Bioprosthetic valves, from a porcine donator, are recommended for dogs. These valves are cross-linked with glutaraldehyde and polyepoxy compounds (Takashima *et al.*, 2007), reducing the thrombogenicity and calcification with increased durability (Okoshi *et al*., 1990). Their surgical implantation requires a CPB (Takashima *et al.*, 2007).

Takashima *et al.* (2007) reported the short-term performance of porcine aortic valves, aseptically obtained from a slaughterhouse and close in dimensions with canine mitral valves. The dogs implanted were perfectly healthy. The valves were treated according to the therein described protocol (Takashima *et al*., 2007). To perform the surgery, the dogs were placed in CPB, and the assess was made by means of a thoracotomy in the fifth intercostal space, on the left side, which complicated the CPB procedure (Takashima *et al.*, 2007). All the mitral valve and *chordae tendinae* were withdrawn by an incision in the left atrium and the bioprosthetic valve was set down in the mitral annulus (Takashima *et al*., 2007). The left coronary cusp was aimed to the left ventricle outflow tract (Takashima *et al.*, 2007). As the first days after surgery are the more critical ones, the function of the bioprosthetic valve was evaluated seven days after surgery. The implanted valve was considered normal, with no acute regurgitation of the implanted valve or thrombogenesis (Takashima *et al.*, 2007). As a result, in a short-time evaluation, these valves seems to be efficient in dogs, using the technique described (Takashima *et al.*, 2007).

Beside the replacement, the valve can also be repaired. The circumferential mitral annuloplasty and artificial *chordal* replacement are important procedures that grant durability and long-time clinical advances, being one of the preferential mitral valve repair techniques, with the assess made in the left atrium (Uechi, 2012). Another advantage is that there is no need for antithrombotic therapy, making this surgery a feasible and important option for dogs affected by MR resulting from MMVD (Mizuno & Uechi, 2020; Uechi, 2012). This surgery requires a non-beating heart with no blood in the surgical field, achieved by means of a cardioplegia (Uechi, 2012).

In a situation of *chordae tendinae* rupture, the best surgical approach is the *chordae* replacement (Uechi, 2012). The most difficult part of the procedure is the assessment of the correct length of the *chordae*, which should equal the contralateral *chordae*. In the absence of equal sized *chordae*, the coaptation of the valve is not efficient (Uechi, 2012). This difficulty can be surpassed by placing the *chordae* in a beating heart, using techniques that do not require CPB (Borgarelli *et al*., 2017). One of

the most effective methods is the use of a double-armed expanded-polytetrafluoroethylene (ePTFE) suture. This will be placed in the lateral side of the septal portion of the leaflet and the craniolateral papillary muscle, returning to the mitral leaflet, terminating this way the implantation of one *chordae* (Uechi, 2012). On the papillary muscle a pledget should be used, to decrease the risk of rupture (Uechi, 2012). The procedure should be repeated two more times, one in the medial side of the septal leaflet and caudomedial papillary muscle and another in the mid-side of the mural leaflet and caudomedial papillary muscle (Uechi, 2012). The length of the *chordae tendinae* are corrected by means of a temporary Alfieri stitch (Uechi, 2012).

More recently, novel surgical techniques have been reported, characterized by being less invasive, simpler and with no need of CPB, being performed in a beating heart (Borgarelli *et al*., 2017). One example of these techniques is the Harpoon Medical TSD-5 device, capable of repair the mitral valve by transapical approach. The repair requires the implantation of ePTFE artificial *chordae* (Borgarelli *et al.*, 2017). Borgarelli *et al*. (2017) studied the efficacy of this device, implanting the artificial *chordae* in six healthy Beagles. They reach concluded that this device represents a possible way to replace the ruptured *chordae tendinae* and that thirty days after surgery, initial signs of the synthetic chords endothelization could be seen (Borgarelli *et al.*, 2017). Nonetheless, in dogs that present a critical annular dilatation, this procedure is not recommended, because there is no annuloplasty performed (Borgarelli *et al*., 2017).

The annuloplasty should be performed, to enhance the durability of the procedure (Gillinov *et al.*, 2009). A prosthetic device helps to maintain the valve coaptation and decrease the risk of post-surgery annular dilatation (Gillinov *et al*., 2009). However, the prosthetic devices are too big for smaller breeds (Uechi, 2012), which are in fact the more frequently affected by the disease (Keene *et al*., 2019). In such cases, the best solution is the use of a prosthetic ring made of ePTFE material, that can be shaped in size according to the size of the dog (Uechi, 2012). Regardless of the annuloplasty technique used, the physiological motions of the mitral valve should be preserved (Uechi, 2012). Uechi (2012), suggested as the best approach, the placement of a plication suture, with pledgets, in the cranial and caudal commissure of the annulus and then 5-0 sutures in the annulus passed through a strip of ePTFE material. Both sutures should be tightened to seat a annuloplasty ring (Uechi, 2012). This method is also important, since it reduces the risk of thromboembolism because of contact of cardiac blood with an extern material (Mizuno & Uechi, 2020; Mizuno *et al.*, 2013).

After the valve repair, the dog should undergo an improvement of the clinical signs (Uechi, 2012). The murmur should decrease significantly, VHS on the thoracic radiographs should be smaller and mitral regurgitation on the echocardiography should became less pronounced (Uechi, 2012). A major advantage of this surgery is the reduced need of medical therapy (Uechi, 2012)

The ValveClamp (Hongyu Medical Technology, Shanghai, China) is a new trans-catheter edge-to-edge mitral valve device, firstly experimented in a porcine model, and performed in a beating heart (Liu *et al*., 2020; Pan *et al.*, 2019). Liu *et al.* (2020) performed a study regarding the utility of such device in

dogs diagnosed with MR as the result of MMVD, all in B1 stage (Liu *et al.*, 2020). The protocol was the same used in the porcine model (Pan *et al*., 2019), involving an access in the apex of the heart and introduction the device until the left atrium. The device was then pulled back, so the proximal extremity could enter the left ventricle. After this, the two clamps of the device were tightened, in a way that the mitral leaflets were both entrapped. If the valve was correctly placed, the device guide was retreated and the clamps maintained in place (Liu *et al.*, 2020; Pan *et al.*, 2019). The results of the procedure, evaluated three months after surgery, were the absence of audible murmur in all the dogs and no mitral regurgitation in 87.5% of the dogs (Liu *et al*., 2020). This outcome, suggests that this technique is applicable to dogs with MMVD, as an effective clinical treatment, at least in dogs in this B1 stage (Liu *et al.*, 2020).

Another option of surgery is the decrease in left atrial pressure (LAP) by means of an acquired atrial septal defect, reported by Allen *et al.* (2021) in dogs at advanced stage of MMVD and left-sided CHF (Allen *et al*., 2021).This defect was made with the use of angiography, and guided by transoesophageal echocardiography (Allen *et al*., 2021). The final result was a decrease in LAP, with an right atrium pressure rise, but with no clinical complications (Allen *et al.*, 2021). The clinical importance of this procedure is the potentially improved quality and expectancy of life, and the feasibility of the surgery (Allen *et al*., 2021).

8.3 Anaesthetic Management

The anaesthetic protocol should be chosen individually, considering all the medical characteristics of the animal. Despite having MMVD, the dogs with this disease can present other comorbidities (Grubb *et al*., 2020; Mama & Ames, 2016). The dog should be closely monitored during all the procedure. Bradycardia and situations of hypo or hypertension should be avoided, because the heart with mitral degeneration is not able to use compensatory mechanisms in these conditions (Haskins, 2011).

An important parameter to consider is the rate of fluid therapy. The normal rate in healthy dogs is 5mL/kg/hr, but this should be decreased in case of cardiac patients (Grubb *et al.*, 2020).

If the dog is medicated with Pimobendan or Furosemide, the animal should continue them in the day of the anaesthesia. Nonetheless, ACEIs should be paused on the intervention day (Grubb *et al.*, 2020)

Nonetheless, there are some categories of drugs that should, if possible be avoided. For example α2-agonists increase the resistance of the systemic vascular system, which can affect the mitral regurgitation, promoting its increasing (Mama & Ames, 2016).

Situation of elective surgeries in dogs in situations of severe heart failure should be delay (Mama & Ames, 2016).

The maintenance during the surgical procedure, can be made with an inhalant agent, but only in mild to moderate disease patients (Mama & Ames, 2016). Whenever possible, locoregional anaesthesia should be used, in an attempt to reduce the drugs used for pain control (Haskins, 2011).

9 Prognosis

The leading predictor of cardiac death is a left atrial enlargement, even if mild (Borgarelli *et al.*, 2012).

It is reasonable to assume that more severe mitral regurgitations are associated with poorest prognosis, with the risks of congestive cardiac failure and death in younger ages. Nonetheless this was never clearly demonstrated (Sargent *et al.*, 2015).

On echocardiography, important prognostic indicators are left atrial and ventricular enlargement and increased E wave velocity. The severity of the valve prolapse, and consequent MR is also important (Hezzell, 2018). Visualization of atypical mitral morphology and function are also indispensable to assess the prognosis (Sargent *et al*., 2014).

Increased mortality in dogs with MMVD is associated with mitral E wave velocity (>1.2m/s), intensity of valve prolapse and a flail mitral leaflet (Borgarelli *et al.*, 2008; Sargent *et al.*, 2014). The presence of increased mitral E wave velocity with associated cough, are a strong predictor of heart failure (Borgarelli *et al.*, 2012), as are episodes of syncope and a LA:Ao > 1.7 (Borgarelli *et al.*, 2008).

The time elapsed until the onset of CHF is also an important feature in delaying the cardiac related death or euthanasia (Bernay *et al.*, 2010; Boswood *et al.*, 2016, 2018).

The Epic Study, from Boswood *et al.* (2016) showed that dogs treated with Pimobendan have 15 additional months until the onset of CHF or cardiac related death, when compared to dogs receiving placebo. The dogs receiving placebo had a median of 766 days until CHF and the Pimobendan group a median of 1228 days (Boswood *et al*., 2016).

There are some key points that both the owner and the assistant veterinary should be watchful, to extend the time until the onset of CHF, besides the medical therapy as follows: the sodium intake should be decreased, the exercise should be restricted and finally procedures with general anaesthesia and treatment with glucocorticoids should only be made in case there are no alternatives (Bonagura & Twedt, 2009).

Final Remarks

Although some facts are, currently, very well established, further studies will be necessary to the total understanding of the disease. The predisposition is well established, with dogs under 20kg of body weight, males and some breeds like the CKCS been identified as more predisposed than other individuals (Keene et al., 2019). Neverthless no definite conclusion about the aetiology has been confirmed to the present day (Bionda *et al.*, 2020; Birkegård *et al.*, 2016; Ljungvall *et al*., 2013; Madsen *et al.,* 2011; Markby *et al*., 2020; Menciotti & Borgarelli, 2017; Parker & Kilroy-Glynn, 2012).

The aetiology of this disease is still under study, nonetheless a number of possibilities have been reported, such as, the two *loci* identified in the CKCS, the small pool of individual that reproduce in one breed, the role of the serotonin and a number of genes that have been identified as a possible explanation for the onset of the disease (Bionda *et al.*, 2020; Birkegård *et al*., 2016; Ljungvall *et al.*, 2013; Madsen *et al.*, 2011; Markby *et al*., 2020; Menciotti & Borgarelli, 2017; Parker & Kilroy-Glynn, 2012).

The events that lead to the last stage of the disease, the congestive heart failure, are also, well established (Hezzell, 2018), and the medical treatment options for the delay of this stage, have been, in the last years the aim of many studies (Borgarelli *et al.*, 2020; Boswood *et al*., 2016, 2018).

The main area for further research, is possibly the surgical treatment. Despite the number of techniques described and the reported studies (Allen *et al.*, 2021; Borgarelli *et al*., 2017; Liu *et al.*, 2020; Menciotti & Borgarelli, 2017; Mizuno & Uechi, 2020; Mizuno *et al*., 2013; Takashima *et al*., 2007; Uechi, 2012), it is still a procedure that is only carried out in a reduced number of veterinary centres. The main underlying reasons are the high surgical cost and the need of specialized teams (Borgarelli *et al.*, 2017).

Considering that MMVD is the most common heart disease in dogs, it is essential to continue to pursue and study additional strategies to delay clinical signs and animal death, as well as improve the quality of life of affected dogs.

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