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Diagnostic Utility of Pentraxin-3 Compared With N-terminal-prohormone of B-Type Natriuretic Peptide (Nt-probnp) and Tumour Necrosis Factor-α (Tnf-α) in Dogs With Degenerative Mitral Valve Disease

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DIAGNOSTIC UTILITY OF PENTRAXIN-3 COMPARED WITH N-TERMINAL-PROHORMONE OF B-TYPE NATRIURETIC PEPTIDE (NT-ProBNP) AND TUMOUR NECROSIS FACTOR-A (TNF-A) IN DOGS WITH DEGENERATIVE MITRAL VALVE DISEASE

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ABSTRACT

Pentraxin-3 (PTX3) is a novel biomarker of interest in cardiovascular disease and injury. Despite this, there is limited literature on PTX3 as a veterinary biomarker. Another ubiquitous inflammatory marker, tumour necrosis factor-alpha (TNF-α), has been studied extensively in dogs with DMVD, with mixed findings. The N-terminal-prohormone of B-type natriuretic peptide (NT-proBNP) is a routinely used biomarker of canine cardiac dysfunction. This study compared the diagnostic utility of PTX3 with TNF-α and NT-proBNP in dogs with DMVD. Serum NT-proBNP, PTX3, and TNF-α were measured in 50 client-owned dogs diagnosed and staged according to the ACVIM stages A-D. Echocardiographic variables, radiographic vertebral heart score (VHS) and vertebral left atrial size (VLAS), systolic blood pressure (SBP), and quality of life (QoL) scores were assessed. The Kruskal-Wallis test was used to compare PTX3, TNF-α, and NT-proBNP levels between different stages of DMVD, and the Jonckheere-Terpstra test assessed whether serum biomarker concentrations showed a significant ordered trend across progressively worsening DMVD across stages. Spearman's correlation was used to assess the associations between biomarker levels, disease stage, QoL scores, SBP, radiographic, and echocardiographic parameters. Concentration of PTX3 and TNF-α did not differ significantly across DMVD stages, while only NT-proBNP showed significant variation between stages ($p=0.02$). Jonckheere-Terpstra trend test revealed a significant ordered trend in serum NT-proBNP ($p<0.01$), but not PTX3 and TNF-α across DMVD stages. NT-proBNP correlated significantly with DMVD stage, QoL score, VHS, VLAS, and echocardiographic variables including LVIDdn, LA:Ao ($p<0.01$), and EF%, FS% ($p=0.02$). NT-proBNP remains a reliable biomarker for diagnosis, disease monitoring, and prognosis assessment in canine DMVD. Future studies employing larger longitudinal cohorts will be essential to better elucidate the role of inflammatory markers in canine heart disease.

Keywords: Pentraxin-3, NT-proBNP, DMVD, mitral valve, inflammation

Introduction

Degenerative mitral valve disease (DMVD) is the most common acquired cardiac condition in dogs, characterised by progressive myxomatous degeneration of the mitral valve leaflets and chordae tendineae. This structural deterioration leads to mitral regurgitation, left atrial and ventricular volume overload, and eventual cardiac remodelling. Over time, these changes can result in the onset of congestive heart failure (CHF), significantly impacting morbidity and mortality in affected dogs (Keene et al. 2019). The aetiology and progression of DMVD, especially concerning the role of inflammation in its pathophysiology, warrant further investigation.

Currently, the N-terminal-prohormone of B-type natriuretic peptide (NT-proBNP) is routinely used in practice to support the diagnosis of heart disease in dogs (Oyama et al. 2008; Oyama et al. 2013; Boswood et al. 2008). NT-proBNP production is stimulated by myocardial stretch secondary to volume overload (Oyama et al. 2009).

TNF-α is a pro-inflammatory cytokine produced by activated macrophages, which is involved in proliferation, differentiation, necrosis, and apoptosis (Parameswaran and Patial 2010). An increased TNF-α expression has been observed in the atrium and ventricle of dogs with advanced DMVD and in dogs with CHF (Fonfara et al. 2013; Nemec-Svete et al. 2021). In contrast, other studies reported no significant association with disease stage or cardiac remodelling indices (Rubio et al. 2020). Despite these inconsistencies, inflammation was increasingly recognised as a contributor to DMVD progression (Aupperle and Disatian 2012), and TNF-α was being explored as both a therapeutic target and biomarker, with agents such as pimobendan shown to reduce its myocardial expression in experimental models (Iwasaki et al. 1999).

PTX3 is an acute-phase protein that plays a broader role in inflammation and extracellular matrix remodelling (Garlanda et al. 2018). Unlike hepatocyte-derived CRP, PTX3 is produced locally at sites of tissue injury and responds earlier, making it a potentially more sensitive early indicator of cardiac inflammation. Increased PTX3 expression or receptor activity at the cardiac valves or the endocardium may contribute to the progression of DMVD. As PTX3 is produced during local cardiac inflammation, rising serum PTX3 concentrations could represent a sensitive early biomarker of disease progression in DMVD.

In human medicine, a multi-marker strategy has been adopted to identify and estimate the prognosis of cardiopathy (Zethelius et al. 2008; Bayes-Genis et al. 2015). In contrast, no validated multi-marker panel currently exists for canine DMVD. The identification of reliable predictors of DMVD progression and adverse cardiac outcomes in dogs remains an unmet clinical need. Given that inflammation plays an emerging role in DMVD progression, cytokine biomarkers such as pentraxin (PTX3) and tumour necrosis factor-α (TNF-α) represent possible candidates for a multi-marker panel in dogs. Therefore, this study compared serum PTX3, TNF-α, and NT-proBNP concentrations across ACVIM disease stages in dogs with DMVD, and assessed the correlations with clinical, echocardiographic, and quality of life parameters.

Compliance with Ethical Standards

The study protocol and procedures were conducted following the approval of the Institutional Animal Care and Use Committee (UPM/IACUC/AUP-R014/2019) of Universiti Putra Malaysia (UPM). Informed consent was obtained from the dog owners with dogs diagnosed with DMVD before recruitment.

Diagnosis, inclusion, and exclusion criteria of DMVD dogs

According to the American College of Veterinary Internal Medicine (ACVIM) guidelines for staging dogs with DMVD (Keene et al. 2019), all recruited dogs were further categorised into groups (Stage A, B1, B2, C, and D). Dogs in stage A had a genetic predisposition to DMVD but no identifiable structural disease at the time of sampling. Dogs in stages B1 and B2 exhibited no clinical signs (pre-clinical) but had an audible murmur. Dogs in stage B1 had no evidence of cardiomegaly, while dogs in stage B2 had evidence of cardiomegaly. Dogs in stages C and D had clinical signs of congestive heart failure (CHF), with stage D dogs being in the end-stage and refractory to treatment.

Inclusion criteria for predisposed breeds (Stage A) included unremarkable physical examination findings, normal blood profile (haematology and serum biochemistry) with no underlying comorbidities, normal radiographic and echocardiographic findings, and negative screening for heartworm disease.

The DMVD dogs fulfilled the criteria as follows: 1) adult or senior dogs weighing <15 kg; 2) had not received any treatment for the diagnosis of DMVD; 3) had no significant comorbidities based on routine blood test; 4) a

systolic murmur grade II to VI localised to the mitral valve region; 5) normal lungs for dogs with pre-clinical DMVD or abnormal lung sound with evidence of interstitial and/or alveolar patterns suggestive of cardiogenic pulmonary oedema for dogs with DMVD stage C and D; 6) radiographic and echocardiographic imaging with complete findings supporting the diagnosis of DMVD, including cardiomegaly and evidence of mitral valve changes (i.e. mitral leaflet thickening, with or without prolapse or ruptured chordae) respectively. Dogs with tricuspid insufficiency assumed to be secondary to DMVD were also included. Dogs were excluded if they had any concurrent cardiac or non-cardiac condition at the time of inclusion.

Baseline data collection

Baseline data were recorded for all dogs. Information on history, clinical signs, physical examination, auscultation, radiology, complete haematology, serum biochemistry, systolic blood pressure, cardiac biomarkers, heartworm disease status using SNAP 4Dx Plus test kit (IDEXX Laboratories, Inc., USA), and echocardiography was obtained.

Clinical signs and quality of life (QoL)

Patient history was collected based on owner-reported observations regarding appetite, demeanour, exercise tolerance, coughing, and increased respiratory effort, which were scored accordingly (Table i). All evaluations, diagnoses, and treatments were conducted by a single licensed veterinarian with extensive experience in veterinary cardiology. The severity of clinical signs in dogs diagnosed with DMVD stages C and D was assessed using an ordinal scoring system adapted from previous studies (Table i), reflecting the worsening QoL with increasing total scores. A second veterinarian independently carried out the QoL scoring using a modified system adapted from Häggström et al. (2013).

The original instrument includes scores for nocturnal dyspnoea, pulmonary oedema, and modified New York Heart Association (NYHA) heart failure classification (White and Myers, 1921). In the present study, these were excluded to focus on clinical signs readily recognisable by owners that are directly associated with day-to-day patient well-being.

Auscultation

Heart rate, rhythm, pulse deficits, and murmur grade (I–IV) at the mitral valve region were recorded as previously described (Gompf, 1988). Respiratory rate and lung sounds were also documented.

Averaged systolic blood pressure (SBP) measurement

The dog was gently restrained, and SBP was obtained using an automated oscillometric device (CARESCAPE™ V100 Vital Signs Monitor, GE Healthcare, USA) in a quiet room with limited distractions. An appropriately sized cuff was placed around the mid-antebrachium, and an average of five systolic blood pressure measurements was used for analysis.

Blood sampling

Approximately 3 ml of blood was collected via venipuncture and immediately stored in plain and ethylenediaminetetraacetic acid K2EDTA tubes (BD Vacutainer®, Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Complete blood count and serum biochemical analyses were performed using a ProCyte Dx Haematology Analyzer and a Catalyst One Chemistry Analyzer, respectively (IDEXX Laboratories, Inc., Westbrook, ME, USA) to rule out any comorbidities. The remaining blood samples were refrigerated temporarily at 4°C and centrifuged within 1 hour of collection.

Measurement of serum biomarkers

Serum NT-proBNP was measured immediately using the Vcheck V200 analyser (Bionote, Korea). The test strip was analysed immunochromatographically by the machine and the results presented immediately as a quantitative value of the analyte concentration. The remaining serum and plasma were then stored at -80°C until further analysis for TNF-α and PTX3. Commercial cytokine ELISA kits were used to measure TNF-α (ab193687, Abcam, Cambridge, MA, USA) and PTX3 (MBS046136, Mybiosource, San Diego, CA, USA) concentrations in the serum of all dogs at each time point. The detection limit of TNF was 2 pg/mL, and the intra- and inter-assay coefficient of variability (CV) were <10 and <15%, respectively. For PTX3, the minimum detection limit is 0.25 ng/mL, with

intra- and inter-assay CVs of <15%. Absorbance was assessed at 450 nm with a microplate reader (Tecan Infinite F50; Tecan Group Ltd., Männedorf, Switzerland).

Radiography

The dogs were gently restrained to obtain images in right lateral and dorsoventral positioning. The images were assessed for signs of congestive heart failure such as pulmonary oedema, blood vessel enlargement, and abnormalities of the cardiac silhouette or surrounding structures (Keene et al. 2019). The Vertebral Heart Score (VHS) and Vertebral Left Atrial Size (VLAS) were measured as previously described (James 2000; Malcolm et al. 2018).

Echocardiography

Dogs were gently restrained and positioned on right and left lateral recumbency for echocardiography. Transthoracic echocardiography was performed using a MyLab X90VET ultrasound machine and a P2 phased-array transducer (5–13 MHz) (Esaote SpA, Genoa, Italy) to assess cardiac function and structure. Two-dimensional images were obtained in the parasternal short-axis view, from which M-mode tracings were recorded. Measurements of interventricular septal (IVS) thickness, left ventricular posterior wall (LVPW) thickness, and left ventricular internal dimensions at end-diastole (LVEDD) and end-systole (LVESD) were taken digitally from the M-mode images and averaged over five consecutive cardiac cycles. The left atrial-to-aortic root ratio (LA:Ao), left ventricular fractional shortening, and left ventricular internal diastolic diameter normalised for body weight (LVIDdn) were also recorded. The E and A wave measurements and velocity of transvalvular blood flow at the mitral valve were obtained in the left parasternal apical view. Images were analysed by an experienced clinician (KKH).

Statistical Analysis

Data collected were tabulated in Microsoft Excel and descriptively analysed using MedCalc version 19.4 and 23.2.1 (MedCalc Software, Belgium), and results were presented as frequencies and percentages. Based on the Shapiro-Wilks test, the continuous data were not normally distributed; therefore, data were reported as median and interquartile range, and non-parametric tests were used. Serum biomarker concentrations below the assay detection limit were substituted with half the lower limit of detection (LOD/2) for continuous analysis (Hornung and Reed 1990). For PTX3, values below 0.25 ng/mL were substituted with 0.125 ng/mL, and for NT-proBNP, values below 500 pmol/L were substituted with 250 pmol/L. Concentrations above the upper limit of detection were assigned the upper LOD value. The Kruskal-Wallis test was used to compare PTX3, TNF-α, and NT-proBNP levels across DMVD stages, with post-hoc pairwise comparisons performed using Conover's test. The Jonckheere-Terpstra test assessed whether serum biomarker concentrations followed a meaningful ordered increase with advancing disease severity. Spearman's correlation assessed the relationship between biomarker levels and stage of disease, QoL scores, SBP, VHS, VLAS, LA:Ao, EF%, FS%, and LVIDdn. A p-value of <0.05 was considered significant.

Results

A total of 50 dogs were recruited. Nine healthy dogs were classified into stage A. Seven dogs were in stage B1, 11 dogs in stage B2, 13 dogs in stage C, and 10 dogs in stage D. The majority of dogs were recruited were male (32/50, 64%), and the most common breed was the Shih Tzu (n=15, 30%), the mean age of dogs ranged from 1 to 16 years (mean 9.72 years) and body weight ranged from 3.0 to 15.0 kg (mean 7.37 kg; Table ii).

Since serum TNF-α concentrations were below the assay detection limit in the majority of samples across all time points, TNF-α was analysed as a binary variable (detectable vs undetectable) using chi-squared test across disease stages (Table iii). Continuous analysis using LOD/2 substitution was subsequently performed but interpreted with caution given the high proportion of substituted values.

No significant association between TNF-α detectability (BDL vs detectable) and disease stage. The serum level of TNF-α was below the detection limit for majority of the dogs (68% of all cases). The distribution of detectable vs undetectable TNF-α is similar across all disease stages (A, B1, B2, C, D), meaning TNF-α does not discriminate between disease stages at this time point (Table iii).

Serum NT-proBNP concentrations differed significantly across DMVD stages and demonstrated a significant positive trend with disease progression (Kruskal-Wallis: H= 12.15, p<0.01; J onckheere-Terpstra: test statistic= 681.00, z= 3.19). NT-proBNP increased progressively from stage A (17.94) through B1 (21.21), B2 (22.14), C (25.77) and stage D (38.65), indicating higher NT-proBNP concentrations in more advanced disease stages. In contrast, no significant differences or monotonic trends were identified for TNF-α (H= 0.53, J-T= 482.5, z = -0.21) or PTX3 (H= 3.36, J-T= 429.50, z= -1.12). Mean ranks varied minimally across stages, and although not statistically significant, the highest ranks were in stage B1 (28.1 and 33.9 respectively) and lower in later stages, including stage D (24.4 and 23.4 respectively). The median PTX3 was also highest in stage B1 dogs at 0.94 ng/mL, as compared to other stages (Table iv).

Overall, NT-proBNP showed the clearest relationship with disease progression, whereas TNF-α and PTX3 demonstrated substantial interindividual variability and less reliable patterns across stages. One dog in stage D with serum NT-proBNP >10,000 pmol/L was excluded from the NT-proBNP graph (Fig. i) for visual clarity. The value was included in all statistical analyses as it may represent true biological variability and therefore remains important for accurate statistical interpretation.

NT-proBNP levels had moderate positive correlations with DMVD stage (ρ= 0.46, p= <0.01), QOL score (ρ= 0.46, p= <0.01), VHS (ρ= 0.40, p= <0.01), VLAS (ρ= 0.40, p= <0.01), LVIDdn (ρ= 0.41, p= <0.01), EF% (ρ= 0.33, p= 0.02), and LA/Ao (ρ= 0.57, p= <0.01). TNF-α levels showed no significant correlations with most clinical variables, except for a weak negative correlation with VLAS (ρ= -0.29, *p*=0.048). PTX3 was not significantly associated with any of the measured variables (Table v).

Discussion

DMVD represents a major cause of acquired cardiac morbidity and is a leading cause of congestive heart failure (CHF) in dogs (Keene et al. 2019; Noordin et al. 2023). Although its structural and haemodynamic consequences are well recognised, the mechanisms driving disease progression are not yet fully understood; inflammation may play a role in valvular degeneration and cardiac remodelling associated with DMVD (Sakarin 2023; Grzeczka et al. 2025).

Among the evaluated biomarkers, NT-proBNP showed the clearest association with DMVD progression, with concentrations increasing consistently across disease stages. Conversely, TNF-α and PTX3 did not demonstrate significant stage-dependent trends and exhibited considerable inter-individual variability. The negative Jonckheere-Terpstra z-statistic reflected a trend opposite to the hypothesised direction for PTX3, largely due to comparatively higher ranks in stage B1 than in the more advanced stages. A similar but less pronounced pattern was observed for TNF-α. This is an interesting observation suggesting that both markers may indicate transient inflammatory activity during earlier phases of DMVD rather than progressive increases with advancing disease severity.

Multiple human studies have demonstrated PTX3 to be a useful prognostic biomarker in heart failure with both reduced and preserved ejection fraction (Kotooka et al. 2008; Suzuki et al. 2008; Matsubara et al. 2011; Matsubara et al. 2014). Unlike hepatocyte-derived CRP, PTX3 is produced locally by stromal and myeloid cells, including endothelial cells, macrophages, and neutrophils, in response to IL-1β, TNF-α, or microbial stimuli (Ristagno 2019). It also peaks earlier than CRP (at 6-8 h vs. 24-48 h), reflecting its role in early innate immunity, and can be rapidly released from neutrophil granules upon tissue injury or infection (Jaillon 2007). Beyond acute inflammation, PTX3 supports matrix remodelling and shows cardioprotective effects in murine myocardial infarction by reducing leukocyte infiltration, tissue damage, apoptosis, and complement activation (Salio 2008). PTX3 has previously shown a weak correlation with BNP but outperforms it in predicting adverse outcomes (Suzuki et al. 2008). Serial measurements over three months showed an independent association with heart failure severity, and both baseline levels and their changes successfully predict mortality (Latini et al. 2012). The role of inflammation in heart failure nonetheless remains incompletely understood, with elevated cytokines and C-reactive proteins recognised as poor prognostic indicators (Ristagno et al. 2019).

The absence of an increasing PTX3 trend with the severity of canine DMVD as seen in this study may reflect its nature as an acute-phase reactant rather than a chronic marker of cardiac remodelling. Following acute myocardial infarction in humans, plasma PTX3 peaks within 8 hours and returns to baseline within 3 days (Latini et al. 2004; Ristagno et al. 2019). In the context of chronic disease such as canine DMVD, without continuous production, sampling may not capture transient elevations. Elevated PTX3 in early-stage dogs may represent an initial

inflammatory response to myxomatous valve degeneration, and future research with focused longitudinal sampling of dogs in the preclinical stages are required to assess this. Additionally, inflammatory cell infiltration has been observed in the left atrial myocardium secondary to mitral valve avulsion and atrial injury that occurs most frequently in cases of severe valvular dysfunction (Lee et al. 2015). Such complications did not appear in this study population, suggesting that PTX3 has limited relevance in uncomplicated DMVD, reducing its potential as a biomarker in the early stages.

Stage B1 represents the earliest detectable phase of DMVD, whereby dogs have an audible murmur but lack cardiomegaly or clinical signs, suggesting that myxomatous degeneration exists at the valvular level without yet producing meaningful haemodynamic consequences. Despite not reaching statistical significance, both PTX3 and TNF-α were numerically higher in our population at this stage. Several lines of evidence support the hypothesis that inflammatory activity in DMVD may be most pronounced in this early pre-remodelling window. TNF-α, IL-1β, and IL-6 increased significantly with disease severity in MMVD dogs, with a positive correlation observed between IL-6 and left ventricular diastolic volume, suggesting that cardiac remodelling associated with progressive volume overload may be linked to inflammation (Piantedosi et al. 2022).

A study by Klein et al. (2022) investigating ST2 (IL1RL1) found a significant increase in serum ST2 concentrations from baseline to six-month follow-up specifically in stage B1 dogs and controls, despite no statistically significant difference between disease stages at any single time point. PTX3 and ST2 are both upregulated in response to inflammation and associated with cardiac stress and tissue damage. Although they do not directly interact, both share overlapping pathways in cardiovascular disease, and their co-elevation in conditions such as heart failure and systemic inflammation suggests they may serve as complementary biomarkers. Early-stage inflammatory activity may precede structural remodelling, and stage B1 may represent a window where PTX3 is transiently elevated. While small and unequal group sizes may have contributed to this observation, the trend warrants further investigation.

Pro-inflammatory cytokines (TNF-α, IL-1, and IL-6) and vasoconstrictor cytokines such as endothelin-1 have been implicated in heart failure (Seta et al. 1996). In human patients, elevated circulating TNF-α has been associated with ventricular remodelling, dysfunction, and cardiomyopathy and has been investigated as a potential therapeutic target and biomarker in human cardiology (Feldman et al. 2000). Oral pimobendan has been shown to reduce myocardial TNF-α expression in murine viral myocarditis (Feldman and McNamara 2000).

In this study, there was no statistically significant trend in serum levels of canine PTX3 or TNF-α across the different stages of DMVD. The role of inflammation in DMVD is controversial, though recent studies have supported the importance of inflammatory mediators in the progression of valvular disease to CHF (Aupperle and Disatian 2012). Findings on serum TNF-α in canine cardiovascular disease have been mixed. TNF-α levels were shown to be significantly elevated in dogs with CHF compared to those without it in a cohort including both DMVD and dilated cardiomyopathy patients (Nemec Svete et al. 2021). In contrast, another study found no significant differences in IL-6, TNF-α, or other cytokines across ACVIM stages, nor any correlation between these cytokines and echocardiographic indicators of cardiac remodelling (Rubio et al. 2020).

TNF-α concentrations were frequently below the assay detection limit across all disease stages and time points in this study, precluding meaningful continuous analysis. Elevated myocardial TNF-α expression has been reported in dogs with advanced DMVD and CHF (Fonfara et al. 2013; Nemec Svete et al. 2021); however, serum TNF-α may not accurately represent myocardial inflammatory activity in this disease. Alternatively, the assay sensitivity used in this study may have been insufficient to detect clinically relevant elevations. Furthermore, TNF-α protein is frequently undetectable in serum by ELISA, even when a significant increase in gene expression has been documented, suggesting that serum measurements may not reliably capture true inflammatory activity at the tissue level (Rana et al. 2012).

The weak negative correlation observed between serum TNF-α and VLAS (ρ= -0.29, p= 0.048) should be interpreted with considerable caution. VLAS is a validated radiographic measure of left atrial size that increases positively with advancing DMVD and is strongly correlated with echocardiographic indices of cardiac remodelling (Malcolm et al. 2018). A true biological inverse relationship between circulating TNF-α and left atrial enlargement would be unexpected and is not supported by existing literature in either canine or human cardiovascular disease. More plausibly, this finding reflects a statistical artefact introduced by the high proportion of TNF-α values below the detection limit (68% across all stages). Accordingly, the negative TNF-α-VLAS correlation is most likely a methodological artefact and should not be interpreted as evidence of a true inverse

biological relationship. Future studies using more sensitive multiplex assays would be necessary to reliably characterise any relationship between circulating TNF-α and measures of cardiac remodelling in canine DMVD.

These findings contrast with human studies describing notable elevations in both PTX3 and TNF-α in cardiomyopathy, where PTX3 is a strong predictor of adverse cardiac events and an early marker of heart failure (Suzuki et al. 2008; Mueller et al. 2006; Vasan et al. 2003). This interspecies variance may reflect the different aetiology of heart failure in dogs and humans. In small breed dogs, DMVD is the most common cause of heart failure worldwide (Keene et al. 2019), characterised by gradual myxomatous thickening of the atrioventricular valve over a long period, with minimal inflammatory changes histologically (Markby et al. 2017). In humans, ischaemic cardiomyopathy due to coronary artery disease is the leading precursor to congestive heart failure, followed by hypertension, viral infection, and toxin exposure (Torre-Amione 2005; Malik et al. 2023). Following myocardial infarction, inflammatory cellular infiltration of the myocardium and elevated circulating inflammatory biomarkers are well documented (Torre-Amione 2005).

NT-proBNP stood out as the only biomarker to behave as hypothesised, with serum concentrations rising progressively across disease stages. The mean rank climbed from 17.94 in stage A to 38.65 in stage D. This trend was confirmed by the Jonckheere-Terpstra test, indicating a statistically significant, ordered relationship across the advancing stages of DMVD. Spearman correlation analysis also demonstrated that NT-proBNP showed significant associations with DMVD stage, quality of life score, radiographic parameters (VHS, VLAS), and echocardiographic parameters (LVIDdn, EF%, FS%, and most notably LA:Ao). This last finding carries clinical weight, as LA:Ao ratio is among the major echocardiographic thresholds used to guide treatment decisions (Keene et al. 2019). Taken together, these findings support NT-proBNP as a reliable, multi-dimensional biomarker capable of supporting diagnosis and ongoing monitoring in canine DMVD. However, further research is needed to investigate the possibility that inflammatory cytokines may potentially serve as early-detection biomarkers before structural or haemodynamic changes become apparent.

Conclusion

This study demonstrated that NT-proBNP remains the most reliable biomarker for the diagnosis, staging, and prognostication of DMVD in dogs. NT-proBNP concentrations correlated strongly with clinical severity, echocardiographic indices, and quality-of-life scores. In contrast, serum TNF-α and PTX3 concentrations did not differ significantly between disease stages, nor were they associated with clinical, or echocardiographic outcomes. A modest rise in PTX3 was noted in stage B1 dogs and controls, but its biological significance is unclear. These findings highlight interspecies differences in the role of inflammatory biomarkers, as PTX3 and TNF-α are established indicators of adverse outcomes in human cardiomyopathies but appear to have limited utility in this cross-sectional cohort of canine DMVD.

Further studies with larger cohorts, more frequent follow-up, and more sensitive inflammatory panels are warranted to clarify whether other cytokines may complement NT-proBNP in the management of canine heart disease, an area that warrants dedicated investigation in future studies.

Limitations

This study has several limitations. Recruitment was based on patients presenting to the University Veterinary Hospital, resulting in unequal stage distribution, with relatively few dogs in stage B1. Sex-related differences were not analysed, and the population varied in age. Because dogs were enrolled upon hospital presentation, neither the duration of DMVD nor the time spent in each stage before enrolment was known. Additionaly, in humans, plasma PTX3 peaks within approximately 8 hours following acute myocardial infarction and returns to baseline within 3 days (Latini et al. 2004; Ristagno et al. 2019). Although the circulating half-life of PTX3 has not been established in dogs, these findings may indicate that sampling did not align with peak concentrations. Future research should focus on larger cohorts, particularly dogs with more advanced DMVD, and distinguish between acute and chronic clinical presentations.

The relatively high proportion of TNF-α values below the detection limit in early disease stages may have reduced the precision of estimates; therefore these results should be interpreted with caution In this cohort, TNF-α demonstrated limited utility as a circulating biomarker, largely due to assay sensitivity constraints. Further studies using more sensitive detection methods are warranted to better clarify the role of inflammation in DMVD.

Statements and Declarations

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Conflict of Interest

All the authors declare no conflicts of interest or influence in the outcome of the findings.

Animal Ethics and Consent to Participate Declarations

The study protocol and procedures were conducted following the approval of the Institutional Animal Care and Use Committee (UPM/IACUC/AUP-R014/2019) of Universiti Putra Malaysia (UPM).

Informed consent

Informed consent was obtained from all dog owners before recruitment, including consent for publication.

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Tables and Figures

Table i: Quality of life scoring system

Variables	Score	Description
Appetite	1	Increased
	2	Normal
	3	Decreased (2/3 normal)
	4	Markedly decreased (<2/3 normal)
Demeanour	1	Alert, responsive
	2	Mildly lethargic
	3	Moderately lethargic
	4	Minimally responsive
	5	Unresponsive
Exercise tolerance	1	The dog moved around with ease and was able to exercise fully.
	2	Dog moved around with ease, not able to fully exercise; ability to run reduced.
	3	The dog was less active than normal, moved around a few times per day, avoided long walks.
	4	The dog was inactive and would only get up to eat, drink, or urinate.
Respiratory effort	1	Normal
	2	Mildly increased respiratory rate or effort
	3	Moderately laboured
	4	Severe respiratory distress

Cough	1	None
	2	Occasional (a few times a week)
	3	Frequent (a few times a day)
	4	Persistent (frequently during the day)

Table ii: Signalment of the DMVD dogs in each stage based on sex, breed, age, and bodyweight

DMVD Stages		**A**	**B1**	**B2**	**C**	**D**	Total
n		9 (18%)	7 (14%)	11 (22%)	13 (26%)	10 (20%)	50 (100%)
Sex	Castrated male	2	1	2	6	2	13 (26%)
	Intact male	4	3	5	3	1	19 (38%)
	Spayed female	2	2	4	2	2	12 (24%)
	Intact female	1	1	0	2	2	6 (12%)
Breed	Shih tzu	3	2	3	3	4	15 (30%)
	Mixed	3	2	0	3	2	10 (20%)
	Toy poodle	1	0	1	2	2	6 (12%)
	Miniature Schnauzer	1	2	2	0	0	5 (10%)
	Miniature Pinscher	0	0	0	2	1	3 (6%)
	Pomeranian	0	0	1	2	0	3 (6%)
	Cavalier Spaniel	1	0	0	0	0	1 (2%)
	Dachshund	0	0	1	0	0	1 (2%)
	Maltese	0	0	0	1	0	1 (2%)
	Papillion	0	0	1	0	0	1 (2%)
	Pekingese	0	0	0	0	1	1 (2%)
	Poodle	0	0	1	0	0	1 (2%)
	Silky Terrier	0	1	0	0	0	1 (2%)
	Yorkshire Terrier	0	0	1	0	0	1 (2%)
Age	Range (years)	1-11	2-13	4-14	8-15	8-16	1-16
	Mean	4.8	9.6*	9.7	11.4	12.1	9.7*
	SD	4.0	3.5	3.5	2.1	2.2	3.9
Body weight	Range (kg)	4.8-14.0	5.4-14.0	3.0-13.2	4.0-10.6	4.2-9.2	3.0-15.0
	Mean	8.7	9.2*	7.2	6.6	6.1	7.4*
	SD	3.6	3.6	3.6	3.6	3.6	3.62

(*-p<0.05 = Shapiro-Wilk, reject normality, DMVD= Degenerative Mitral Valve Disease, SD=standard deviation)

Table iii: Detectability of TNF-α serum levels in dogs across different stages of DMVD

DMVD Stage (n)	**A (9)**	**B1 (7)**	**B2 (11)**	**C (13)**	**D (10)**	**Total (50)**	**Chi-Square**
Count	6	4	8	9	6	34	χ^2 (4) = 1.19
% TNF- α BDL	66.7	57.1	72.7	69.2	60.0	68.0	p = 0.88
Count	3	3	3	4	4	16	
% TNF- α detectable	33.3	42.9	27.3	30.8	40.0	32.0	

(DMVD= Degenerative Mitral Valve Disease, TNF-α= tumour necrosis factor alpha, χ^2= Chi-Squared test statistic, BDL= below detection limit)

Table iv: Kruskal Wallis test to assess the difference in serum biomarker levels between dogs in different stages of DMVD.

Biomarker	**A (n=9)**	**B1 (n=7)**	**B2 (n=11)**	**C (n=13)**	**D (n=10)**	**KW H**	**p**	**JT z**	**p**
NT-proBNP (pmol/L)	536.00 (250.00-695.18)[a]	653.00 (335.60-1121.55)[a]	754.20 (250.00-1190.25)[a]	795.30 (250.00-1760.80)[a]	2910.40 (1574.1-3544.40)[b]	12.15	**0.02**	3.19	**<0.01**
TNF-α (pg/mL)	1.00 (1.00-12.16)	1.00 (1.00-67.98)	1.00 (1.00-19.78)	1.00 (1.00-29.71)	1.00 (1.00-17.14)	0.53	0.97	-0.21	0.83
PTX3 (ng/mL)	0.68	0.94	0.65	0.48	0.51	3.36	0.50	-1.12	0.26

	(0.42-1.20)	(0.62-1.50)	(0.29-1.05)	(0.31-0.83)	(0.37-0.72)

*p<0.05; Values with different superscript letters differ significantly (Conover's test, p<0.05); degrees of freedom= 4 throughout. NT-proBNP= N-terminal-prohormone of B-type natriuretic peptide, TNF-α= tumour necrosis factor alpha, PTX3= pentraxin-3, H= Kruskal Wallis H test statistic, corrected for ties, JT = Jonckheere-Terpstra trend test, z= z statistic.

Table v: Correlation between serum biomarker levels and disease stage, QOL score, SBP, radiographic and echocardiographic parameters

Variable [n]	NT-proBNP (ρ, p)	TNF-α (ρ, p)	PTX3 (ρ, p)
Stage of DMVD [50]	0.46, <0.01*	-0.04, 0.8	-0.18, 0.22
QOL [50]	0.46, <0.01*	-0.19, 0.19	-0.16, 0.28
Systolic BP [50]	0.197, 0.10	0.06, 0.6	-0.17, 0.23
VHS [49]	0.40, <0.01*	0.15, 0.30	-0.07, 0.64
VLAS [47]	0.40, <0.01*	-0.29, 0.048*	-0.05, 0.70
LVIDdn [47]	0.41, <0.01*	-0.04, 0.81	0.19, 0.20
EF% [47]	0.33, 0.02*	0.20, 0.19	0.20, 0.17
FS% [47]	0.33, 0.02*	0.14, 0.36	0.19, 0.20
E/A [40]	0.26, 0.11	-0.098, 0.50	0.18, 0.27
LA:Ao [45]	0.57, <0.01*	0.10, 0.50	-0.13, 0.39

(*-p= <0.05; ρ= Spearman's correlation coefficient; n= sample size; NT-proBNP= N-terminal pro-B-type natriuretic peptide; TNF-α= tumour necrosis factor alpha; PTX3= pentraxin-3; DMVD= degenerative mitral valve disease; QOL= quality of life; SBP= systolic blood pressure; VHS= vertebral heart score; VLAS= vertebral left atrial size; LVIDdn= left ventricular internal diameter in diastole, corrected for bodyweight; EF%= ejection fraction; FS%= fractional shortening; E/A= ratio of early (E) to late (A) diastolic transmitral flow velocities; LA:Ao= left atrial-to-aortic ratio.)

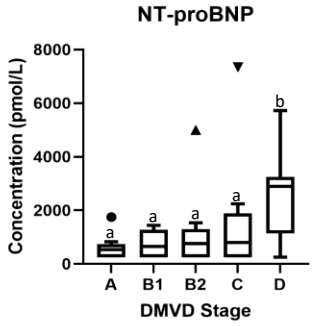


Fig. i Box-and-whisker plot of serum NT-proBNP concentrations (pmol/L) in dogs in different stages of degenerative mitral valve disease (DMVD). The bold line represents the median, the box indicates the interquartile range (IQR), the whiskers extend to the most extreme values within 1.5 × IQR, and symbols represent outliers. Different lowercase letters indicate statistically significant differences between groups according to Conover's post hoc test ($p < 0.05$). One extreme outlier (NT-proBNP >10,000 pmol/L) is not shown to improve visual clarity but was included in all statistical analyses.

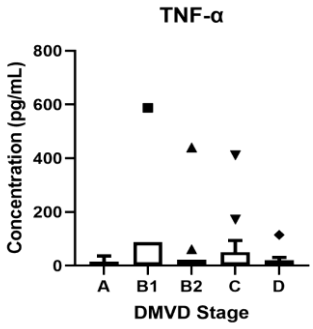


Fig. ii Box-and-whisker plot of serum TNF-α concentrations (pg/mL) in dogs with different ACVIM stages of degenerative mitral valve disease (DMVD). The bold line represents the median, the box indicates the interquartile range (IQR), the whiskers extend to the most extreme values within 1.5 × IQR, and symbols represent outliers.

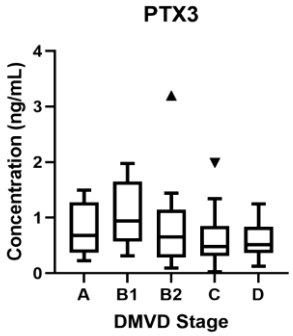


Fig. iii Box-and-whisker plot of serum PTX3 concentrations (ng/mL) in dogs with different ACVIM stages of degenerative mitral valve disease (DMVD). The bold line represents the median, the box indicates the interquartile range (IQR), the whiskers extend to the most extreme values within 1.5 × IQR, and symbols represent outliers.