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Update on degenerative mitral valve disease

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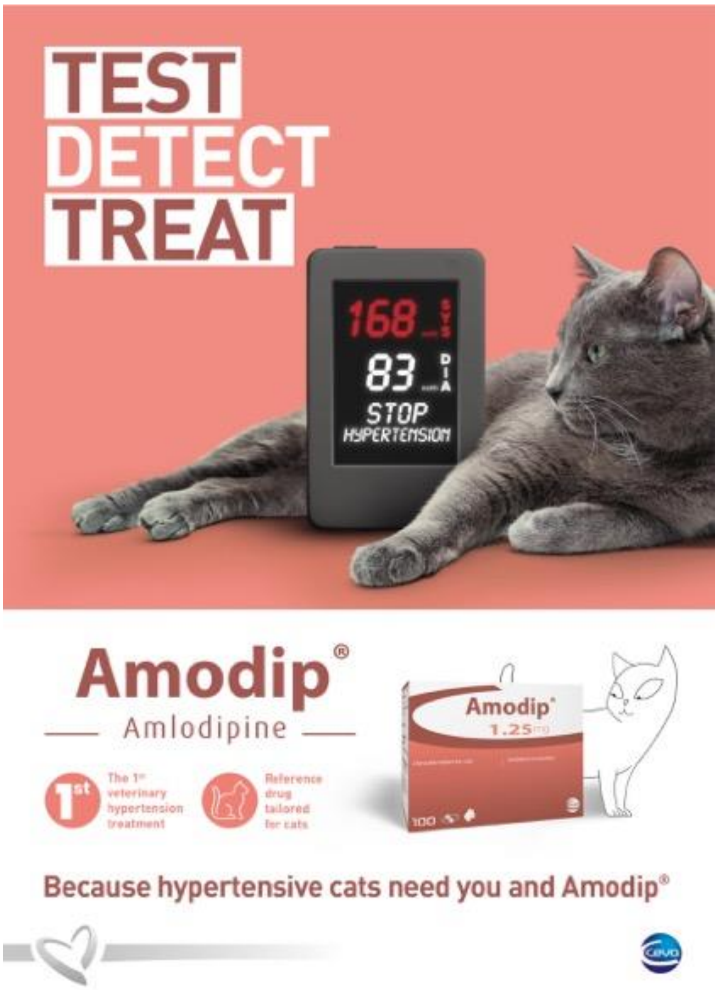
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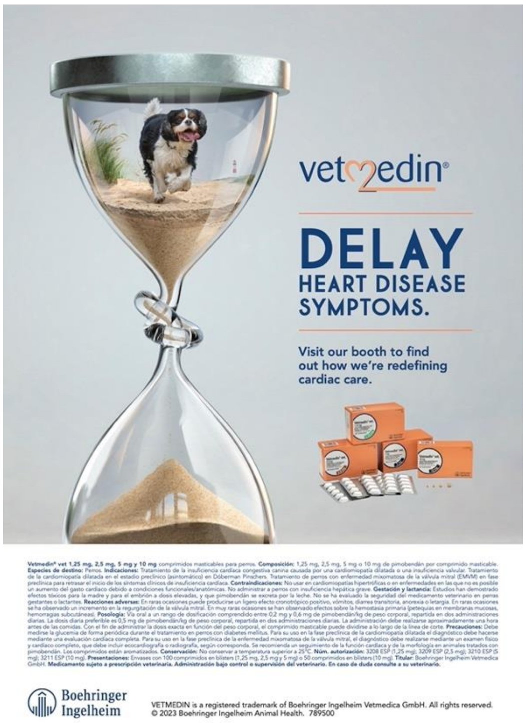
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Pre-congress program
Update on Degenerative Mitral Valve Disease

Time	Title	Speaker
9:00 - 9:30	*Registration*	
9:.30 – 10:10	Update on staging, what is a real B2?	A. Boswood
10:15 – 10:55	Predictors of outcome and progression in canine MMVD: Results from LOOK-Mitral study	M. Borgarelli
11:00 – 11:30	Coffee break	
11:30 – 12:10	Mitral valve disease: are we on the cusp?	J. Culshaw
12:15 - 12:55	Mitral valve morphologic analysis using echocardiography	G. Menciotti
13:00 - 14:00	Lunch	
14:00 - 14:40	Beyond the valve: why does mitral valve disease progress?	M. Hezzell
14:45 - 15:25	Update on Biomarkers and use in myxomatous mitral valve disease	J. Dukes-McEwan
15:30 - 16:00	Coffee break	
16:00 – 16:40	The usefulness of 3D TEE in mitral regurgitation in human	F. Faletra
16:45 – 18:00	Panel discussion	All Speakers
18:00	*End of the meeting*	

Update on staging, what is a real B2?

Adrian Boswood
Professor of Veterinary Cardiology, The Royal Veterinary College, London

Why do we classify patients with disease into stages?

Each patient with a disease is unique and many disease states tend to represent a continuous spectrum from the mildest of disease to the most severe. Any form of staging of disease is necessarily discontinuous; stages are considered distinct and ideally should not overlap. Stages of disease group together patients who share characteristics similar to each other. Those characteristics can be used to distinguish them from other subjects affected by the same disease. There are many ways in which this is convenient. It is particularly useful when it allows practitioners to distinguish groups with clinically useful differences – for instance differences in prognosis, differences in therapy or differences in how a patient's disease should be monitored. There is also a convenience in staging a patient's disease that improves communication between practitioners, for instance communication between cardiologists, or between practitioners and the owners of the patients they examine. One sign of a successful classification system is that it is widely adopted. Of the three classification systems that I have seen used in my career (modified NYHA, ISACHC and ACVIM) the ACVIM system is by far the most widely adopted and well understood – I believe this is a sign of its usefulness and the ease with which it can be understood.

Despite the clear value of this system, in its short lifetime the ACVIM stage B sub-classification has already undergone subtle redefinition and has resulted in considerable debate. The ACVIM classification was first published in 2009 [1] and was based on the classification proposed by the American College of Cardiology and American Heart Association [2]. In the first iteration of the guidelines Stage B1 was defined as consisting of "asymptomatic patients that have ***no*** radiographic or echocardiographic evidence of cardiac remodeling in response to CVHD". The 2019 guidelines [3] stated "Stage B1 dogs are characterized by a spectrum of imaging findings ranging from those with radiographically and echocardiographically normal left atrial [LA] and ventricular [LV] dimensions with normal LV systolic function and normal radiographic vertebral heart or VLAS to those with echocardiographic or radiographic evidence of left atrial and ventricular enlargement that does not meet specific criteria outlined below." This small change means that dogs that

might have been classified as stage B2 in the original 2009 classification are now considered to be B1 in the 2019 classification.
The criteria that were adopted to define what now constitutes a stage B2 dog in the 2019 guidelines [3] are broadly based on the inclusion criteria that were used in the EPIC trial [4] (and the DELAY study [5]). This is a very pragmatic redefinition, particularly if one of the main reasons for grouping dogs together is to identify sub-populations that will benefit from a specific therapy.
The use of the EPIC criteria, their combination and their method of acquisition has led to discussion and debate which can broadly be considered under several headings

- How and why were the EPIC criteria chosen?
- Do all dogs meeting those criteria or only some of them benefit from therapy?
- Do any dogs not meeting those criteria benefit from therapy?
- Are the EPIC criteria too lenient and therefore might dogs without cardiac enlargement end up on therapy?
- Are the EPIC criteria too strict and therefore are dogs that might benefit from therapy being denied it?
- Are there more effective criteria that could be used?

<u>How and why were the EPIC criteria chosen?</u>
The purpose of the EPIC inclusion criteria was to identify dogs with enlarged hearts secondary to MMVD. This was the population that we hypothesized would benefit from pimobendan therapy. At the time the only published study with a similar aim was the VetProof trial [6] which had used an LA:Ao ≥ 1.6 as the sole echocardiographic heart-size criterion (in addition to radiographic cardiomegaly, but no proposed radiographic cut-off). We were wary of using a single criterion – and particularly one prone to erroneous measurement and therefore included a second echocardiographic criterion, LVIDDN ≥ 1.7, based on a cut-off derived from a study of prognosis in dogs with MMVD [7]. This was combined with a VHS score of 10.5 – a relatively lenient score but one intended to improve specificity (i.e. make it less likely that a dog without cardiomegaly would be enrolled) when combined with the two other heart size criteria. It was never intended that any single heart size criterion would be used in isolation and we considered at the time that the LA:Ao criterion, of the three, was probably the most stringent.
<u>Do all dogs meeting those criteria or only some of them benefit from therapy?</u>

The EPIC study was designed to have adequate power to show a difference between groups on the basis of the estimated size of the treatment effect. It was not designed to have sufficient statistical power to allow reliable well-powered analyses of sub-populations recruited to the study. We were however able to show that there was no “interaction” between LA:Ao or LVIDDN and the treatment effect as estimated by the hazard ratio [4]. What that means is that dogs across the spectrum of heart size recruited to the study appear to benefit equally in terms of hazard reduction – relative risk reduction in the study appears to be the same irrespective of size, although the absolute hazard is greater for dogs with bigger hearts.

Do any dogs not meeting those criteria benefit from therapy?
This is perhaps the simplest question to answer – we don’t know, because they weren’t in the study!

Are the EPIC criteria too lenient and therefore might dogs without cardiac enlargement end up on therapy?
There have been several studies published that claim or appear to have shown that the upper limits of reference intervals for LA:Ao and LVIDDN of dogs without cardiac enlargement (or normal dogs) exceed the cut-offs used for identification of stage B2 dogs. There is therefore concern that dogs without cardiac enlargement may end up receiving treatment from which they will not benefit (or theoretically, by which they may be harmed). There is a difference between the upper limit of a reference interval and a diagnostic cut-off. By definition, the “specificity” for the identification of cardiomegaly of the upper limit of a reference interval will be 97.5% i.e. 97.5% of normal dogs will be below that limit. When deriving a cut-off there is always a trade off between specificity and sensitivity and therefore a cut-off may be lower than the upper limit of a reference interval. If the consequences of false negatives and false positives have roughly similar impact – then diagnostic cut-offs are usually optimised to approximately balance false negatives and false positives. It is also the case that when two cut-offs (or more) must be exceeded there is a built in “double-check” in the process which tends to prevent single erroneous or extreme measurements resulting in false positives.

Are the EPIC criteria too strict and therefore are dogs that might benefit from therapy being denied it?
To some extent this question has the same answer as the one above about dogs not meeting the entry criteria – we don’t know. However – data from a study we recently published describing a large population of dogs at stage B of MMVD [8] suggested that up to 25% of dogs fall into an

equivocal category where one echocardiographic heart size measurement exceeds the relevant EPIC inclusion criterion and the other does not. These findings tend to be borne out by other descriptions of large populations of stage B dogs [9]. Some of these dogs may be showing quite marked enlargement of their atrium or ventricle (relative to values previously obtained from the same dog or relative to proposed upper reference intervals) and yet they should not receive treatment according to the current staging criteria. With patients like these, as with many patients that fall outside populations precisely defined by clinical trial inclusion criteria, it may fall to the judgement of the individual clinician as to whether therapy should be initiated.

Are there more effective criteria that could be used?

I think we must distinguish frustration with measurement-based classifications in general, from frustration with the specific criteria that we currently use.

There are multiple sources of variability in any measurement. These can roughly be summarised as

- Variation between individuals (some of which, in dogs, may be associated with breed)
- Variation within individuals – biological variation due to factors such as hydration, heart rate, respiration etc.
- Variation attributable to methods of image acquisition.
- Variation attributable to methods of image measurement.
- Variation as a consequence of disease.

As clinicians we are most interested in variation as a consequence of disease and would like to be able to confidently conclude that when the measurement in a specific individual exceeds a given point, that individual's heart is enlarged. Unfortunately, no biological measurement will ever be free of other confounding sources of variation. The more reproducibly a measurement can be acquired and the greater the proportion of variability that can be attributed to disease the better that measurement is likely to be – but you will never be able to eliminate uncertainty, particularly in individuals with measurements adjacent to the cut-offs.

It is possible that more effective measurements will be identified in future and we should always strive to be better – however, given that the current cut-offs are pragmatic and based on there being a clinical trial that demonstrates effectiveness of treatment in a population of dogs identified that way, it will be necessary to show that new methods of measurement more effectively identify a population of dogs that benefit from treatment and not just that the new methods are more accurate or reproducible. This will be a considerable challenge.

References

1. Atkins, C., et al., *Guidelines for the diagnosis and treatment of canine chronic valvular heart disease.* J Vet Intern Med, 2009. **23**(6): p. 1142-50.
2. Hunt, S.A., et al., *ACC/AHA Guidelines for the Evaluation and Management of Chronic Heart Failure in the Adult: Executive Summary A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Revise the 1995 Guidelines for the Evaluation and Management of Heart Failure): Developed in Collaboration With the International Society for Heart and Lung Transplantation; Endorsed by the Heart Failure Society of America.* Circulation, 2001. **104**(24): p. 2996-3007.
3. Keene, B.W., et al., *ACVIM consensus guidelines for the diagnosis and treatment of myxomatous mitral valve disease in dogs.* J Vet Intern Med, 2019. **33**(3): p. 1127-1140.
4. Boswood, A., et al., *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, 2016. **30**(6): p. 1765-1779.
5. Borgarelli, M., et al., *DELay of Appearance of sYmptoms of Canine Degenerative Mitral Valve Disease Treated with Spironolactone and Benazepril: the DELAY Study.* J Vet Cardiol, 2020. **27**: p. 34-53.
6. Atkins, C.E., et al., *Results of the veterinary enalapril trial to prove reduction in onset of heart failure in dogs chronically treated with enalapril alone for compensated, naturally occurring mitral valve insufficiency.* J Am Vet Med Assoc, 2007. **231**(7): p. 1061-9.
7. Moonarmart, W., et al., *N-terminal pro B-type natriuretic peptide and left ventricular diameter independently predict mortality in dogs with mitral valve disease.* J Small Anim Pract, 2010. **51**(2): p. 84-96.
8. Wilshaw, J., et al., *Accuracy of history, physical examination, cardiac biomarkers, and biochemical variables in identifying dogs with stage B2 degenerative mitral valve disease.* J Vet Intern Med, 2021. **35**(2): p. 755-770.
9. Franchini, A., et al., *The Longitudinal Outcome Of Canine (K9) myxomatous mitral valve disease (LOOK-Mitral registry): Baseline characteristics.* J Vet Cardiol, 2021. **36**: p. 32-47.

MMVD – are we on the cusp?

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Introduction

If myxomatous mitral valve disease (MMVD) is the commonest heart disease in the dog, then it is also the commonest source of cardiac-derived case material, research material, and, let's face it, income. These features are prerequisites for securing funding for comprehensive, meaningful, and statistically rigorous laboratory studies. Frustratingly, though, while we bask in the success of the largest clinical studies conducted in veterinary medicine, relatively few centres are research-active in MMVD at the experimental level. My own contribution to MMVD research is, as yet, an incomplete story that incorporates discoveries relating to valvular function, pathological remodelling and contributions to morbidity and mortality from the vasculature and systemic disease. I hope that this talk illustrates the benefits of straying from the paths of veterinary and cardiac disease, and that translational research is bidirectional.

Significant advances have been made over the past three decades in our management of canine MMVD. Since we can be confident of a diagnosis of MMVD based on signalment and stethoscope, most of the early advances were in therapeutics rather than diagnostics. Indeed, when agents such as pimobendan first came on the scene, they were rather sceptically viewed because image-based evidence of systolic dysfunction was lacking. Over time, and as echocardiographic modalities advanced, a greater body of evidence supported the use of therapies that were already commonly prescribed. This approach of "placing the cartwheel before the horse" may have been particularly disadvantageous to funding the research required to understand and characterise the molecular pathogenesis of MMVD. However, significant further advances in delaying the development of stage C MMVD or prolonging quality of life once stage C is reached are going to be difficult to achieve by prescribing classes of agent already currently available to us. Dogs still die prematurely because of MMVD despite diuretics, RAAS antagonism and pimobendan. I believe that only through applying the laboratory and translational research of past and present colleagues at R(D)SVS, and other research centres, will we trial effectively and responsibly the latest agents licensed for use in people.

Mitral innervation

Back in 2005, when I started at the R(D)SVS, there was a strong body of opinion that myxomatous degeneration was initiated by valvular trauma. This theory, still held by many today, was attractive because it was consistent with the relationship between MMVD and ageing, and the areas of coaptation at the valvular tips where myxomatous changes are first observed and typically where they are most severe. Furthermore, it tapped into the notion that the mitral valve was a dynamic rather than a static structure that remodelled in response to changes in intracardiac haemodynamics and transvalvular stresses and shear forces, much the same way that other tissues do, such as bone. Work in sheep and rabbits had shown that the mitral valve would "unfurl" and stretch/lengthen in response to annular stretch caused by volume overload, and that haemorrhagic lesions appeared throughout the mitral valve apparatus following direct cervical vagosympathetic stimulation. This led me to hypothesise that the canine mitral valve was innervated and that disruption to this innervation increased the trauma that initiated myxomatous degeneration. Immunohistochemistry of mitral valves from adult dogs detected fairly dense innervation, predominantly sympathetic and within the proximal third of the mitral leaflet, coursing through the extension of cardiac muscle from the annulus and its supplying vasculature [1]. Additionally, mitral leaflets from older dogs had lost muscle, vasculature and innervation, and these had been replaced with adipose tissue. The study was underpowered for determining whether or not this was linked myxomatous degeneration and the age-independent features of mitral valvular muscle loss and its mechanical robustness remain to be determined.

Image analysis

Meanwhile, Prof. Brendan Corcoran and collaborators at the National University of Ireland had provided further evidence of remodelling in response to trauma through electron microscopic studies that served to define the surface morphological phenotype of MMVD [2, 3]. A key feature was loss of endothelial integrity and exposure of the subendothelial lamina. Neighbouring reactive endothelial cells appeared poised to divide and move to repair the exposed zone. Conventional microscopy, transcriptomics and immunohistochemistry undertaken by Richard Han [4, 5] identified proliferation and differentiation of normally quiescent mesenchymal valvular interstitial cells (VICs, the cells that produce the mitral matrix) into a more smooth muscle cell phenotype (α smooth muscle actin +ve) that migrated to the valvular tip and the initial site of myxomatous degeneration. Mojtaba Hadian demonstrated that coincidental with the altered valvular interstitial phenotype, there was a shift in the balance of matrix deposition and metalloproteinase activity that

led to a reduction in total collagen content but also a loss of post translational collagen processing , an increase in immature collagen content and myofibrillar disarray [6]. While an attempt to shore up the mitral valve through VIC proliferation and repair at a thickened tip appears appropriate, this loss of collagenous integrity within the valve is a little more difficult to understand and it remains to be determined whether this is a maladaptive response or a true primary feature of the disease. However, molecular phenotyping of these features by Fox Lu and Tina Liu [7-9], and more subtle characterisation of VIC differentiation have charted a course to possibly identifying new therapeutic targets.

Endothelin, the kidney and diabetes

I found the loss of endothelial integrity and migration of VICs intriguing. Endothelial cell division and migration is tightly regulated in a paracrine/autocrine manner by endothelin (ET-1). But the downstream effects of ET-1 are determined by the receptor subtype (A or B) that it binds to. A good example of this is the role ET-1 plays in the regulation of blood pressure (BP). In the vasculature, endothelial cells exposed to shear stress release ET-1, which stimulates nitric oxide release from endothelial cells via ET_B receptors. This in turn promotes endothelial-dependent vasodilation and is crucial to buffering the powerful vasoconstrictive effect of ET-1 through ET_A receptors on vascular smooth muscle cells (an interesting quirk of ET-1 is that it is the most powerful vasoconstrictor ever identified and yet it promotes vasodilation). In the kidney, ET-1 promotes natriuresis, again via ET_B receptors and nitric oxide release. Nitric oxide then inhibits sodium transport (absorption) through the epithelial sodium channel (ENaC). *Pedersen et al* [10] had already shown that the progression of MMVD in cavalier King Charles spaniels was associated with a reduction in circulatory levels of nitric oxide metabolites, and had characterised the expression of ET_B receptors in porcine mitral valves following stretch [11]. This provided further supporting evidence of a role for ET-1 in MMVD pathogenesis. And so I embarked on a PhD to learn more about this little 21-amino acid peptide. The project I designed was to investigate the role of ET-1 in the regulation of salt balance in a rat model of diabetes. At first sight, this might appear as detached from MMVD as you could hope to get, but it afforded me the opportunity to familiarise myself with the interplay between vascular function and sodium excretion in the kidney and the impact this could have on cardiovascular health. Type 1 diabetes bears striking similarities to congestive heart failure (CHF), with its incessant drive towards sodium and water reabsorption from the distal nephron, and this, in combination with loss of nitric oxide signalling, promotes premature death through nephropathy and cardiovascular disease such as atheromatous plaque formation and arterial calcification. Lesions appear in arteries in MMVD

too, such as the myxomatous lesions in pulmonary arteries [12], and there is increased risk of thrombosis and platelet dysfunction in CHF and particularly cavalier King Charles spaniels [13, 14].

One of the first findings from my PhD (funded by Kidney Research UK) was that Type 1 diabetes increases sodium and water retention by interfering with the kidney's normal mechanism of enhanced sodium and water excretion that is normally activated when BP is acutely increased (pressure natriuresis) [15]. Although Type 1 diabetics produce less urine under haemodynamic stress, I showed that the relationship between urinary sodium excretion and urinary excretion of ET-1 (a marker of renal ET-1 activity) was unchanged, and that interfering with one of the new generation of endothelin A receptor antagonists produced a marked sodium retentive effect in non-diabetic rats [16]. Back at the clinic, the cardiology team collected urine samples from dogs with cardiac disease ranging from stages B-C. When we measured urinary ET-1 in these samples, we found that urinary ET-1 levels increased in dogs with stage B2 MMVD. The conclusion is that there is an exquisitely sensitive relationship between renal ET-1 signalling and MMVD even when left-sided cardiac output is relatively normal. This raised the possibility that MMVD might represent a disruption to systemic ET-1 signalling. Liz Bode and I looked at whether other systemic metabolic disruptions accompanied cardiac disease, and particularly MMVD. She focused on cortisol [17]. Cortisol, like RAAS but contrary to ET-1, drives sodium and water retention through ENaC. This is via the glucocorticoid receptor, which is co-localised to the mineralocorticoid receptor (MR) that aldosterone binds to (and spironolactone blocks). Circulatory levels of cortisol are an order of magnitude greater than aldosterone levels, so in order to ensure that ENaC activity is under aldosterone control, the receptor-bound enzyme 11β HSD2 converts active cortisol to inactive cortisone. Metabolic syndrome in people, and the associated increased cardiovascular risk, have been linked with loss of 11β HSD2 activity and unhindered sodium transport through ENaC. Although we identified important regulatory changes in the metabolism of cortisol in the face of CHF, we couldn't demonstrate loss of 11β HSD2 protection of MR. This suggested that ET-1 rather cortisol may be of more relevance to our MMVD patients. Further work characterising this relevance is required, but embarking on pharmacological interference may be difficult to fund because the licensing of ET receptor antagonists in people stalled at about the time I completed my PhD.

SGLT2 inhibition

Therefore, in the latter part of my PhD and in post doc work, I decided to focus more on localising the disruption in sodium transport in Type 1 diabetes. Pressure natriuresis is predominantly dependent on

downregulation of sodium transport in the proximal tubule (where 70% of filtered sodium is immediately reabsorbed). An important transporter within the proximal tubule is the sodium-glucose transporter 2 (SGLT2) channel, which is responsible for re-absorbing almost all of renally filtered glucose. Over the last 10 years, use of SGLT2 inhibitors (SGLT2i) in patients with Type 2 diabetes, and subsequently non-diabetic patients, has dramatically reduced cardiovascular mortality and hospitalisations following cardiovascular events. This has been achieved in people already receiving standard cardiovascular medications. The exact mechanism underpinning these benefits is not entirely understood. Along with my fellow post doc, Natalie Jones, we demonstrated that SGLT2 has no effect on pressure natriuresis either in diabetic or non-diabetic rats [18]. Indeed we showed that compensatory distal tubular sodium reabsorption negated the natriuretic effect of blocking SGLT2. On the face of it, this will appear to be very detached from MMVD. But what this means is that benefits of SGLT2 blockade to cardiovascular health in CHF are not solely derived from improved natriuresis. Similar conclusions with previous agents have led to paradigm shifts in heart failure theory- from haemodynamics to neurohumoral and remodelling hypotheses. SGLT2i has led to the advent of metabolomics, in which modifying the cardiovascular system's fuel source directs cells to a pro-survival phenotype.

Senescence

At this stage, my research and Brendan Corcoran's research started to merge. Over the last 10 years, members of his research team, including Greg Markby and Paul Tang, have been characterising the molecular phenotype of MMVD in more detail [19, 20]. Other research centres are broadly in agreement that a key regulator of VIC activation, expression of α smooth muscle actin, excessive matrix production and collagen dysgenesis, is transforming growth factor beta (TGF β). By defining the canonical (SMAD) and non-canonical (PI3K and JNK) pathways that are important in canine MMVD valve preps and cell cultures, Brendan's team has shown that the accumulation of VICs is linked with a shift of VICs into the senescent state, a failure to clear them from the valve, and the production of poor quality extracellular matrix [21]. Under the influence of mTOR, aVICs enter the senescent process and stop clearing their own aged and damaged mitochondria (mitophagy). Blocking mTOR with rapamycin re-activates autophagy and allows cells to remove their abnormal mitochondrial components but apoptosis of the accumulated cells does not resume. A therapeutic strategy might therefore be to block accumulation of abnormal mitochondria in the first place by activating autophagic and mitophagic pathways through starvation. This could, in part, be achieved through strict dietary regulation, or by switching on

starvation responses that enhance ketone production and promote ketones as the primary myocardial fuel source. Rapamycin is currently undergoing clinical development in dogs and cats, but in MMVD, there is now growing evidence that targeting the senescent process through enhancing mitophagy and possibly restricting, stopping or even reversing myxomatous degeneration is a genuine mouth-watering prospect. Since SGLT2i promote the starvation phenotype, even in cells in which there are no SGLT2 receptors (possibly via glucagon), they are gaining momentum as an agent to trial clinically in MMVD dogs.

Endothelial dysfunction

Even so, if we have learned anything from clinical development of our current armorarium, and the development and success or failure of rival drug classes for use in people, such as ET_A receptor antagonists and NEP/AngIIR inhibitors, it is that we require sound experimental understanding of how these agents work and the patient populations in which their beneficial effects are most likely to be observed. Clearly, in people, the beneficial effects of SGLT2i do not come from arresting the myxomatous state, and while appropriate myocardial metabolomics may be the reason, vascular function also improves under their influence. Despite its undisputed contribution to human patient morbidity and mortality, vascular function remains a relatively neglected area of research and therapeutic targeting in veterinary cardiology. While this may reflect the bygone dogmas that “dogs don’t get vascular disease”, and perhaps the slightly more exciting status that the heart holds over the vasculature, studies on laboratory animals and clinical development in equine laminitis and cats suggest strong translational relevance. In MMVD, changes in nitric oxide metabolites [10] and *in vivo* quantitative imaging of flow-mediated vasodilation (mediated through ET-1 and NO release) [22, 23] have charted vascular dysfunction with disease progression. The gold standard (*ex vivo*) assessment of vascular function, routinely performed on laboratory animal arteries, had not been applied before to arteries from pet dogs. Marco Mazzarella has undertaken an MScR at the Roslin Institute, and refined and validated the technique, before identifying vascular dysfunction in renal arteries of older dogs in the form of impaired endothelial (nitric oxide) dependent vasodilation. Further testing of this technique, and in particular in pulmonary arteries, will be undertaken by us, and these data will be compared with echocardiographic assessments of vascular function. As we again establish a link between renal function, vascular function and MMVD, this brings us back to the ET-1 story.

Blood pressure and circadian regulation

While we look at ways of mitigating the myxomatous changes within the valve, the initiating stimulus is still of interest. There have been advances in our understanding of the condition at the genetic level, and past and

present studies investigating valvular geometry and abnormal loading on the valve leaflet, in line with the initial trauma theory. If trauma is important, ET-1 becomes a major player, not just because of the valvular remodelling, but also the accompanying increase in renal ET-1 signalling, renal vascular dysfunction, pulmonary arterial lesions, pulmonary hypertension and increased thrombogenesis that have all been described . The possibility remains that MMVD is a manifestation of a systemic endothelial disorder. It has been long established in people that endothelial dysfunction is associated with increased cardiovascular risk through disruption to regulation of BP. Indeed, hypertension is the single biggest contributor to the global disease burden. An early manifestation of this is loss of circadian variation in BP, in part, related to impaired pressure natriuresis following food and water ingestion, and vascular relaxation that is inadequate to accommodate the associated increase in blood volume. We hope, one day soon, to measure BP continually in our patients, via telemetry implants, but in the meantime we can at least establish the lability of circadian variation in cardiovascular regulation. Maria Ines Oliveira [24] has demonstrated that circadian variation in heart rate is robust in healthy dogs and is even maintained in most dogs that develop atrial fibrillation associated with atrial enlargement. So, if circadian variation in heart rate is robust, it does imply that this must be important, just as it is in people, and that its loss could have cardiovascular consequences, of which we are, as yet, unaware.

Conclusion

Despite therapeutic advances, MMVD remains a major cause of morbidity and mortality in our canine population. Future management and treatment strategies will require understanding new drug classes and when to deploy them, and the co-morbidities of vascular and renal dysfunction whose contributions to morbidity and mortality remain undetermined.

References

1. Culshaw, G.J., et al., *Evaluation of innervation of the mitral valves and the effects of myxomatous degeneration in dogs.* Am J Vet Res, 2010. **71**(2): p. 194-202.
2. Corcoran, B.M., et al., *Identification of surface morphologic changes in the mitral valve leaflets and chordae tendineae of dogs with myxomatous degeneration.* Am J Vet Res, 2004. **65**(2): p. 198-206.
3. Black, A., et al., *Ultrastructural morphologic evaluation of the phenotype of valvular interstitial cells in dogs with myxomatous degeneration of the mitral valve.* Am J Vet Res, 2005. **66**(8): p. 1408-14.

4. Han, R.I., et al., *Structural and cellular changes in canine myxomatous mitral valve disease: an image analysis study.* J Heart Valve Dis, 2010. **19**(1): p. 60-70.
5. Han, R.I., et al., *Distribution of myofibroblasts, smooth muscle-like cells, macrophages, and mast cells in mitral valve leaflets of dogs with myxomatous mitral valve disease.* Am J Vet Res, 2008. **69**(6): p. 763-9.
6. Hadian, M., B.M. Corcoran, and J.P. Bradshaw, *Molecular changes in fibrillar collagen in myxomatous mitral valve disease.* Cardiovasc Pathol, 2010. **19**(5): p. e141-8.
7. Lu, C.C., et al., *Gene network and canonical pathway analysis in canine myxomatous mitral valve disease: a microarray study.* Vet J, 2015. **204**(1): p. 23-31.
8. Yang, C.H., et al., *Canine tissue-specific expression of multiple small leucine rich proteoglycans.* Vet J, 2012. **193**(2): p. 374-80.
9. Lu, C.C., et al., *Developmental pathways and endothelial to mesenchymal transition in canine myxomatous mitral valve disease.* Vet J, 2015. **206**(3): p. 377-84.
10. Pedersen, H.D., et al., *Decreased plasma concentration of nitric oxide metabolites in dogs with untreated mitral regurgitation.* J Vet Intern Med, 2003. **17**(2): p. 178-84.
11. Pedersen, L.G., et al., *Increased expression of endothelin B receptor in static stretch exposed porcine mitral valve leaflets.* Res Vet Sci, 2007. **82**(2): p. 232-8.
12. Karlstam, E., et al., *Pulmonary artery lesions in cavalier King Charles spaniels.* Vet Rec, 2000. **147**(6): p. 166-7.
13. Nielsen, L.A., et al., *Platelet function in dogs: breed differences and effect of acetylsalicylic acid administration.* Vet Clin Pathol, 2007. **36**(3): p. 267-73.
14. Tarnow, I., et al., *Hemostatic biomarkers in dogs with chronic congestive heart failure.* J Vet Intern Med, 2007. **21**(3): p. 451-7.
15. Culshaw, G.J., et al., *Impaired pressure natriuresis and non-dipping blood pressure in rats with early type 1 diabetes mellitus.* J Physiol, 2019. **597**(3): p. 767-780.
16. Culshaw, G., et al., *The acute pressure natriuresis response is suppressed by selective ETA receptor blockade.* Clin Sci (Lond), 2021. **136**(1): p. 15-28.
17. Bode, E.F., et al., *Glucocorticoid metabolism and the action of 11 beta-hydroxysteroid dehydrogenase 2 in canine congestive heart failure.* Vet J, 2020. **258**: p. 105456.
18. Jones, N.K., et al., *Sodium-glucose cotransporter 2 inhibition does not improve the acute pressure natriuresis response in rats with type 1 diabetes.* Exp Physiol, 2023. **108**(3): p. 480-490.

19. Markby, G.R., et al., *Comparative transcriptomic profiling of myxomatous mitral valve disease in the cavalier King Charles spaniel.* BMC Vet Res, 2020. **16**(1): p. 350.
20. Markby, G.R., et al., *Comparative Transcriptomic Profiling and Gene Expression for Myxomatous Mitral Valve Disease in the Dog and Human.* Vet Sci, 2017. **4**(3).
21. Tang, Q., et al., *TGF-beta-induced PI3K/AKT/mTOR pathway controls myofibroblast differentiation and secretory phenotype of valvular interstitial cells through the modulation of cellular senescence in a naturally occurring in vitro canine model of myxomatous mitral valve disease.* Cell Prolif, 2023. **56**(6): p. e13435.
22. Moesgaard, S.G., et al., *Flow-mediated vasodilation measurements in Cavalier King Charles Spaniels with increasing severity of myxomatous mitral valve disease.* J Vet Intern Med, 2012. **26**(1): p. 61-8.
23. Jones, I.D., et al., *Ultrasonographic measurement of flow-mediated vasodilation in dogs with chronic valvular disease.* J Vet Cardiol, 2012. **14**(1): p. 203-10.
24. Oliveira, M.I., et al., *Validation of heart rate spot-check protocol to measure circadian variation and heart rate in healthy dogs and dogs with atrial fibrillation.* J Vet Cardiol, 2022. **43**: p. 41-54.

Mitral valve morphologic analysis using echocardiography

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While the pathogenesis and natural history of canine myxomatous mitral valve disease are well described [1], the etiopathogenesis of the most common canine cardiac disease is still uncertain. The disease is characterized by several derangements at the level of the extracellular matrix, the most prominent being the increased production of glycosaminoglycans, expression of proteins that are responsible for matrix degeneration (metalloproteases) [2]. Physiologically, the mitral valve annulus is shaped as a hyperbolic paraboloid – a morphology commonly defined as “saddle shaped”. Levine et al has extensively investigated the impact of this morphology under several aspects. First and foremost, the definition of mitral valve prolapse was revolutionized [3]. The newly defined mitral annular morphology revealed that echocardiographic views not including the left ventricular outflow tract (i.e. four-chamber views) would unequivocally cause an overestimation of portions of mitral leaflets bulging into the left atrium, therefore causing a misdiagnosis of mitral valve prolapse. Following the publication of his work, the prevalence of mitral valve prolapse in humans drastically declined. Evidence from in vitro, computational, and indirect clinical experience, suggests that abnormal mechanical valvular stress can play a role in the etiopathogenesis of myxomatous valvular degeneration. Particularly, in the context of mitral valve repair, it was found that reconstruction of the mitral annulus (annuloplasty) using artificial rings that were flat, was associated with more early surgical failures and an overall worse prognosis, compared to annuloplasties aimed at restoring a more physiologic saddle-shaped annulus [4]. Upon further computational investigation, it was postulated that this may be secondary to increase valvular stress caused by a flat mitral annulus. Finite element analysis studies have indeed confirmed that having a flatter mitral valve annulus causes increased stress at the level of the mitral leaflets [5]. Furthermore, Lacerda et al. have effectively demonstrated that applying abnormal stress on canine mitral valve leaflets causes expression of myxomatous proteins [6]. Therefore, there is evidence that links abnormal mitral valve morphology, increased leaflet stress, and myxomatous valvular degeneration. The advent and technological improvements of three-dimensional echocardiography, have allowed studying the mitral valve morphology of dogs in its most physiologic form, i.e. non-invasively, in a beating heart without the need for sedation or anesthesia. With this technology, the entire volume of the heart can be acquired and post-

processed in order to obtain detailed information about structures with complex three-dimensional shapes. Using this approach, our group has previously confirmed that the mitral valve annulus of healthy dogs is also “saddle-shaped” and therefore a diagnosis of mitral valve prolapse should follow guidelines similar to the ones used in people, i.e. use views that include the left ventricular outflow tract (sometimes commonly named “five-chambers” views) [7]. With the same technology we have then showed that dogs affected by mitral regurgitation secondary to myxomatous valvular degeneration have a mitral valve that is flatter and more circular compared to healthy dogs [8]. Furthermore, in attempt to support a causative relationship of mitral valve morphology in the myxomatous process, we had compared the mitral valves of a group of healthy Cavalier king Charles Spaniels (CKCS), a breed predisposed to early development of myxomatous changes, to a group of dogs of other breeds. Interestingly, we found that CKCS had a valve that resembled the one of dogs affected by myxomatous valvular degeneration, even before developing any degree of valvular regurgitation [9]. In attempt to investigate whether this different valvular morphology is determined at birth, or whether it is a change occurring early during growth in this breed, we are now investigating the mitral valvular morphology in a group of puppies. In this study we are enrolling 21 CKCS puppies (14-22 weeks old) and 21 puppies of other breeds. We will then compare the valvular morphology between these two groups. Furthermore we are performing follow-up echocardiographic examination in the CKCS puppies every 8 weeks, until they reach adulthood or develop more than trivial mitral valve regurgitation. Although data has been collected only from a small number of dogs, our preliminary results, presented at this ESVC pre-congress, show that CKCS puppies have a high prevalence (~30%) of very small, often intermittent jets of mitral valve regurgitation. When looking at 22-weeks old CKCS we also learned that allometrically scaled echocardiographic measurements obtained by a large group of adult dogs are very similar in puppies, despite their small variation in body weight. By using a pediatric volumetric probe, we were able to acquire and analyze three-dimensional datasets from every puppy enrolled in the study. Interestingly, the morphologic valvular variables, in contrast to the conventional 2D ones, did not appear significantly related to body weight. When compared to a historical group of adult CKCS however, we found that the valve of the puppies have smaller annuli and leaflets while maintaining the same ratios and angles. While the results are preliminary this may suggest that the valvular shape is indeed determined at birth and does not change between puppies and adult dogs. Instead, when compared to adults of breeds other than CKCS, the annulus of CKCS puppies is more circular, a finding that was present also in our previous

studies in adults. Should we confirm these findings when the enrollment will be completed, further investigations aimed at identifying early morphologic determinants of myxomatous valvular degeneration could have important breeding implications.

References

1. Borgarelli M, Buchanan JW. Historical review, epidemiology and natural history of degenerative mitral valve disease. J Vet Cardiol [Internet]. 2012 Mar [cited 2012 Nov 25];14(1):93–101. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22386588
2. Aupperle H, Disatian S. Pathology, protein expression and signaling in myxomatous mitral valve degeneration: comparison of dogs and humans. J Vet Cardiol [Internet]. 2012 Mar [cited 2012 Nov 25];14(1):59–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22364722
3. Levine RA, Triulzi MO, Harrigan P, Weyman AE. The relationship of mitral annular shape to the diagnosis of mitral valve prolapse. Circulation [Internet]. 1987 Apr 1;75(4):756–67. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3829339
4. Jensen MO, Jensen H, Smerup M, Levine R a., Yoganathan AP, Nygaard H, Hasenkam JM, Nielsen SL. Saddle-shaped mitral valve annuloplasty rings experience lower forces compared with flat rings. Circulation. 2008;118.
5. Jensen MO, Jensen H, Levine RA, Yoganathan AP, Andersen NT, Nygaard H, Hasenkam JM, Nielsen SL. Saddle-shaped mitral valve annuloplasty rings improve leaflet coaptation geometry. J Thorac Cardiovasc Surg [Internet]. 2011 Sep;142(3):697–703. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0022522311000535
6. Lacerda CMR, Kisiday J, Johnson B, Orton EC. Local serotonin mediates cyclic strain-induced phenotype transformation, matrix degradation, and glycosaminoglycan synthesis in cultured sheep mitral valves. AJP: Heart and Circulatory Physiology [Internet]. 2012;302(February 2012):H1983–90. Available from: http://www.physiology.org/doi/10.1152/ajpheart.00987.2011
7. Menciotti G, Borgarelli M, Aherne M, Häggström J, Ljungvall I, Lahmers SM, Abbott JA. Assessment of mitral valve morphology using three-dimensional echocardiography. Feasibility and reference values. Journal of Veterinary Cardiology [Internet]. 2016 Jun;18(2):156–67. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1760273416000084
8. Wesselowski S, Borgarelli M, Menciotti G, Abbott J. Echocardiographic anatomy of the mitral valve in healthy dogs and dogs with myxomatous mitral valve disease. Journal of Veterinary Cardiology [Internet]. 2015;17(2):97–106. Available from: http://linkinghub.elsevier.com/retrieve/pii/S176027341500020X

9. Menciotti G, Borgarelli M, Aherne M, Camacho P, Häggström J, Ljungvall I, Lahmers SM, Abbott JA. Comparison of the mitral valve morphologies of Cavalier King Charles Spaniels and dogs of other breeds using 3D transthoracic echocardiography. J Vet Intern Med [Internet]. 2018 Sep;32(5):1564–9. Available from: http://doi.wiley.com/10.1111/jvim.15252

Beyond the Valve: Why Does Mitral Valve Disease Progress?

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Introduction

Veterinary cardiologists are only too familiar with myxomatous mitral valve disease (MMVD); as the most common cardiac disease in dogs, it constitutes 75-80% of our canine case load.[1] Classification of MMVD most commonly uses the American College of Veterinary Internal Medicine (ACVIM) scheme, from stage A (at risk of future MMVD), through stages B1 (evidence of preclinical MMVD without cardiomegaly), B2 (evidence of preclinical MMVD with cardiomegaly) and C (congestive heart failure (CHF) secondary to MMVD) to stage D (refractory CHF secondary to MMVD). However, the marked phenotypic variation seen in this disease means that prognosis remains challenging to predict. At one end of the phenotypic spectrum are the dogs that develop a murmur relatively early in life, rapidly develop cardiomegaly and die due to CHF at a young age (e.g. 6-7 years old). At the other are dogs that die aged 15-16 years old with stage B1 MMVD. Nevertheless, it remains unclear what drives these differences in disease phenotype. This was one of the key questions that motivated me to undertake a PhD in 2008 and has continued to perplex me ever since.

Pathophysiology of MMVD

Mitral valve disease is characterised by progressive myxomatous valvular degeneration, resulting in abnormal collagen organisation and glycosaminoglycan accumulation. This degeneration is associated with mitral valve prolapse and regurgitation. Eccentric hypertrophy develops to compensate for the decrease in forward stroke volume (FSV) resulting from mitral regurgitation. Over time, this volume overload may result in the development of left atrial and ventricular dilation (i.e. stage B2 MMVD). We know that a dog's risk of developing CHF significantly increases once cardiomegaly develops, so progression from stage B1 to B2 is a clinically significant milestone. The question is, why do some dogs remain in B1 for many years while other progress to stage B2 over a matter of months?

A simple explanation might be that the rate at which the atrium and ventricle dilate is determined entirely (or almost entirely) by the rate of myxomatous degeneration of the mitral valve. After all, it is logical to

assume that more severe degeneration will result in a larger regurgitant orifice and hence a larger regurgitant stroke volume (RSV). In the absence of a compensatory increase in total stroke volume (TSV), any increase in RSV will result in an equivalent decrease in FSV.[2] This decrease in FSV will trigger homeostatic mechanisms to increase the circulating volume and thereby increase end-diastolic volume, enabling an increase in TSV to compensate for the volume of blood regurgitated into the left atrium. This increase in left ventricular end-diastolic volume will trigger the development of eccentric hypertrophy to normalise ventricular wall stress. Additionally, a combination of the increase in RSV and the increase in venous return due to increased circulating volume result in an increase in the volume of blood in the left atrium and therefore trigger a similar process of atrial dilation. Unfortunately, the development of prolapse and regurgitation increase valvular shear stress, which is likely to result in further pathological changes in the leaflets, while the mitral valve annulus dilates as a result of eccentric hypertrophy, further impairing coaptation and thereby increasing RSV yet further. Mitral regurgitation therefore begets mitral regurgitation, creating a vicious cycle that results in increasingly rapidly developing eccentric hypertrophy as the valvular pathology becomes more severe. After all, we know that the rate of change of left atrial and ventricular measurements is higher in dogs that die due to MMVD and increases as dogs approach congestive heart failure.[3,4] We also know that echocardiographic estimates of regurgitant orifice size are associated with prognosis in dogs with MMVD.[5] It should come as no surprise, therefore, that a wealth of research exists investigating the mechanisms and pathways responsible for myxomatous valvular degeneration, as this process is clearly important in determining disease progression and prognosis.

Beyond the Valve

Nevertheless, in human patients with MMVD, the relationship between severity of mitral regurgitation and the degree of left ventricular dilatation is not straightforward. For example, left ventricular dysfunction can develop in human patients with only mild or moderate mitral regurgitation in the absence of worsening regurgitation.[6] Additionally, the presence of left ventricular replacement fibrosis is associated with mitral valve apparatus changes and left ventricular dilatation not explained by volume overload in human patients with only trace-mild mitral regurgitation.[7] So, perhaps phenotype is not solely determined by differences in the pathophysiological processes occurring in the mitral valve after all. For example, there may be differences in the way the ventricle responds to volume overload between individuals that are at least partially responsible for differences in the rate of development of cardiomegaly. As in humans,

it is possible that, in the presence of the same degree of mitral regurgitation, some dogs respond by developing a greater degree of eccentric hypertrophy and/ or develop hypertrophy more rapidly than others. It is possible, therefore, that factors beyond the valve contribute to differences in phenotype.

1. The Influence of Genetics

Canine MMVD is inherited as a polygenic threshold trait; this complex mode of inheritance makes the genetic basis of the disease challenging to investigate. Some variability in typical phenotypes can be seen at the breed level. For example, Cavalier King Charles spaniels (CKCS) present relatively frequently with the most severe phenotypes, while other breeds, such as the Jack Russell terrier, are more likely to be mildly affected, even at an advanced age. Nevertheless, some CKCS will have stage B1 MMVD at 15 years of age, so generalisations cannot be made about all individuals in any particular breed.

The most straightforward hypothesis might be that phenotypic variation is genetically “pre-programmed”, such that all differences in disease severity between dogs can been explained by different combinations of alleles in genes important in valvular and extra-valvular health. For example, depending on genotype, an individual might be more or less likely to develop significant ventricular fibrosis as a feature of their ventricular remodelling response to the development of mitral regurgitation. Nevertheless, it is more likely that environmental modifiers and epigenetic effects modify phenotype, increasing complexity yet further and creating additional challenges to understanding the genetic basis of canine MMVD.

I have been investigating the genetic basis of MMVD in CKCS with Lucy Davison and Adrian Boswood and their groups at the Royal Veterinary College (RVC) in London since 2018. To date, the project has comprised 3 phases:

- Phase 1: whole genome sequencing of 12 CKCS (6 with the most severe phenotypes and 6 with the least severe phenotypes, using stored DNA samples from a population with known outcome data) plus 48 non-CKCS breeds at low risk of MMVD.
- Phase 2: follow up genotyping using custom targeted sequencing (TWIST Bioscience) of 10,000 genetic variants in dogs of a variety of breeds.
- Phase 3 (currently in progress): follow up genotyping using targeted genotyping by sequencing (Flex-Seq, LGC Biosearch technologies) of 15,000 genetic variants in 480 dogs (CKCS plus some CKCS crosses).

To date, we have identified ~2,000 genetic variants that are breed-distinct in CKCS. Of these, at least 100 variants are associated with phenotype severity. Work is ongoing to develop genetic risk scores and to understand how these variants contribute to disease pathogenesis and pathophysiology. Of course, the question remains as to what we will do with the information once we understand the contribution of genetics to the development of MMVD. As MMVD demonstrates a polygenic mode of inheritance, is essentially ubiquitous in CKCS, and this breed has limited genetic diversity, breeding MMVD out of CKCS will never be a realistic goal (unless we take the drastic approach of banning their breeding, of course). Alternative options include selective breeding for milder phenotypes, as is already being attempted in Scandinavia; however, it is important to recognise the risk of inadvertently selecting for genes involved in other disease processes via this approach. Alternatively, it might be possible to edit disease-associated alleles to directly reduce phenotypic severity. Identification of the genes and pathways involved in MMVD pathogenesis also has the potential to identify novel therapeutic targets, which are needed to improve outcomes. Finally, the development of genetic risk scores opens up the possibility of precision medicine for the future, in which therapy can be targeted to specific patient genotypes. However, much work will still need to be done to understand the environmental modifies and epigenetic factors that probably influence MMVD phenotype.

2. The Renin-Angiotensin-Aldosterone Cascade and Fibrosis

Left ventricular remodelling is a complex process, believed to be mediated in part by activation of the renin-angiotensin-aldosterone system (RAAS). In human patients, clinical benefits of mineralocorticoid (aldosterone) receptor blockade[8] have been suggested to be conferred through a decrease in aldosterone-mediated interstitial fibrosis.[9] In a canine experimental model of atrial fibrillation, spironolactone inhibited the development of atrial dilatation and fibrosis.[10] In canine MMVD survival times are negatively correlated with myocardial fibrosis scores at post mortem.[11] Additionally, excess aldosterone is known to activate the immune system; inflammation has been implicated in the pathophysiology of MMVD.

Urinary aldosterone to creatinine ratio (UAC) estimates 24 hour aldosterone production and is therefore more reflective of RAAS activation than measurement of the more labile plasma concentration.[12] Circulating N-terminal procollagen type III (PIIINP) is a marker of collagen type III turnover and hence extracellular matrix (ECM) turnover.[13] In human patients, is considered a marker of myocardial fibrosis.[14] Intramural

arteriosclerosis and interstitial fibrosis are common histopathological findings in dogs with MMVD.[11] The severity of arteriosclerosis and fibrosis are associated with systolic dysfunction in dogs with MMVD,[11] and these changes are most evident in the papillary muscles.[15]

During my PhD, which was supervised by Adrian Boswood and Jonathan Elliott, I investigated relationships between serum PIIINP, UAC and fibrosis, both in a longitudinal study of dogs with MMVD (based at the RVC) and a population of dogs that had undergone *post mortem* examination (collected by Torkel Falk). We found that serum PIIINP and, therefore, it is believed collagen type III turnover, decreased with increasing measurements of left ventricular size in dogs with naturally occurring MMVD. UAC increased with increasing rates of change of left ventricular measurements, suggesting that aldosterone concentrations may increase around times of active ventricular remodelling rather than continuously throughout the course of the disease. In a small randomised, placebo-controlled, double-blinded parallel grouped pilot study of spironolactone in dogs with advanced preclinical MMVD, left atrial ($P=0.002$) and ventricular ($P=0.005$) dimensions increased over time in the placebo group, but not the spironolactone group; however, the change did not differ significantly between groups. Our results also suggested that there might also be breed-specific differences in the pathophysiological response to mitral regurgitation in dogs, with CKCS having higher PIINP and UAC, even when controlling for disease stage. Does this provide support for the idea that the remodelling response is not uniform across all dogs? Cavalier King Charles spaniels often appear to respond differently from other breeds in studies of MMVD, e.g. in the QUEST study, CKCS had longer survival times than other breeds, despite typically being thought of as a breed which tends to have earlier onset and more rapidly progressive disease.[16]

We also demonstrated that serum PIIINP concentrations are correlated with myocardial fibrosis in dogs, although the relationship appears to be weaker than that observed in human patients with cardiomyopathies. Although both left ventricular internal dimensions, normalised for body weight (LVIDdN) and serum PIIINP concentrations increased with increasing fibrosis scores, serum PIIINP concentrations decreased with increasing LVIDdN. As LVIDdN increases with progression of MMVD, this suggests that a complex interplay between increasing fibrosis and decreasing collagen type III turnover might be occurring as the disease progresses.

3. <u>Endothelial Damage and Dysfunction</u>

The endothelial layer covering the mitral valve itself is damaged and denuded as part of the process of myxomatous degeneration; endothelial damage is therefore inherent to MMVD. However, there is also evidence that systemic endothelial dysfunction develops in dogs with MMVD and becomes progressively more severe with progression of MMVD. During my PhD, I worked with Ian Jones (supervised by Virginia Luis Fuentes) on a project in which dogs with MMVD enrolled in the longitudinal study underwent measurement of flow mediated vasodilation (FMD), an indirect assessment of global endothelial function.[17] This study demonstrated that FMD was diminished in dogs with MMVD and decreased with increasing LVIDdN, suggesting that global endothelial function deteriorates with progression of MMVD. This begs the question, why might a disease process that affects the cardiac valves cause endothelial dysfunction in peripheral arteries?

One possible explanation for this is that the endothelial glycocalyx, a gel-like layer lining the entire circulatory system, is damaged in dogs with MMVD. The endothelial glycocalyx has many important physiological roles, including regulation of vascular permeability, shielding endothelial cells from circulating blood cells, regulation of thrombosis and coagulation and regulation of vascular tone. Endothelial glycocalyx damage can, therefore, result in leakage of macromolecules from the vasculature, initiation of inflammation (via increased leucocyte adhesion), platelet activation and decreased vasodilation in response to shear stress (as seen in the reduced FMD response in dogs with MMVD described above). Causes of endothelial glycocalyx damage include volume overload, sympathoadrenal activation, inflammation, excessive shear stress, shock, ischaemia-reperfusion, sepsis and hyperglycaemia. Of these, the first four have been documented in dogs with MMVD.

In volume overload, atrial and ventricular myocardial cells release atrial and B-type natriuretic peptides (ANP and BNP, respectively). These neurohormones are important for homeostatic regulation of volume status, counteracting the effects of the RAAS. I am particularly interested in the natriuretic hormones from a research perspective, having investigated the clinical utility of N-terminal pro-B-type natriuretic peptide (NT-proBNP) in dogs and cats with heart disease since starting my PhD. In human patients and guinea pig models, ANP and BNP cause shedding of the endothelial glycocalyx via a cyclic GMP-linked proteolytic pathway. As it is well established that ANP and BNP are released in dogs with MMVD and that measurements increase with disease severity, this provides a plausible (although unconfirmed) mechanism whereby volume overload may result in endothelial glycocalyx damage, resulting in generalised endothelial dysfunction. The endothelial glycocalyx is highly challenging to study, as it is very fragile and easily damaged during

sample processing. Marco Mazzarella is currently undertaking a PhD in which he is using a novel technique for the longitudinal *in vivo* assessment of endothelial glycocalyx depth in dogs with MMVD (stages A-D). We hope that over the next few years we will start to understand the contribution of endothelial glycocalyx damage to the pathophysiology of MMVD in dogs.

Sadly, but predictably, over the 15 years I have been trying to understand remodelling in MMVD I have yet to answer the question posed in the title; I hope you don't feel short-changed by my failure. Philosophically, of course, "why" is generally one of the hardest questions to answer – if you drill down into any problem far enough, we usually run out of answers eventually (as anyone who has ever had a conversation with a 5 year old will tell you). Nevertheless, for me, the quest to understand why things happen is the most interesting and exciting reason to undertake research. I have no doubt that there will continue to be enough perplexing questions to answer in MMVD to keep me occupied for the rest of my career (and possibly beyond).

References

1. Kittleson MD, Kienle RD. Myxomatous atrioventricular valvular degeneration. In: Kittleson MD, Kienle RD, eds. Small Animal Cardiovascular Medicine, 1st ed. St. Louis: Mosby; 1998:297-318.
2. Hezzell MJ. Pathology and prognosis of canine myxomatous mitral valve disease. In Practice 2018;40:3-6.
3. Lord P, Hansson K, Kvart C, et al. Rate of change of heart size before congestive heart failure in dogs with mitral regurgitation. Journal of Small Animal Practice 2010;51:210-218.
4. Hezzell MJ, Boswood A, Moonarmart W, et al. Selected echocardiographic variables change more rapidly in dogs that die from myxomatous mitral valve disease. Journal of veterinary cardiology : the official journal of the European Society of Veterinary Cardiology 2012;14:269-279.
5. Sargent J, Muzzi R, Mukherjee R, et al. Echocardiographic predictors of survival in dogs with myxomatous mitral valve disease. Journal of veterinary cardiology : the official journal of the European Society of Veterinary Cardiology 2015.
6. Suri RM, Aviernos JF, Dearani JA, et al. Management of less-than-severe mitral regurgitation: should guidelines recommend earlier surgical intervention? Eur J Cardiothorac Surg 2011;40:496-502.
7. Constant Dit Beaufils AL, Huttin O, Jobbe-Duval A, et al. Replacement Myocardial Fibrosis in Patients With Mitral Valve Prolapse: Relation to

Mitral Regurgitation, Ventricular Remodeling, and Arrhythmia. Circulation 2021;143:1763-1774.
8. Pitt B, Zannad F, Remme WJ, et al. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. N Engl J Med 1999;341:709-717.
9. Pitt B. Do diuretics and aldosterone receptor antagonists improve ventricular remodeling? J Card Fail 2002;8:S491-493.
10. Zhao J, Li J, Li W, et al. Effects of spironolactone on atrial structural remodelling in a canine model of atrial fibrillation produced by prolonged atrial pacing. Br J Pharmacol 2010;159:1584-1594.
11. Falk T, Jonsson L, Olsen LH, et al. Associations between cardiac pathology and clinical, echocardiographic and electrocardiographic findings in dogs with chronic congestive heart failure. Vet J 2010;185:68-74.
12. Gardner SY, Atkins CE, Rausch WP, et al. Estimation of 24-h aldosterone secretion in the dog using the urine aldosterone:creatinine ratio. Journal of veterinary cardiology : the official journal of the European Society of Veterinary Cardiology 2007;9:1-7.
13. Jensen LT. The aminoterminal propeptide of type III procollagen. Studies on physiology and pathophysiology. Dan Med Bull 1997;44:70-78.
14. Klappacher G, Franzen P, Haab D, et al. Measuring extracellular matrix turnover in the serum of patients with idiopathic or ischemic dilated cardiomyopathy and impact on diagnosis and prognosis. Am J Cardiol 1995;75:913-918.
15. Falk T, Jonsson L, Olsen LH, et al. Arteriosclerotic changes in the myocardium, lung, and kidney in dogs with chronic congestive heart failure and myxomatous mitral valve disease. Cardiovasc Pathol 2006;15:185-193.
16. Haggstrom J, Boswood A, O'Grady M, et al. 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 2008;22:1124-1135.
17. Jones ID, Fuentes VL, Boswood A, et al. Ultrasonographic measurement of flow-mediated vasodilation in dogs with chronic valvular disease. Journal of veterinary cardiology : the official journal of the European Society of Veterinary Cardiology 2012;14:203-210.

Update on biomarkers and use in myxomatous mitral valve disease in dogs

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Biomarkers are characteristics that are objectively measured as indicators of health, disease, or a response to an exposure or intervention, including therapeutic interventions (FDA definition). https://www.fda.gov/science-research/focus-areas-regulatory-science-report/focus-area-biomarkers
Cardiac biomarkers are blood-based substances which can be assayed to give an indication of cardiac or cardiovascular health or disease. Most commonly used in clinical veterinary and cardiology specialist practice are:

- N-terminal proBNP (brain natriuretic peptide) (NTproBNP); synthesised and released from ventricular cardiomyocytes under conditions of myocardial wall stress.
- Troponin I: marker of cardiomyocyte injury (leakage marker from cardiomyocytes).

Myxomatous mitral valve disease (MMVD) is a valvular disease associated with volume overload with left sided chamber dilatation in presence of significant mitral regurgitation and therefore increased myocardial wall stress; increasing NTproBNP reflects this. In contrast, during phases of compensatory remodelling, there is no or minimal cardiomyocyte injury, so Troponin I is less useful for diagnosis or screening for the condition (in contrast to cardiomyopathies).

N-terminal pro-B-type natriuretic peptide

Role of NTproBNP in screening for MMVD

In primary care practice, where there may be no easy access for echocardiography, the significance of an acquired heart murmur consistent with mitral regurgitation can be assessed with NTproBNP. It can also be used to differentiate between a cardiac versus noncardiac cause of clinical signs, such as coughing. If concentration is normal, watchful monitoring of the murmur grade and serial evaluation of NT-proBNP (e.g. 6 monthly) can be considered. If values are increased (e.g. >900 pmol/L) further assessment and staging the MMVD based on the ACVIM consensus statement (Keene et al., 2019) is indicated; echocardiography and / or thoracic radiographs should be carried out, via referral if necessary. With NTproBNP values of >1500 pmol/L, there is

increased risk of developing CHF within 6 – 12 months (Chetboul et al 2009; Serres et al. 2009, Borgarelli et al 2021)
If reliable echocardiography is available, NTproBNP level correlates with echo variables including LA/Ao, regurgitant fraction and normalised diastolic LV diameter or volume (Chetboul et al 2009) and so the addition of NTproBNP may not provide additional information.

Can NTproBNP be used to stage MMVD?
The increase in NTproBNP concentration with worsening stage of MMVD has been long recognised (Häggström et al 2000). There are certainly statistically significant population differences for dogs with different stages of MMVD. However, there is considerable overlap between groups, especially between ACVIM Stage B1 and B2 (Wolf et al, 2013), which means in primary care practice, the NTproBNP result cannot be used to determine when to start pimobendan. It is useful at identifying congestive heart failure as the cause of respiratory distress (cut-off >2447 pmol/L) (Fox et al. 2015). Higher values in MMVD dogs in Stage B indicate dogs which are more likely to progress (Mattin et al 2019a).
N-terminal proBNP concentration was associated with Stage B2, but predictive value improved when used in in conjunction with other physical examination and /or biochemical variables in a predictive model using multivariable logistic regression (Wilshaw et al. 2021). These authors (HAMLET study) proposed a future app which would be useful in primary care practice.

What limits the value of NTproBNP assay?
There can be considerable day to day biological variability of NTproBNP concentration, but usually not sufficient to affect decision making (Winter et al. 2017a). There are also some breed influences (Misbach et al 2013; Sjöstrand et al 2014 Gomart et al, 2020). There is a positive association between NTproBNP and age, independent of disease severity (Mattin et al. 2019b). Renal function (Miyagawa et al., 2013, Pelander et al 2017), exercise (Wall et al. 2018) and systemic hypertension (Jang et al. 2023) may also influence results. In some cases, NTproBNP can be increased for no identifiable reason (Misbach et al. 2013).

Which NTproBNP assay?
N-terminal proBNP assays are species specific. There is sample degradation after taking the sample, and with freeze-thaw cycles etc. recognised in the past (Hezzell et al. 2015). IDEXX now have developed a 2nd generation assay (Cahill et al. 2015), considered more stable, but the recommendation is that it should be received by the lab within 24 – 48 hours (Cardiopet). EDTA plasma is preferred as most stable (Cahill et al.

2015), and the sample should be centrifuged and separated as soon as possible after the taking sample. In recognition of the issue in sample degradation for the 1st generation assay in the past, the lab provided special protease inhibitor tubes, to put the EDTA plasma sample into prior to shipping.
The NT-proBNP assay is offered by other commercial labs, but it should be noted that this is the first generation assay and prone to sample degradation; they do not provide protease inhibitor tubes.
Another advantage of the 2nd generation assay is that the upper limit of quantification is ≥10,000 pmol/L (1st generation ≥3,000) so there is broader dynamic range. Comparison of the first and second assays showed similar results, so similar cut-offs from original publications can still be used (Cahill et al. 2015).
NTproBNP would be much more useful if available as a patient side point-of care test, especially in an animal with clinical signs. So far, there is no SNAP test similar to the feline assay for use by veterinarians. However, there is a recent point of care test (V-check) with good correlation with the IDEXX assay, although the lower limit of quantification is 650 pmol/L (Harr et al. 2022)

Can we use NT-proBNP to guide Treatment?
In human cardiology, UK NICE guidelines recommend that general practitioners use NTproBNP to assess every patient with suspected heart failure, with guidance when and how urgently to refer to a cardiologist based on the results (2023 update):
https://www.nice.org.uk/guidance/qs9/chapter/Quality-statement-1-N-terminal-pro-B-type-natriuretic-peptide-measurement#rationale
However, current NICE guidelines about monitoring treatment for all type of heart failure on recommend "NT-proBNP as part of a treatment optimisation protocol only in a specialist care setting for people aged under 75 who have heart failure with reduced ejection fraction and an eGFR above 60 ml/min/1.73 m^2" (2018) https://www.nice.org.uk/guidance/ng106/chapter/Recommendations#monitoring-treatment-for-all-types-of-heart-failure .
A Cochrane review compared the approach of NTproBNP (or BNP) guided therapy to conventional management and monitoring of congestive heart failure, based on symptoms. It suggested that there was a reduced relative risk (RR) for heart failure mortality and heart failure admissions in patients with NP guided therapy (RR 0.84 and 0.70 respectively), although this was less clear for all-cause mortality (RR 0.87) or all-cause admissions (RR 0.93) (McLellan et al. 2016). The benefit of NTproBNP guided therapy in reducing hospitalisations is only significant in patients less than 75 years old (Davarzani et al 2017). Continued high NT-pro-

BNP levels predicts poor quality of life in human patients (Zelenak et al. 2019).
Veterinary cardiology has not so far embraced serial monitoring of canine NTproBNP, possibly because of the cost. However, decrease in NTproBNP concentration (to <965 pmol/L) in response to treatment is a good prognostic indicator (Wolf et al. 2012). Rate of increase of NTproBNP is associated with death (Hezzell et al. 2012). Serial monitoring to guide treatment was proposed to be potentially useful a decade ago (Oyama et al. 2013). Hezzell and colleagues (2018) reported on intensification of treatment in dogs with recently decompensated CHF based on NTproBNP concentration (>1500 pmol/L), showing NTproBNP could be reduced. This study was just over 21 days, so longer-term follow up and direct comparison between dogs monitored and treated based on clinical signs such as sleeping respiratory rate (SRR) as well as those based on NTproBNP concentration is indicated. Increase in SRR is an early indicator of further decompensation, but intervening based on serial NTproBNP concentration may actually prevent this decompensation.

Troponin I
Troponin I, a marker of heart muscle cell injury, is usually not increased in early (pre-symptomatic) stages of MMVD. However, Troponin I increases in more advanced disease, and it correlates with clinical status (Polizopoulou et al 2014; Chan et al 2019). Persistently high or increasing values indicate poor prognosis (Hezzell et al. 2012). However, treatment may result in decrease of Troponin I concentration (Polizopoulou et al 2014; Chan et al. 2019). Surprisingly, in the study by Chan and colleagues (2019), greater decrease in Troponin I over time was associated with worse prognosis.
The reason for increase in Troponin I in a non-myocardial disease may reflect the remodelling changes associated with volume overload. This may result in cardiomyocyte death and replacement fibrosis. Furthermore, arteriosclerosis has been reported in MMVD, resulting in ischaemic injury and fibrosis. In a longitudinal study of dogs with MMVD and subsequent post-mortem examination, the last measured Troponin I concentration was found to correlate with the severity of myocardial fibrosis on histopathology (Falk et al. 2013). Troponin I concentration also correlates with the acute phase protein, CRP, in dogs with MMVD (Ljungvall et al 2010), with CRP associated with severity of disease; further evidence of cardiac disease and congestive heart failure being associated with inflammation (Polizopoulou et al. 2015).
Troponin I may increase with systemic disease affecting the myocardium, so increased concentration is not specific for cardiac disease. Some studies also show an association with ageing (Ljungvall et al 2010).

Troponin I therefore predicts all mortality rather than just specifically cardiac mortality (Hezzell et al 2012). There is also considerable biological variability in Troponin I in both healthy dogs and those with MMVD. It has been recommended that only changes in concentration of >110% in dogs with MMVD are likely to reflect changes in status during serial monitoring; smaller changes may merely reflect this biological variation (Winter et al. 2017b).

Which Troponin I assay?

Troponin I is not species specific, so there are a range on assays available. In healthy dogs, the standard Immulite assay is often below the limit of detection. Therefore, use of the standard assays is not recommended, but they can be useful (and have a wider range) when levels are high. There are a range of high- or ultra- sensitivity assays now commercially available and they are more likely to be useful for serial monitoring, as long as the biological variability is noted (Winter et al. 2017b). They will have different reference ranges, lower and upper limits of detection, and range of values.

Use of both cardiac biomarkers together?

Hezzell and colleagues (2012) showed that, when excluding echocardiographic variables, high sensitivity Troponin I and NTprobBNP (as well as heart rate and age) were all independently associated with survival. If both NTproBNP and high sensivity Troponin I were above cut-off values (>0.025 ng/mL and >524 pmol/L respectively), there was reduced survival compared to just one being increased and better survival when both biomarkers were below these cut-offs. This study showed that Troponin I was independently associated with survival when echocardiography variables were included, whereas NTproBNP was associated with the echo variables.

It makes sense that use of both cardiac biomarkers provides additional information, as they reflect different things.

MicroRNAs

MicroRNAs (or miRNAs) are small, non-coding RNAs (about 22 nucleotides long). They are transcribed in the same way as mRNA, but when processed (by endonucleolytic cleavages), the silence or down-regulate the expression of their gene targets. They are therefore important in numerous biological processes, and may be altered in various pathophysiological processes. MicroRNAs can be found in tissue (e.g. heart valves; Yang et al 2018) and can also be circulating. They can be cell-free in the plasma or within exosomes (microvesicles) (Yang et al 2017). If free in the plasma, they are protein bound with argonaute-2 or

high density lipoproteins. They are very stable, so could be an ideal biomarker if specific for a certain disease or stage of a disease such as MMVD. An excellent review article about the role of miRNAs in veterinary cardiovascular disease has recently been published (Reis-Ferreira et al 2022). Nomenclature is not intuitive. Most are preceded by “miR”, with the capitalisation indicating the mature form of the miRNA, whereas “mir” is indicative of a precursor or primary miRNA (pre-miRNA and pri-miRNA). This is a newer nomenclature system, and many miRNAs have been renamed to adhere to this system, something which must be considered with older literature. However, some older sequences, preceded by “let” or “lin”, have been left unaltered for historical reasons. There then follows a number sequential to the order of discovery. A final suffix may be included, which may represent slight differences in sequence, with the number may be followed by a letter (a, b etc) or hyphenated number (-1, -2, etc), or may indicate sequence direction (5p or 3p). There may be a species specific prefix (e.g. cfa for *Canis familiaris* if canine), however miRNAs conserved between species will all carry the same main sequential numerical identifier. Due to the rapidly evolving nature of the field and constant identification of new miRNAs, future revisions and further standardisation of the current nomenclature is likely.

Plasma or serum miRNAs associated with MMVD include:

1. In Dachshunds with various stages of MMVD: miR-30b differed in Dachshunds with MMVD; down-regulated in Stage B compared with Stage A dogs (marker of preclinical MMVD). miR-133b was down-regulated in Stage C compared with Stage A dogs (so possible marker of CHF) (Hulanicka et al 2014)
2. In a study looking at differential expression of miRNAs between dogs of various breeds with MMVD and controls (Li et al. 2015), 7/11 miRNAs showed decreased expression in MMVD (Stage B or C/D compared with controls: cfa-miR-302d, cfa-miR-380, cfa-miR-874, cfa-miR-582, cfa-miR-490, cfa-miR-329b, and cfa-miR-487b. 4/11 showed increased expression in dogs with B or C compared with controls: cfa-miR-103, cfa-miR-98, cfa-let-7b, and cfa-let-7c). There were six which showed different expression between Stages B1/B2 and C/D: cfa-miR-582, cfa-miR-487b, cfa-miR-103, cfa-miR-98, cfa-let-7b.
3. When 9 healthy dogs were compared with 8 dogs with MMVD and CHF, of 326 miRNAs identified, principle component analysis (PCA) identified 47% of differential expression for component 1 and only an additional 9% for component 2. Of the 326 miRNAs, 5 canine specific miRNAs were significantly upregulated in CHF dogs; miR-

133, miR-1, miR-139, cfa-let7e, miR-125a. There were 88 miRNAs down regulated in CHF (Jung & Bohan 2018)

4. In a study with 72 dogs with various heart diseases (35 with MMVD), and 10 healthy dogs, cfa-miR-130b was up-regulated in Stage B MMVD, but this is down-regulated again in Stages C/D, without any significant difference from control dogs. cfa-miR-130b was claimed to be more accurate (based on ROC AUC) than NT-proBNP for discriminating dogs with heart diseases from healthy dogs (but only Stage B2 MMVD) (Ro et al 2021).
5. CKCS specifically with Stage B1 MMVD, in different age categories, were addressed (Bagardi et al. 2022). MiR-30b-5p was significantly higher in CKCS with Stage B1 compared with stage A; with a trend to increasing up-regulation in Stage B1 if dogs were older (Bagardi et al 2022). Why this is different from the finding in Dachshunds (see no. 1 above) is not certain. However, miR-30b-5p could identify Stage B1 confirmed on echocardiography, but without any audible murmur.
6. Ghilardi et al (2022) further studied 35 CKCS with MMVD in different stages and the predictive value of miR-30b-5p (PRIME study). This showed that miR-30b-5p levels declined with advancing disease, evidenced by LV diameters or volumes, both in diastole and systole, LA/Ao, mitral regurgitation severity, MINE score, heart murmur grade. Higher miR-30b-5p therefore indicated more stable MMVD and less severe progression. However, this decline does show that miR-30b-5p is a marker of early MMVD, not advanced disease.

Compared to most biomarkers, there are no units of measurement and miRNAs are simply given as severity of down- or up-regulation (expression profiling) compared with control animals or an internal control such as an endogenous or added miRNA.

The plethora of different miRNAs presented in the available literature likely indicates that no single one can be a gold-standard cardiac biomarker, but a more integrated approach is required for diagnosis and stage of the disease, such as principle component analysis.

This expression profiling approach of multiple miRNA markers is currently in development by a UK-based veterinary diagnostics company (MI:RNA Ltd, Edinburgh, www. mirna-diagnostics.com). In summary, a multiplexing detection assay is used to describe the unique profile or ‘fingerprint’ based on up to 30 miRNA markers for a particular patient’s sample, for which disease status can then be classified with bioinformatic modelling analysis based on a well described reference population. The modelling analysis has a continually improving supervised AI component, ensuring the accuracy of classification improves as the reference population increases,

harnessing the power of so called ‘big data’. Results from a MMVD focused study are under submission, and the company has already applied the technology to infectious livestock disease, Johne’s disease (https://doi.org/10.1101/2023.07.07.548088).

Other cardiac biomarkers

Copeptin

Copeptin in the C-terminal fragment of pre-pro-vasopressin, which is much more stable than assaying vasopressin. It was found to be the best predictor of mortality, superior to NTproBNP (Düngen et al. 2018). Other studies have confirmed this, especially as an indicator of outcome in heart failure patients, recently reviewed in a metanalysis (Zimodro et al. 2022). Although salivary copetin has been investigated in dogs with separation anxiety, the author is unaware of any copeptin publications about heart failure in dogs.

Galectin-3

This is over-expressed in heart failure. It is produced by macrophages involved in tissue and fibrous remodelling. High levels predict morbidity in humans (need for hospitalisation) and mortality. It is inferior to NTproBNP in humans, but can be useful in risk stratification (Castiglione et al 2022). It has been investigated in dogs and is increased in dogs with MMVD, reflecting myocardial fibrosis (Sakarin et al. 2016; Lee et al. 2021). However, another study did not identity changes in Galectin-3 in dogs (Klein et al. 2022).

Others

A number of other biomarkers have been used both in the diagnosis of heart failure and risk stratification in humans (Castiglione et al 2022). These include Adrenomedullin (or fragment of its precursor – MR-proADM) and others. **Soluble suppression of tumorigenesis-2 (SsST2** – a marker of inflammation in heart failure) (Myre et al. 2022) has been investigated in dogs (interleukin-1 receptor like 1 protein (ST2) -which has a transmembrane (ST2L as well as the soluble form (sST2) (Klein et al. 2022)), but without significant differences from controls (Klein et al. 2022). **Osteopontin** modulates myocardial remodelling (hypertrophy) as well as remodelling of the extracellular matrix and is proinflammatory (Mamarzhakyov et al 2022). **Cartilage intermediate layer protein-1 (CILP1)** has recently been recognised as being important in human heart disease, and is of prognostic value in heart failure (Wang et al. 2022). A very recent study showed serum levels increased with stage of MMVD in dogs (Kim et al. 2023). It is an extracellular matrix protein and is involved in myocardial fibrosis.

A panel of biomarkers may be useful in the future in veterinary cardiology in risk stratification.

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References

- Bagardi, M., et al. (2022). "Circulating MiR-30b-5p is upregulated in Cavalier King Charles Spaniels affected by early myxomatous mitral valve disease." PLoS One **17**(7): e0266208.
- Borgarelli, M., et al. (2021). "The predictive value of clinical, radiographic, echocardiographic variables and cardiac biomarkers for assessing risk of the onset of heart failure or cardiac death in dogs with preclinical myxomatous mitral valve disease enrolled in the DELAY study." J Vet Cardiol **36**: 77-88.
- Cahill, R. J., et al. (2015). "Analytical validation of a second-generation immunoassay for the quantification of N-terminal pro-B-type natriuretic peptide in canine blood." J Vet Diagn Invest **27**(1): 61-67.
- Castiglione, V., et al. (2022). "Biomarkers for the diagnosis and management of heart failure." Heart Fail Rev **27**(2): 625-643.
- Chan, I. P., et al. (2019). "Serial measurements of cardiac troponin I in heart failure secondary to canine mitral valve disease." Vet Rec **185**(11): 343.
- Chetboul, V., et al. (2009). "Association of plasma N-terminal pro-B-type natriuretic peptide concentration with mitral regurgitation severity and outcome in dogs with asymptomatic degenerative mitral valve disease." J Vet Intern Med **23**(5): 984-994.
- Davarzani, N., et al. (2017). "N-Terminal Pro-B-Type Natriuretic Peptide-Guided Therapy in Chronic Heart Failure Reduces Repeated Hospitalizations-Results From TIME-CHF." J Card Fail **23**(5): 382-389.
- Düngen, H. D., et al. (2018). "Prognostic performance of serial in-hospital measurements of copeptin and multiple novel biomarkers among patients with worsening heart failure: results from the MOLITOR study." ESC Heart Fail **5**(2): 288-296.

- Falk, T., et al. (2013). "Cardiac troponin-I concentration, myocardial arteriosclerosis, and fibrosis in dogs with congestive heart failure because of myxomatous mitral valve disease." J Vet Intern Med **27**(3): 500-506.
- Fox, P. R., et al. (2015). "Relationship of plasma N-terminal pro-brain natriuretic peptide concentrations to heart failure classification and cause of respiratory distress in dogs using a 2nd generation ELISA assay." J Vet Intern Med **29**(1): 171-179.
- Ghilardi, S., et al. (2022). "Prospective pilot study on the predictive significance of plasma miR-30b-5p through the study of echocardiographic modifications in Cavalier King Charles Spaniels affected by different stages of myxomatous mitral valve disease: The PRIME study." PLoS One **17**(12): e0274724.
- Gomart, S., et al. (2020). "Long-term biological variability and the generation of a new reference interval for plasma N-terminal pro-B-type natriuretic peptide in Labrador retrievers." J Small Anim Pract **61**(6): 368-373.
- Häggström, J., et al. (2000). "Relationship between different natriuretic peptides and severity of naturally acquired mitral regurgitation in dogs with chronic myxomatous valve disease." J Vet Cardiol **2**(1): 7-16.
- Harr, K. E., et al. (2022). "Analytical validation of a novel point-of-care immunoassay for canine N-terminal pro-brain natriuretic peptide analysis." Vet Clin Pathol **51**(3): 398-407.
- Hezzell, M. J., et al. (2012). "The combined prognostic potential of serum high-sensitivity cardiac troponin I and N-terminal pro-B-type natriuretic peptide concentrations in dogs with degenerative mitral valve disease." J Vet Intern Med **26**(2): 302-311.
- Hezzell, M. J., et al. (2015). "The effects of storage conditions on measurements of canine N-terminal pro-B-type natriuretic peptide." J Vet Cardiol **17**(1): 34-41.
- Hezzell, M. J., et al. (2018). "Effect of prespecified therapy escalation on plasma NT-proBNP concentrations in dogs with stable congestive heart failure due to myxomatous mitral valve disease." J Vet Intern Med **32**(5): 1509-1516.
- Hulanicka, M., et al. (2014). "Plasma miRNAs as potential biomarkers of chronic degenerative valvular disease in Dachshunds." BMC Vet Res **10**: 205.
- Jang, I. S., et al. (2023). "N-terminal pro-B-type natriuretic peptide levels in normotensive and hypertensive dogs with myxomatous mitral valve disease stage B." Ir Vet J **76**(1): 3.

- Jung, S. and A. Bohan (2018). "Genome-wide sequencing and quantification of circulating microRNAs for dogs with congestive heart failure secondary to myxomatous mitral valve degeneration." Am J Vet Res **79**(2): 163-169.
- Keene, B. W., et al. (2019). "ACVIM consensus guidelines for the diagnosis and treatment of myxomatous mitral valve disease in dogs." J Vet Intern Med **33**(3): 1127-1140.
- Kim, H. J., et al. (2023). "Can cartilage intermediate layer protein 1 (CILP1) use as a novel biomarker for canine myxomatous mitral valve degeneration levels or not?" BMC Vet Res **19**(1): 59.
- Klein, S., et al. (2022). "Evaluation of new and old biomarkers in dogs with degenerative mitral valve disease." BMC Vet Res **18**(1): 256.
- Lee, G. W., et al. (2021). "Circulating Galectin-3 Evaluation in Dogs With Cardiac and Non-cardiac Diseases." Front Vet Sci **8**: 741210.
- Li, Q., et al. (2015). "Expression Profiling of Circulating MicroRNAs in Canine Myxomatous Mitral Valve Disease." Int J Mol Sci **16**(6): 14098-14108.
- Ljungvall, I., et al. (2010). "Cardiac troponin I is associated with severity of myxomatous mitral valve disease, age, and C-reactive protein in dogs." J Vet Intern Med **24**(1): 153-159.
- Mamazhakypov, A., et al. (2022). "Clinical and Molecular Implications of Osteopontin in Heart Failure." Curr Issues Mol Biol **44**(8): 3573-3597.
- Mattin, M. J., et al. (2019)a. "Factors associated with disease progression in dogs with presumed preclinical degenerative mitral valve disease attending primary care veterinary practices in the United Kingdom." J Vet Intern Med **33**(2): 445-454.
- Mattin, M. J., et al. (2019)b. "Prognostic factors in dogs with presumed degenerative mitral valve disease attending primary-care veterinary practices in the United Kingdom." J Vet Intern Med **33**(2): 432-444.
- McLellan, J., et al. (2016). "B-type natriuretic peptide-guided treatment for heart failure." Cochrane Database Syst Rev **12**(12): CD008966.
- Myhre, P. L., et al. (2022). "Changes in cardiac biomarkers in association with alterations in cardiac structure and function, and health status in heart failure with reduced ejection fraction: the EVALUATE-HF trial." Eur J Heart Fail **24**(7): 1200-1208.
- Miyagawa, Y., et al. (2013). "Relationship between glomerular filtration rate and plasma N-terminal pro B-type natriuretic peptide

concentrations in dogs with chronic kidney disease." Vet J **197**(2): 445-450.

- Misbach, C., et al. (2013). "Basal plasma concentrations of N-terminal pro-B-type natriuretic peptide in clinically healthy adult small size dogs: effect of body weight, age, gender and breed, and reference intervals." Res Vet Sci **95**(3): 879-885.
- Oyama, M. A., et al. (2013). "Clinical usefulness of an assay for measurement of circulating N-terminal pro-B-type natriuretic peptide concentration in dogs and cats with heart disease." J Am Vet Med Assoc **243**(1): 71-82.
- Pelander, L., et al. (2017). "Cardiac Troponin I and Amino-Terminal Pro B-Type Natriuretic Peptide in Dogs With Stable Chronic Kidney Disease." J Vet Intern Med **31**(3): 805-813.
- Polizopoulou, Z. S., et al. (2014). "Serial analysis of serum cardiac troponin I changes and correlation with clinical findings in 46 dogs with mitral valve disease." Vet Clin Pathol **43**(2): 218-225.
- Polizopoulou, Z. S., et al. (2015). "Correlation of serum cardiac troponin I and acute phase protein concentrations with clinical staging in dogs with degenerative mitral valve disease." Vet Clin Pathol **44**(3): 397-404.
- Reis-Ferreira, A., et al. (2022). "Emerging Roles of MicroRNAs in Veterinary Cardiology." Vet Sci **9**(10).
- Ro, W. B., et al. (2021). "Identification and Characterization of Circulating MicroRNAs as Novel Biomarkers in Dogs With Heart Diseases." Front Vet Sci **8**: 729929.
- Sakarin, S., et al. (2016). "Galectin-3 in cardiac muscle and circulation of dogs with degenerative mitral valve disease." J Vet Cardiol **18**(1): 34-46.
- Serres, F., et al. (2009). "Plasma N-terminal pro-B-type natriuretic peptide concentration helps to predict survival in dogs with symptomatic degenerative mitral valve disease regardless of and in combination with the initial clinical status at admission." J Vet Cardiol **11**(2): 103-121.
- Sjöstrand, K., et al. (2014). "Breed differences in natriuretic peptides in healthy dogs." J Vet Intern Med **28**(2): 451-457.
- Wall, L., et al. (2018). "Clinical use of submaximal treadmill exercise testing and assessments of cardiac biomarkers NT-proBNP and cTnI in dogs with presymptomatic mitral regurgitation." PLoS One **13**(6): e0199023.
- Wang, C., et al. (2022). "Prognostic value of cartilage intermediate layer protein 1 in chronic heart failure." ESC Heart Fail **9**(1): 345-352.

- Wilshaw, J., et al. (2021). "Accuracy of history, physical examination, cardiac biomarkers, and biochemical variables in identifying dogs with stage B2 degenerative mitral valve disease." J Vet Intern Med **35**(2): 755-770.
- Winter, R. L., et al. (2017a). "Biologic variability of N-terminal pro-brain natriuretic peptide in healthy dogs and dogs with myxomatous mitral valve disease." J Vet Cardiol **19**(2): 124-131.
- Winter, R. L., et al. (2017b). "Biologic variability of cardiac troponin I in healthy dogs and dogs with different stages of myxomatous mitral valve disease using standard and high-sensitivity immunoassays." Vet Clin Pathol **46**(2): 299-307.
- Wolf, J., et al. (2012). "Lowered N-terminal pro-B-type natriuretic peptide levels in response to treatment predict survival in dogs with symptomatic mitral valve disease." J Vet Cardiol **14**(3): 399-408.
- Wolf, J., et al. (2013). "The diagnostic relevance of NT-proBNP and proANP 31-67 measurements in staging of myxomatous mitral valve disease in dogs." Vet Clin Pathol **42**(2): 196-206.
- Yang, V. K., et al. (2017). "Circulating exosome microRNA associated with heart failure secondary to myxomatous mitral valve disease in a naturally occurring canine model." J Extracell Vesicles **6**(1): 1350088.
- Yang, V. K., et al. (2018). "Dysregulation of valvular interstitial cell let-7c, miR-17, miR-20a, and miR-30d in naturally occurring canine myxomatous mitral valve disease." PLoS One **13**(1): e0188617.
- Zelenak, C., et al. (2019). "Early NT-proBNP and MR-proANP associated with QoL 1 year after acutely decompensated heart failure: secondary analysis from the MOLITOR trial." Biomark Med **13**(17): 1493-1507.
- Zimodro, J. M., et al. (2022). "Role of copeptin in diagnosis and outcome prediction in patients with heart failure: a systematic review and meta-analysis." Biomarkers **27**(8): 720-726.

The usefulness of 3D TEE in mitral regurgitation in human

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Introduction

There are multiple fortunate circumstances that make 3D TOE the “ideal” technique for mitral valve (MV) imaging. First, the nearly perpendicular angle of incidence between the pyramidal beam and mitral leaflets. Hence, the 3D image of the valve is reconstructed mostly using axial resolution with the highest spatial resolution (see section of physics). Second, the short distance between the valve and the TOE transducer coupled with the absence of ribs and lungs. Both circumstances allow the use of high frequency transducers without artefacts and 3D image distortion.
Third, the ability of 3D TOE volumetric data set to be rotated in any direction depicting the valve from a countless number of perspectives, the most popular being the view from overhead (the so-called surgical view). A correct interpretation of the wide spectrum of MV diseases requires the knowledge of the 3D TOE features of normal MV anatomy.

Normal MV anatomy

The main components of the mitral valve (MV) apparatus are the mitral annulus (MA), the anterior (AML) and posterior (PML) mitral leaflets, the chordae tendineae, and the papillary muscles (PMs). These components work together with a delicate balance of spatial and temporal coordination, preventing systolic leakage and allowing an unrestricted diastolic inflow.
3D TOE offers impressive, high-quality images of the normal MV anatomy.

Mitral valve regurgitation

Introduction

Mitral valve regurgitation (MVR) is currently the most common disorder in developed countries affecting 2-3% of the general population. Moreover, due to aging and growth of the population, the prevalence of MVR is likely to further increase in the coming decades.

Mitral valve regurgitation

MVR can be classified as ***primary*** or ***organic,*** defined as a primary abnormality of the MVV apparatus and ***secondary*** or ***functional***, defined as a disease of the atrium (atriogenic MVR) or ventricle (ventricular MVR) causing MVR which is not caused by intrinsic MV disease but rather secondary to changes in the atrium or ventricle

Primary (organic, degenerative) MR

The primary MR, includes a wide spectrum of pathological conditions affecting primarily MV leaflets and chordae tendineae. This lesion corresponds at type II Carpentier functional classification. Four morphological phenotypes based on the progression of redundancy of the valve tissue have been described[21]: 1) fibroelastic deficiency, 2) fibroelastic deficiency plus, 3) Forme fruste and 4) Barlow disease. While 2D TTE/TOE is essential as the first line imaging technique, 3D TOE provides exquisite images of these four phenotypes

Chronic secondary (Functional) MR

Chronic secondary(functional) MR is usually due to an imbalance between two competing forces: trans-mitral closure forces which are generated by intraventricular pressure and tethering forces exerted by the displaced PMs. Two subgroups of patients can be distinguished on the basis of the tethering patterns, different degree of local and global LV remodelling and characteristics of the regurgitant jet: the asymmetric tethering patten and the “symmetric” tethering pattern[22]. These lesions correspond to Carpentier IIIB functional classification. Isolated LA dilatation due to long standing atrial fibrillation (AF) may cause functional MR. This phenotype has been named “atrial” functional MR[24] and corresponds to Carpentier type I functional classification.

In both cases the leaflets move apart each other resulting in incomplete coaptation and regurgitation.

2D TOE *is essential* for defining the pathophysiological mechanism, while 3D TOE may add supplementary qualitative/quantitative data.