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# How accurate are NT-proBNP, ANP, and cTnI levels in diagnosing dogs with myxomatous mitral valve disease?

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**Abstract**

**Background:** Myxomatous mitral valve disease (MMVD) is prevalent in dogs. Specialized diagnostics (radiography and echocardiography) may be unavailable in some veterinary settings. Cardiac biomarkers offer potential alternatives.
**Aim:** This study evaluated the diagnostic value of *N*-terminal fragments of pro-brain natriuretic peptides (NT-proBNPs), atrial natriuretic peptides (ANPs), and cardiac troponin I (cTnI) levels in dogs with MMVD.
**Methods:** 69 dogs with MMVD (asymptomatic and symptomatic) and 19 healthy controls were assessed. Biomarker levels were measured using commercial kit rapid tests.
**Results:** Our results showed that the median NT-proBNP level in the symptomatic group was higher than those in the asymptomatic ($p < 0.001$) and control ($p < 0.001$) groups. Moreover, the median NT-proBNP level in the asymptomatic group was higher than that in the control group ($p < 0.001$). The cTnI level in the control group was lower than those in the asymptomatic ($p = 0.039$) and symptomatic ($p = 0.001$) groups. No statistically significant difference in the cTnI level was noted between the asymptomatic and symptomatic groups. The best cutoff value of the NT-proBNP level to differentiate the normal controls from dogs with MMVD with or without congestive heart failure was > 505.65 pmol/l [sensitivity, 76.8%; specificity, 89.5%; and area under the curve (AUC), 0.862]. The suggested cutoff value of the NT-proBNP level to differentiate symptomatic MMVD from asymptomatic MMVD was >787.65 pmol/l (sensitivity, 78.38%; specificity, 72.55%; and AUC, 0.792).
**Conclusion:** NT-proBNP and cTnI may serve as point-of-care tests for dyspneic dogs, aiding MMVD assessment where specialized diagnostics are limited.
**Keywords:** Myxomatous mitral valve disease, cardiac disease, canine, dog, biomarker.

## Introduction

Myxomatous mitral valve disease (MMVD) is the most common cardiac disease affecting dogs, especially in small- to medium-sized dog breeds, and can gradually develop congestive heart failure (Ettinger *et al.*, 2017). MMVD is characterized by gradual deterioration of the mitral valves, leading to mitral regurgitation and subsequent enlargement of the left atrium and left ventricle (LV). The survival time after the occurrence of CHF is approximately 6–14 months because of disease progression (Ettinger *et al.*, 2017).

Diagnosis and staging of MMVD according to the American College of Veterinary Internal Medicine (ACVIM) consensus guidelines are generally based on clinical signs, thoracic radiography, and echocardiography (Keene *et al.*, 2019). Echocardiography is particularly useful for identifying mitral valvular lesions and assessing the severity of MMVD. Biomarkers, which are indicators of biological processes performed by the body, have also been studied for their potential to diagnose and predict prognosis in dogs with MMVD (Bettencourt *et al.*, 2004; Riengvirodkij and Sakcamduang, 2021; Valli *et al.*, 1999). The *N*-terminal fragments of pro-atrial and pro-brain natriuretic peptides (NT-proANPs and NT-proBNPs, respectively) and cardiac troponins have

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been suggested as useful biomarkers in this proposal (Oyama *et al.*, 2009). Atrial natriuretic peptides (ANPs) and brain natriuretic peptides (BNPs) are released by myocardial tissue response to volume or pressure overload and consecutive increased atrial and ventricular wall stretch (Hori *et al.*, 2020). The mean serum ANP levels increased in an advanced stage of MMVD (Khaki *et al.*, 2022). Myofibrillar proteins called cardiac troponins control the calcium-mediated interaction of actin and myosin filaments in cardiac myocytes. Troponins are released into the bloodstream as a result of myocardial injury (van der Laarse, 2002). The cardiac troponin I (cTnI) level increases in dogs with cardiac disease, regardless of myocyte damage. Although the level varies among dogs with similar disease severity, the cTnI level increases with disease severity in dogs with MMVD (Ljungvall *et al.*, 2010).

Circulating biomarkers, such as cardiac troponins, and NT-proBNP, can be measured using various techniques with varying measurement times, ranging from minutes to days (Häggström *et al.*, 2000; Oyama *et al.*, 2009). These biomarkers are useful in differentiating dogs with MMVD from healthy dogs, as well as in staging MMVD in dogs (Häggström *et al.*, 2000; Oyama *et al.*, 2009). These biomarker measurements may be particularly useful in private practice settings where echocardiography is not readily available.

The ability of cardiac biomarkers to distinguish between dogs with CHF and those with subclinical MMVD remains a matter of debate. To evaluate the diagnostic reliability of the NT-proBNP, ANP, and cTnI levels in dogs with MMVD, this study aimed to determine the accuracy of these markers. We also aimed to assess the diagnostic potency of these cardiac biomarkers, echocardiographic measures, and radiographic findings.

## Materials and Methods

### *Dogs*

Privately owned dogs were enrolled at the Prasu Arthorn Veterinary Teaching Hospital, Faculty of Veterinary Science, Mahidol University, Thailand. This study was conducted prospectively between December 2018 and October 2022 and included dogs of any sex, breed, and weight as long as they were at least 5 years old. Dogs were eligible for inclusion if affected by MMVD at different ACVIM stages and diagnosed and classified according to the ACVIM consensus guideline (Keene *et al.*, 2019). Healthy dogs were included as the controls for comparative purposes. Dogs were considered healthy in the absence of any signs of illness on clinical examination and clinicopathological abnormalities on complete blood count (CBC) and serum biochemistry profile.

All enrolled dogs underwent complete physical examination, including history taking and evaluation of the cardiorespiratory system by thoracic auscultation to detect the presence of a heart murmur as well as the type, intensity (grades I–VI), and location of the murmur.

For each enrolled dog, thoracic radiographs were taken in the right lateral and ventrodorsal or dorsoventral positions to evaluate the radiographic distribution of pulmonary edema and differentiate MMVD stage C from stage B2. The VHS measurement was performed using an adjustable clipper, according to the method of measurement as originally described (Buchanan, 2000). The standard six-lead recording systems using the Kenz Cardico 601 (Kenz, Japan), including limb leads I, II, and III and aVR, aVL, and aVF, were used to conduct electrocardiography (ECG). The six-lead ECG findings were recorded for approximately 10 seconds for each dog. Additional 1-minute lead II recordings were supplied if any dogs had arrhythmias to assess the heart rhythm. All metrics, including the heart rate, recording intervals, amplitudes, and vasovagal tone index, were obtained from the lead II recording (Moonarmart et al., 2012).

Echocardiographic examinations were performed by one well-trained investigator using the GE Vivid E9 ultrasound machine (GE, Norway) with a multifrequency sector transducer: 4.5–12-MHz and 4–8 MHz probes for small- and medium-sized dogs, respectively. The procedure was performed in awake unsedated dogs with continuous ECG recordings throughout the process. The echocardiographic examination included a complete two-dimensional and M-mode examination, color flow Doppler examination, and pulsed-wave and continuous-wave Doppler examinations. The following parameters were evaluated: left atrial-to-aortic root (LA/Ao) ratio, normalized left ventricular internal diameter in the diastolic phase (NLVIDd), normalized left ventricular internal diameter in the systolic phase (NLVIDs), fractional shortening, ejection fraction, and mitral valve *E*-wave velocity (E).

Based on body weight (BW), the NLVIDd and NLVIDs were calculated using the following formulas: NLVIDd = LVIDd (cm)/(BW [kg])$^{0.294}$ and NLVIDs = LVIDs (cm)/(BW [kg])$^{0.315}$ (Cornell *et al.*, 2004).

During the study, 5 ml of blood was collected from either the jugular or saphenous vein of the enrolled dogs for routine hematology and serum biochemistry analysis. The first part of the sample was collected in an ethylenediaminetetraacetic acid tube for a CBC test. The second part was collected in a heparinized tube for serum biochemistry analysis, including alanine transaminase (ALT), alkaline phosphatase (ALP), creatinine, blood urea nitrogen (BUN), total protein (TP), and albumin. The CBC test and biochemistry analysis were performed to exclude the dogs that have concurrent systemic diseases such as renal disease, hepatic disease, hormonal disease, and significant gastrointestinal disease that could interfere with cardiac biomarkers in the studied dogs. The serum NT-proBNP, cTnI, and serum ANP levels were determined using the canine NT-proBNP immunoassay test (Bionote®), Vcheck cTnI immunoassay test kit (Bionote®), and ANP enzyme immunoassay kit (Detect X®), respectively, following the manufacturer's

instructions (https://www.bionote.com/vcheckcanine-nt-probnp, https://irpcdn.multiscreensite.com/20fad1a2/files/uploaded/Dianotech%20Fluorescence%20Quatitative%Analyer.pdf, and https://www.arborassays.com/documentation/inserts/K071-H.pdf, respectively). The enrolled dogs were divided into the following three groups:

- **Group asymptomatic MMVD:** referred to dogs affected by MMVD with no clinical signs of CHF (asymptomatic patients). No radiographic evidence of CHF, including pulmonary edema and/or venous congestion, was detected. Asymptomatic dogs without echocardiographic signs of left-sided cardiac remodeling were considered to be in stage B1. Asymptomatic dogs with echocardiographic signs of left-sided cardiac remodeling (LA/Ao ratio of ≥1.6 and NLVIDd of ≥1.7) were considered to be in stage B2.

- **Group symptomatic MMVD:** It refers to dogs affected by MMVD that had echocardiographic evidence of MVD and clinical signs of CHF (symptomatic patients), identified by clinical examination and radiographic evidence of pulmonary edema and/or pulmonary venous congestion.

- **Group normal:** It refers to normal healthy control dogs with no heart disease. Dogs in this group included those with no history or clinical signs of cardiorespiratory diseases such as coughing, dyspnea, exercise intolerance, cyanosis, or syncope. No heart murmurs or crackle lung sound was detected by auscultation. Each animal was inspected by thoracic radiography, ECG, and echocardiography to ensure that it had a normal heart condition. The age of enrolled dogs in this group matched that of the others.

***Exclusion criteria***

This study excluded dogs with significant concurrent systemic diseases, such as renal, hepatic, hormonal, or gastrointestinal disease, based on the previous history, clinical examination, and laboratory test results mentioned earlier. Dogs with any congenital or acquired heart diseases other than MMVD detected by echocardiography were also excluded from this study.

***Statistical analysis***

Statistical analysis was conducted using the computerized statistical software IBM SPSS Statistics for Windows, Version 24.0 (Armonk, NY). The normality of the distribution was tested using the nonparametric Kolmogorov–Smirnov tests. The results are presented as means ± standard deviations for normally distributed variables and medians with interquartile ranges for non-normally distributed variables. A $p$-value of <0.05 was considered significant. The difference in sex distribution was determined using chi-square. The groups were compared using the Kruskal–Wallis test. If statistical significance was obtained between groups from the Kruskal–Wallis test, the Mann–Whitney $U$ test with Bonferroni correction multiple comparisons was used for the post hoc analysis.

The receiver operating characteristic (ROC) curves were constructed to determine the sensitivity, specificity, area under the curve (AUC), and recommended cutoff values for the ANP, NT-proBNP, and cTnI levels. The Spearman correlation coefficient was used to evaluate the correlation between the echocardiography parameters and cardiac biomarkers.

The logistic regression analysis was used to identify parameters associated with the clinical presenting signs in dogs with MMVD. The odds ratios (ORs) were calculated to assess the strength of the associations between parameters and the likelihood of having clinical presenting signs of the disease. Subsequently, the statistically significant clinical variables from univariate logistic regression were included in the backward stepwise method of multivariate logistic regression to determine the independent predictors.

***Ethical approval***

All dogs were enrolled with informed consent from their owners, and this study was approved by the Mahidol University–Institutional Animal Care and Use Committee of the Faculty of Veterinary Science (No. MUVS-2018-06-32 and MUVS-2019-05-29).

## Results

The study population consisted of 19 healthy dogs and 69 dogs with MMVD. Of the dogs with MMVD, 32 (46.3%) and 37 (53.6%) were included in the asymptomatic and symptomatic MMVD groups, respectively. The distribution of breeds is illustrated in Table 1, and the demographic distributions of the three groups are displayed with comparisons in Table 2. No statistically significant differences in age, BW, and sex were noted between groups. In the asymptomatic MMVD group, 5 (15.6%) of 32 dogs had ACVIM stage B1, whereas 27 (84.4%) had ACVIM stage B2.

No statistically significant differences in body temperature and heart rate were noted between groups. However, the heart rate measured from ECG was significantly lower in the asymptomatic group than in the normal control ($p = 0.006$) or symptomatic MMVD ($p = 0.008$) group (Table 2).

The cardiac size was measured using the vertebral heart score (VHS) from the thoracic radiograph and echocardiographic measurement of the LA and LV sizes. The LA/Ao ratio, NLVIDd, NLVIDs, and E wave were significantly higher in the symptomatic group than in the asymptomatic and normal control groups ($p < 0.001$, $p < 0.001$, $p = 0.037$, and $p < 0.001$, respectively; Table 3). Likewise, these variables in the asymptomatic group were higher than those in the normal control group ($p < 0.001$, $p < 0.001$, $p = 0.001$, and $p < 0.001$, respectively; Table 3).

**Table 1.** Breed distribution in each group.

| Breeds | Normal group | Asymptomatic MMVD group | Symptomatic MMVD group | Total |
|---|---|---|---|---|
| Chihuahua | 11 | 6 | 7 | 24 |
| Poodle | 2 | 6 | 12 | 20 |
| Mixed | 1 | 4 | 9 | 14 |
| Shih tzu | 1 | 5 | 6 | 12 |
| Pomeranian | 2 | 6 | 3 | 11 |
| Miniature | 1 | 1 | - | 2 |
| Yorkshire terrier | - | 2 | - | 2 |
| Jack Russel | - | 1 | - | 1 |
| Dachshund | - | 1 | - | 1 |
| Pug | 1 | - | - | 1 |

**Table 2.** Physical examination of dogs in the MMVD and control groups.

| Variables | Normal group | Asymptomatic MMVD group | Symptomatic MMVD group | *p*-value |
|---|---|---|---|---|
| Number | 19 | 32 | 37 | |
| Sex (male/female) (%) | 7/12 (36.8/63.2) | 23/9 (71.8/28.1) | 21/16 (56.8/43.2) | 0.050 |
| Age (year) | 10 [8, 13] | 10.8 [8.2, 13] | 10 [7, 12.6] | 0.694 |
| Body weight (kg) | 4.8 [3.6, 6.7] | 5.4 [4.2, 7.2] | 5.6 [4.1, 7.4] | 0.533 |
| Temperature (°F) | 101.4 [100.6, 102] | 101.05 [100.40, 101.55] | 101.2 [100.9,101.8] | 0.354 |
| Heart rate (bpm) | 140 [112, 168] | 140 [120, 160] | 140 [124, 160] | 0.507 |

The statistically significant differences within each group were tested using the chi-square, Kruskal–Wallis test and Mann–Whitney *U* test with Bonferroni correction.

From hematological data, the white blood cell (WBC) and neutrophil counts in the asymptomatic MMVD group were significantly lower than those in the symptomatic group ($p = 0.009$ and $p = 0.013$, respectively). The platelet count in the asymptomatic MMVD group was significantly lower than those in the control ($p = 0.011$) and symptomatic MMVD ($p = 0.036$) groups. The eosinophil count in the control group was significantly lower than that in the symptomatic MMVD group ($p = 0.044$). However, the median values of all hematological parameters of the three groups were within the normal limits (Table 4).

The BUN level and BUN-to-creatinine ratio in the symptomatic group were higher than those in the asymptomatic ($p = 0.002$, Table 5) and normal control ($p < 0.001$, Table 5) groups.

Dogs with MMVD had significantly higher creatinine levels than the control group ($p = 0.001$ for the asymptomatic group and $p < 0.001$ for the symptomatic group). The BUN-to-creatinine ratio was significantly lower in the control group than in the symptomatic MMVD group ($p < 0.001$); however, no significant difference was noted with that in the asymptomatic group (Table 2). Other serum biochemistry parameters, including ALT, ALP, albumin, and TP were not different between groups, and the median values of each variable were within the normal limits (Table 5).

The median NT-proBNP level in the symptomatic group was higher than those in the asymptomatic ($p < 0.001$, Table 2) and normal control ($p < 0.001$, Table 6) groups. Moreover, the median NT-proBNP level in the asymptomatic group was higher than that in the normal control group ($p < 0.001$, Table 2). The cTnI level in the normal control group was lower than those in the asymptomatic ($p = 0.039$, Table 6) and symptomatic ($p = 0.001$, Table 6) groups. No statistically significant difference in the cTnI was noted between the asymptomatic and symptomatic groups (Table 6).

No significant difference in the ANP levels was noted between groups (Table 6).

**Table 3.** Radiographic, electrocardiographic, and echocardiographic findings of dogs in the MMVD and control groups.

| Variables | Normal group | Asymptomatic MMVD group | Symptomatic MMVD group | *p*-value |
|---|---|---|---|---|
| Heart (ECG) (bpm) | 137 [123, 137][a,d] | 116.5 [97.25, 137.5][a,c,d,f] | 135 [115.50, 157][c,f] | 0.006 |
| VVTI | 7.37 [6.85, 8.72] | 8.00 [7.04, 10.18] | 7.66 [6.25, 8.63] | 0.171 |
| VHS | 10 [10,10.2][a,b,d,e] | 11 [10.5, 11.5][a,c,d,f] | 11.5 [11.1, 12.65][b,c,e,f] | <0.001 |
| LA:Ao | 1.23 [1.15, 1.38][a,b,d,e] | 1.87 [1.72, 2.10][a,c,d,f] | 2.22 [2.0, 2.77][b,c,e,f] | <0.001 |
| NLVIDd | 1.36 [1.26, 1.42][a,b,d,e] | 1.76 [1.63, 1.87][a,c,d,f] | 2.02 [1.80, 2.26][b,c,e,f] | <0.001 |
| NLIVDs | 0.71 [0.63, 0.85][a,b,d,e] | 0.89 [0.76, 1.02][a,c,d] | 0.99 [0.81, 1.18][b,c,e] | <0.001 |
| FS | 43.47 [38.59, 50.10] | 46.67 [41.92, 51.89] | 48.28 [43.54, 52.96] | 0.129 |
| E value | 0.66 [0.61, 0.77][a,b,d] | 1.10 [0.95, 1.21][a,c,d,f] | 1.42 [1.24, 1.70][b,c,f] | <0.001 |

The statistically significant differences within each group were tested using the Kruskal–Wallis test and Mann–Whitney *U* test with Bonferroni correction.

[a] Statistically significant difference compared with the normal controls and asymptomatic dogs with MMVD ($p < 0.05$).
[b] Statistically significant difference compared with the normal controls and symptomatic dogs with MMVD ($p < 0.05$).
[c] Statistically significant difference compared with the asymptomatic and symptomatic dogs with MMVD ($p < 0.05$).
[d] Statistically significant difference compared with the normal controls and asymptomatic dogs with MMVD ($p < 0.025$).
[e] Statistically significant difference compared with the normal controls and symptomatic dogs with MMVD ($p < 0.025$).
[f] Statistically significant difference compared with the asymptomatic and symptomatic dogs with MMVD ($p < 0.025$).

MMVD, myxomatous mitral valve disease; HR(ECG); heart rate measured by electrocardiogram; VVTI, vasovagal tonus index; VHS, vertebral heart score; LA/Ao ratio, left atrial-to-aortic root ratio; NLVIDd, normalized left ventricular internal diameter in the diastolic phase; NLVIDs, normalized left ventricular internal diameter in the systolic phase; FS, fractional shortening; *E* value, peak velocity of early diastolic transmitral flow.

The ROC curve analyses were plotted to calculate the cutoff values for reliably predicting different clinical stages. The best cutoff value of the NT-proBNP level to differentiate the normal controls from dogs with MMVD with or without CHF was >505.65 pmol/l [sensitivity, 76.8%; specificity, 89.5%; and area under the curve (AUC), 0.862] (Fig. 1A). Similar differentiation on cTnI suggested that the cutoff value was 0.075 ng/ml (sensitivity, 76.8%; specificity, 57.9%; and AUC, 0.725) (Fig. 1A).

The suggested cutoff value of the NT-proBNP level to differentiate symptomatic MMVD from asymptomatic MMVD was >787.65 pmol/l (sensitivity, 78.38%; specificity, 72.55%; and AUC, 0.792) (Fig. 1B).

***Logistic regression***

**Univariate logistic regression**

The univariate logistic regression of each variable was analyzed to predict symptomatic from asymptomatic dogs with MMVD. The results are shown in Table 3.

The variables including NT-proBNP, BUN-to-creatinine ratio, VHS, heart rate by ECG, WBC, neutrophils, NLVIDd, and NLVIDs were statistically significantly associated with the likelihood of being symptomatic dogs with MMVD, whereas the remaining variables did not show statistically significant associations (Table 7).

**Multivariable logistic regressions**

In the multivariate logistic regression, two variables were significantly associated with the ORs of symptomatic dogs: NLVIDd and BUN-to-creatinine ratio. Dogs with a 0.01-unit increase in the NLVIDd had an approximately 4.2% increase in the likelihood of developing symptomatic CHF (OR, 1.042; 95% confidence interval [CI], 1.008–1.078; $p = 0.016$). In addition, dogs with a unit increase in the BUN-to-creatinine ratio had an approximately 17.2% increase in the likelihood of developing symptomatic signs (OR, 1.172; 95% CI, 1.058–1.298; $p = 0.002$) (Table 8).

**Table 4.** Hematological parameters of dogs in the MMVD and control groups.

| Variables | Normal group | Asymptomatic MMVD group | Symptomatic MMVD group | *p*-value |
|---|---|---|---|---|
| WBC (cells/µl) | 10,090 [8,010, 12,720] | 9755 [7,622, 11,777][c,f] | 11730 [9,500, 14,545][c,f] | 0.032 |
| Neutrophils (cells/µl) | 7,769 [6,247, 9,297] | 7345 [6,039, 9,013][c,f] | 9152 [7,180, 10,900][c,f] | 0.029 |
| Monocytes (cells/µl) | 462 [272, 735] | 424 [258, 669] | 491 [242,734] | 0.863 |
| Lymphocytes (cells/µl) | 1770 [1,442, 2,026] | 1656 [1293, 1990] | 1956 [1469,2483] | 0.228 |
| Eosinophils (cells/µl) | 154 [99, 276][b] | 258 [78, 497] | 318 [131, 513][b] | 0.157 |
| Basophils (cells/µl) | 0 | 0 | 0 | 1.000 |
| Neutrophils/lymphocyte ratio | 4.11 [3.60, 5.20] | 4.53 [3.56, 5.18] | 4.62 [3.61, 6.12] | 0.606 |
| Hematocrit (%) | 47.7 [44.6, 51.9] | 47.8 [43.8, 50.7] | 47.5 [43.1, 49.9] | 0.575 |
| Hemoglobin (g/dl) | 16.4 [15.3, 17.7] | 15.9 [14.7, 17.2] | 15.9 [14.2, 16.9] | 0.384 |
| Platelet ($10^3$/µl) | 404 [344, 451][a,d,e] | 289 [216, 366][a,c,d] | 336 [250, 422][c] | 0.015 |
| Plasma protein (g/dl) | 8.4 [8.0, 9.0] | 8.6 [8.2, 9.1] | 9.0 [8.0, 9.5] | 0.819 |
| MCV (fl) | 70.7 [67.8, 72.0] | 69.2 [67.2, 71.7] | 69.6 [67.5, 71.6] | 0.436 |
| MCH (pg) | 24.0 [22.9, 24.5] | 23.3 [22.6, 24.0] | 23.3 [22.7, 24.1] | 0.300 |
| MCHC (g/dl) | 34.1 [ 33.1, 34.6] | 33.6 [32.7, 34.0] | 33.5 [32.9, 34.2] | 0.270 |
| RDW (%) | 12.7 [12.3, 13.7] | 12.7 [12.3, 13.5] | 12.7 [12.2, 13.4] | 0.931 |

The statistically significant differences within each group were tested using the Kruskal–Wallis test and Mann–Whitney *U* test with Bonferroni correction.

[a] Statistically significant difference compared with the normal controls and asymptomatic dogs with MMVD ($p < 0.05$).

[b] Statistically significant difference compared with the normal controls and symptomatic dogs with MMVD ($p < 0.05$).

[c] Statistically significant difference compared with the asymptomatic and symptomatic dogs with MMVD ($p < 0.05$).

[d] Statistically significant difference compared with the normal controls and asymptomatic dogs with MMVD ($p < 0.025$).

[e] Statistically significant difference compared with the normal controls and symptomatic dogs with MMVD ($p < 0.025$).

[f] Statistically significant difference compared with the asymptomatic and symptomatic dogs with MMVD ($p < 0.025$).

MMVD, myxomatous mitral valve disease; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width.

A subanalysis of multiple logistic regression was also performed in several models to provide useful information as follows.

**Hematology and serum biochemistry**

In the multivariate logistic regression analysis of only hematology and serum biochemistry in dogs with MMVD, the BUN-to-creatinine ratio was found to be significantly associated with the OR of symptomatic dogs.

This study found that dogs with a unit increase in the BUN-to-creatinine ratio had an approximately 9.8% increase in the likelihood of developing symptomatic

**Table 5.** Biochemistry parameters of dogs in the MMVD and control groups.

| Variables | Normal group | Asymptomatic MMVD group | Symptomatic MMVD group | *p*-value |
|---|---|---|---|---|
| ALT (U/l) | 47 [35, 70] | 52 [39.5, 123.25] | 56 [36.5, 128.5] | 0.491 |
| ALP (U/l) | 68 [30, 266] | 69 [41, 152] | 64 [43.5, 167.5] | 0.878 |
| BUN (mg/dl) | 16 [12, 18][a,b,d,e] | 20 [15, 29.75][a,c,d,f] | 32 [21.5, 44.5][b,c,e,f] | <0.001 |
| Creatinine (mg/dl) | 0.94 [0.85, 1.02][a,b,d,e] | 1.18 [0.97, 1.42][a,d] | 1.21 [1.12, 1.39][b,e] | <0.001 |
| BUN/creatinine ratio | 16.98 [12.37, 19.35][b,e] | 16.41 [13.23, 23.69][c,f] | 24.39 [19.33, 33.72][b,c,e,f] | <0.001 |
| Albumin (g/dl) | 3.4 [3.1, 3.6] | 3.3 [3.0, 3.5] | 3.3 [2.95, 3.4] | 0.286 |
| Total protein (g/dl) | 6.9 [6.7. 7.3] | 7.25 [6.70, 7.57] | 7.0 [6.5, 7.5] | 0.608 |

The statistically significant differences within each group were tested using the Kruskal–Wallis test and Mann–Whitney *U* test with Bonferroni correction.

[a] Statistically significant difference compared with the normal controls and asymptomatic dogs with MMVD ($p < 0.05$).

[b] Statistically significant difference compared with the normal controls and symptomatic dogs with MMVD ($p < 0.05$).

[c] Statistically significant difference compared with the asymptomatic and symptomatic dogs with MMVD ($p < 0.05$).

[d] Statistically significant difference compared with the normal controls and asymptomatic dogs with MMVD ($p < 0.025$).

[e] Statistically significant difference compared with the normal controls and symptomatic dogs with MMVD ($p < 0.025$).

[f] Statistically significant difference compared with the asymptomatic and symptomatic dogs with MMVD ($p < 0.025$).

MMVD, myxomatous mitral valve disease; ALT, alanine transaminase; ALP, alkaline phosphatase; BUN, blood urea nitrogen

**Table 6.** Cardiac biomarkers of dogs in the MMVD and control groups.

| Variables | Normal group | Asymptomatic MMVD group | Symptomatic MMVD group | *p*-value |
|---|---|---|---|---|
| NT-pro-BNP (pmol/l) | 499 [499, 499][a,b,d,e] | 749 [499, 1528][a,c,d,f] | 2159.90 [846.30, 3542.20][b,c,e,f] | <0.001 |
| cTnI (ng/ml) | 0.06 [0.04, 0.12][a,b,e] | 0.125 [0.062, 0.225][a] | 0.17 [0.105, 0.235][b,e] | <0.005 |
| ANP (pg/ml) | 3340 [1804, 4600] | 3565.50 [2962, 6836.75] | 3582 [1650, 6754] | 0.224 |

The statistically significant differences within each group were tested using the Kruskal–Wallis test and Mann–Whitney *U* test with Bonferroni correction.

[a] Statistically significant difference compared with the normal controls and asymptomatic dogs with MMVD ($p < 0.05$).

[b] Statistically significant difference compared with the normal controls and symptomatic dogs with MMVD ($p < 0.05$).

[c] Statistically significant difference compared with the asymptomatic and symptomatic dogs with MMVD ($p < 0.05$).

[d] Statistically significant difference compared with the normal controls and asymptomatic dogs with MMVD ($p < 0.025$).

[e] Statistically significant difference compared with the normal controls and symptomatic dogs with MMVD ($p < 0.025$).

[f] Statistically significant difference compared with the asymptomatic and symptomatic dogs with MMVD ($p < 0.025$).

MMVD, myxomatous mitral valve disease; NT-proBNP, N-terminal fragment of pro-brain natriuretic peptide; cTnI, cardiac troponin I; ANP, atrial natriuretic peptide.

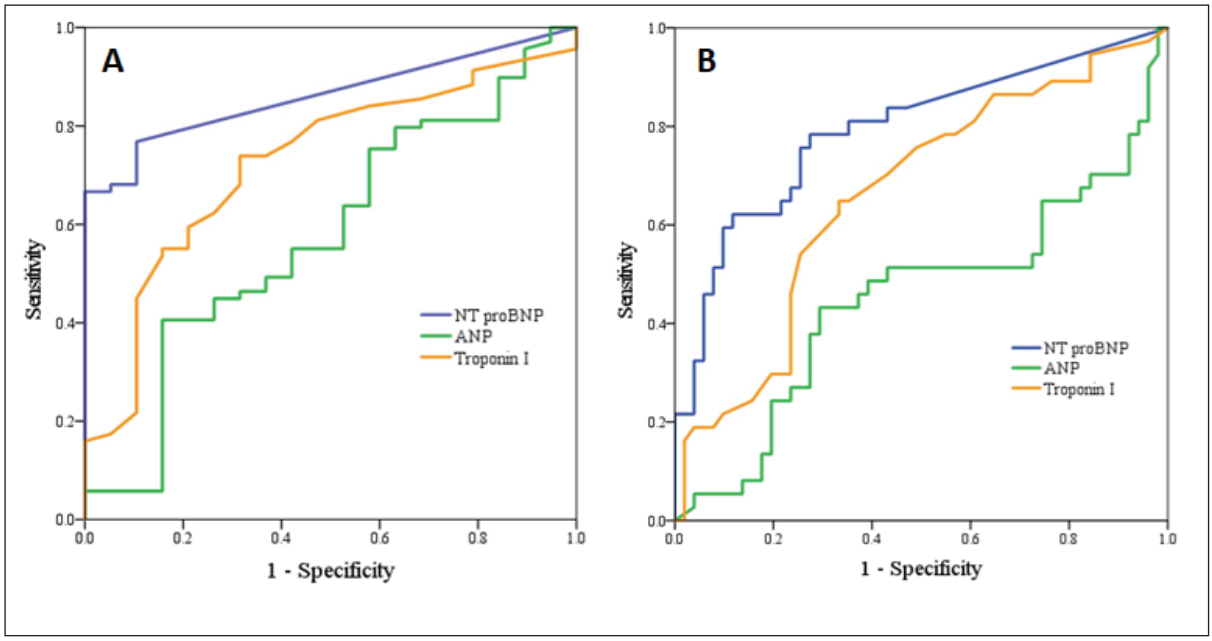


**Fig. 1.** ROC curve for the accuracy of each cardiac biomarker. ROC curve for the accuracy of each cardiac biomarker in the discrimination of MMVD dogs from normal control dogs (A), in the discrimination of symptomatic MMVD dogs from asymptomatic MMVD dogs and normal control dogs (B). NT-proBNP, N-terminal fragment of pro-brain natriuretic peptide; ANP, atrial natriuretic peptide; cTnI, cardiac troponin I.

CHF (OR, 1.098; 95% CI, 1.029–1.171; $p$ = 0.005) (Table 9). This implies that a higher BUN-to-creatinine ratio was positively correlated with a higher likelihood of clinical presenting signs in dogs with MMVD.

**Hematology, serum biochemistry, and cardiac biomarkers**

In the multivariate logistic regression analysis of only hematology, serum biochemistry, and cardiac biomarkers in dogs with MMVD, three variables were found to be significantly associated with the OR of clinical presenting signs: BUN-to-creatinine ratio, neutrophil, and NT-proBNP.

Dogs with a 100-unit increase in the neutrophil had an approximately 2.2% increase in the likelihood of developing symptomatic CHF (OR, 1.022; 95% CI, 1.002–1.043; $p$ = 0.035) (Table 10). In addition, dogs with a unit increase in BUN-to-creatinine ratio had an approximately 9.8% increase in the likelihood of developing symptomatic signs (OR, 1.098; 95% CI, 1.010–1.174; $p$ = 0.027) (Table 10). For every 100-unit increase in the NT-proBNP level, the probability of dogs displaying clinically significant symptoms of MMVD increased by approximately 7.0% (OR, 1.070; 95% CI, 1.014–1.128; $p$ = 0.013) (Table 10).

**Echocardiography parameters**

In the multivariate logistic regression analysis of echocardiography parameters in dogs with MMVD, two variables were found to be significantly associated with the OR of symptomatic dogs, namely, *E* value and LA/Ao ratio.

This study found that dogs with a 0.1-unit increase in the *E* value had an approximately 35.4% increase in the likelihood of developing symptomatic CHF (OR, 1.354; 95% CI, 1.063–1.725; $p$ = 0.014) (Table 11). In addition, dogs with a 0.1-unit increase in the LA/Ao ratio had an approximately 28.1% increase in the likelihood of developing symptomatic signs (OR, 1.281; 95% CI, 1.047–1.568; $p$ = 0.016) (Table 11).

**Echocardiography parameters and cardiac biomarkers**

In the multivariate logistic regression analysis of echocardiography parameters in combination with cardiac biomarkers in dogs with MMVD, two variables were found to be significantly associated with the OR of symptomatic dogs, namely, NLVIDd (multiplied by 100) and NT-proBNP. Dogs with a 0.01-unit increase in the NLVIDd had an approximately 3.6% increase in the likelihood of developing symptomatic CHF (OR, 1.036; 95% CI, 1.003–1.070; $p$ = 0.034). In addition, dogs with a 100-unit increase in the NT-proBNP level had an approximately 5.3% increase in the likelihood of developing symptomatic signs (OR, 1.053; 95% CI, 1.007–1.102; $p$ = 0.024) (Table 12).

**Thoracic radiography parameters and ECG**

In the multivariate logistic regression analysis of thoracic radiography parameters in combination with ECG in dogs with MMVD, only one variable was found to be significantly associated with the OR of symptomatic dogs, namely, VHS (multiplied by 10). Dogs with a 0.1-unit increase in the VHS had

**Table 7.** Univariate logistic regression of factors associated with the presence of dogs showing signs attributable to myxomatous mitral valve degeneration (MMVD).

| Variables | Odds ratio (95% CI) | *p*-value |
|---|---|---|
| Age (years) | 0.941 (0.813–1.089) | 0.417 |
| Sex (female) | 1.947 (0.710–5.337) | 0.195 |
| Temperature (°F) | 1.510 (0.726–3.143) | 0.270 |
| Body weight (kg) | 1.005 (0.895–1.130) | 0.930 |
| Heart rate (bpm) | 1.010 (0.994–1.028) | 0.228 |
| Heart rate (ECG) (bpm) /10 | 1.230 (1.035–1.463) | 0.019 |
| VVTI | 0.743 (0.547–1.009) | 0.057 |
| VHS ×10 | 1.137 (1.051–1.231) | 0.001 |
| LAAo ×10 | 1.381 (1.150–1.659) | 0.001 |
| NLIVDd ×10 | 1.047 (1.021–1.075) | <0.001 |
| NLVIDs ×10 | 1.031 (1.004–1.059) | 0.024 |
| FS (%) | 1.031 (0.961–1.105) | 0.399 |
| E value (m/s) ×10 | 1.547 (1.212–1.975) | <0.001 |
| Hemoglobin (g/dl) | 0.861 (0.664–1.116) | 0.258 |
| HCT (%) | 0.947 (0.866–1.035) | 0.230 |
| RDW (%) | 1.019 (0.575–1.805) | 0.949 |
| MCHC (g/dl) | 1.176 (0.840–1.647) | 0.344 |
| MCH (pg) | 1.122 (0.757–1.663) | 0.567 |
| MCV (fl) | 1.032 (0.895–1.190) | 0.666 |
| Plasma protein (g/dl) | 1.007 (0.643–1.576) | 0.977 |
| WBC (cells/µl) /100 | 1.018 (1.002–1.034) | 0.023 |
| Eosinophils (cells/µl) | 1.000 (0.999–1.002) | 0.720 |
| Monocyte (cells/µl) | 1.000 (0.999–1.002) | 0.596 |
| Neutrophil (cells/µl) /100 | 1.024 (1.004–1.044) | 0.019 |
| N to L ratio | 1.177 (0.847–1.635) | 0.332 |
| Platelet ($10^3$/µl) /1000 | 1.004 (1.000–1.008) | 0.050 |
| Creatinine (mg/dl) | 0.644 (0.234–1.775) | 0.395 |
| Albumin (g/dl) | 0.989 (0.408–2.396) | 0.980 |
| ALP (U/l) | 1.001 (0.998–1.005) | 0.433 |
| ALT (U/l) | 1.001 (0.993–1.008) | 0.882 |
| BUN (mg/dl) | 1.020 (0.992–1.050) | 0.164 |
| BUN to creatinine ratio | 1.103 (1.036–1.175) | 0.002 |
| Total protein (g/dl) | 1.093 (0.746–1.602) | 0.647 |
| NTproBNP (pmol/l) /100 | 1.068 (1.019–1.119) | 0.006 |
| cTnI (ng/ml) | 0.831 (0.440–1.567) | 0.567 |
| ANP (pg/ml) | 1.00 (1.00–1.00) | 0.671 |

Sex (female), male compared to female; bpm, beats per minute; HR(ECG); heart rate measured by electrocardiogram; VVTI, Vasovagal tonus index; VHS, Vertebral heart score; LA:Ao ratio, left atrial-to-aortic root ratio; NLVIDd, left ventricular end-diastolic diameter normalized for BW; NLVIDs, left ventricular end-systolic diameter normalized for BW; FS, fractional shortening; E value, peak velocity of early diastolic transmitral flow; HCT%, hematocrit; RDW, red blood cell distribution width; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; WBC, white blood cells; N to L ratio, Neutrophils to lymphocyte ratio; ALT, alanine transaminase; ALP, alkaline phosphatase; BUN, Blood urea nitrogen; NT-proBNP, N-terminal pro B-type natriuretic peptide; cTnI, cardiac troponin I; ANP, Atrial natriuretic peptide

/10 implies that the odds ratio is the increase in odds per ten unit increase in the value of the measured variable.

/100 implies that the odds ratio is the increase in odds per one hundred unit increase in the value of the measured variable.

/1,000 implies that the odds ratio is the increase in odds per one thousand unit increase in the value of the measured variable.

X10 implies that the odds ratio is the increase in odds per 0.1 unit increase in the value of the measured variable.

**Table 8.** Multivariable logistic regression of all parameters

| Variables | Odds ratio (95% CI) | *p*-value |
|---|---|---|
| E value ×10 | 1.230 (0.981–1.542) | 0.073 |
| NLIVDd ×10 | 1.042 (1.008–1.078) | 0.016 |
| BUN to creatinine ratio | 1.172 (1.058–1.298) | 0.002 |
| VHS ×10 | 1.117 (1.000–1.249) | 0.051 |

*E* value, peak velocity of early diastolic transmitral flow; NLVIDd, left ventricular end-diastolic diameter normalized for BW; BUN, Blood urea nitrogen; VHS, Vertebral heart score.

X10 implies that the odds ratio is the increase in odds per 0.1 unit increase in the value of the measured variable.

**Table 9.** Multivariable logistic regression of hematology and serum biochemistry

| Variables | Odds ratio (95% CI) | *p*-value |
|---|---|---|
| BUN to creatinine ratio | 1.098 (1.029–1.171) | 0.005 |
| Neutrophil (cells/µl) /100 | 1.020 (0.999–1.042) | 0.067 |

BUN, Blood urea nitrogen.

/100 implies that the odds ratio is the increase in odds per one hundred unit increase in the value of the measured variable.

**Table 10.** Multivariable logistic regression of hematology, serum biochemistry and cardiac biomarkers

| Variables | Odds ratio (95% CI) | *p*-value |
|---|---|---|
| BUN to creatinine ratio | 1.098 (1.010–1.174) | 0.027 |
| Neutrophil (cells/µl) /100 | 1.022 (1.002–1.043) | 0.035 |
| NTproBNP (pmol/l) /100 | 1.070 (1.014–1.128) | 0.013 |

BUN, Blood urea nitrogen; NT-proBNP, N-terminal pro B-type natriuretic peptide.

/100 implies that the odds ratio is the increase in odds per one hundred unit increase in the value of the measured variable.

**Table 11.** Multivariable logistic regression of echocardiography parameters.

| Variables | Odds ratio(95% CI) | *p*-value |
|---|---|---|
| *E* value ×10 | 1.354 (1.063–1.725) | 0.014 |
| La/Ao ratio ×10 | 1.281 (1.047–1.568) | 0.016 |

*E* value, peak velocity of early diastolic transmitral flow; LA:Ao ratio, left atrial-to-aortic root ratio.

X10 implies that the odds ratio is the increase in odds per 0.1 unit increase in the value of the measured variable.

**Table 12.** Multivariate logistic regression of echocardiography parameters and cardiac biomarkers.

| Variables | Odds ratio (95% CI) | *p*-value |
|---|---|---|
| La/Ao ratio ×10 | 1.219 (0.983–1.511) | 0.071 |
| NLIVDd ×10 | 1.036 (1.003–1.070) | 0.034 |
| NTproBNP (pmol/l) /100 | 1.053 (1.007–1.102) | 0.024 |

LA/Ao ratio, left atrial-to-aortic root ratio; NLVIDd, normalized left ventricular internal diameter in the diastolic phase; NT-proBNP, N-terminal fragment of pro-brain natriuretic peptide.

X10 implies that the odds ratio is the increase in odds per 0.1 unit increase in the value of the measured variable.

/100 implies that the odds ratio is the increase in odds per one hundred unit increase in the value of the measured variable.

**Table 13.** Multivariable logistic regression of thoracic radiograph parameters and ECG.

| Variables | Odds ratio (95% CI) | *p*-value |
|---|---|---|
| VHS ×10 | 1.133 (1.045–1.228) | 0.003 |
| HR (ECG) (bpm) /10 | 1.207 (1.000–1.457) | 0.051 |

VHS, vertebral heart score; HR(ECG); heart rate measured by electrocardiogram; bpm, beats per minute.

X10 implies that the odds ratio is the increase in odds per 0.1 unit increase in the value of the measured variable.

/10 implies that the odds ratio is the increase in odds per ten unit increase in the value of the measured variable.

an approximately 13.3% increase in the likelihood of developing symptomatic CHF (OR, 1.133; 95% CI, 1.045–1.228; $p = 0.003$) (Table 13).

**Thoracic radiography parameters, ECG, and cardiac biomarkers**

In the multivariate logistic regression analysis of thoracic radiography parameters in combination with ECG and cardiac biomarkers in dogs with MMVD, two variables were found to be significantly associated with the OR of symptomatic dogs, namely, VHS (multiplied by 10) and NT-proBNP. Dogs with a 0.1-unit increase in the VHS had an approximately 12.1% increase in the likelihood of developing symptomatic CHF (OR, 1.121; 95% CI, 1.030–1.221; $p = 0.008$). In addition, dogs with a 100-unit increase in the NT-proBNP level had an approximately 6.1% increase in the likelihood of developing symptomatic signs (OR, 1.061; 95% CI, 1.009–1.116; $p = 0.021$) (Table 14).

**All without echocardiography parameters**

In the multivariate logistic regression of all parameters except echocardiography parameters, two variables were significantly associated with the ORs of symptomatic dogs, namely, BUN-to-creatinine ratio

**Table 14.** Multivariable logistic regression of thoracic radiograph parameters, ECG, and cardiac biomarkers.

| Variables | Odds ratio (95% CI) | *p*-value |
|---|---|---|
| VHS ×10 | 1.121 (1.030–1.221) | 0.008 |
| NT-proBNP × 0.01 | 1.061 (1.009–1.116) | 0.021 |

VHS, Vertebral heart score; NT-proBNP, N-terminal pro B-type natriuretic peptide.

**Table 15.** Multivariable logistic regression of all parameters except echocardiographic parameters.

| Variables | Odds ratio (95% CI) | *p*-value |
|---|---|---|
| NT-proBNP ×0.01 | 1.056 (0.997–1.119) | 0.064 |
| BUNtoCreatinine ratio | 1.135 (1.042–1.237) | 0.004 |
| VHS ×10 | 1.166 (1.055–1.287) | 0.003 |

VHS, Vertebral heart score; NT-proBNP, N-terminal pro B-type natriuretic peptide.

and VHS (multiplied by 10). Dogs with a unit increase in the BUN-to-creatinine ratio had an approximately 13.5% increase in the likelihood of developing symptomatic CHF (OR, 1.135; 95% CI, 1.042–1.237; $p = 0.004$). In addition, dogs with a 0.1-unit increase in the VHS had an approximately 16.6% increase in the likelihood of developing symptomatic signs (OR, 1.166; 95% CI, 1.055–1.287; $p = 0.003$) (Table 15).

## Discussion

The main objective of this study was to evaluate the accuracy of cardiac biomarkers to discriminate symptomatic dogs with MMVD and assess the diagnostic potency of these biomarkers and clinical variables. The study population was representative of the general population of dogs affected by MMVD (old, small-size dog breeds).

The NT-proBNP level was significantly higher in symptomatic dogs with MMVD than in the control and asymptomatic MMVD groups. In dogs with MMVD, the ROC curve analysis revealed that the serum NT-proBNP level could differentiate dogs with CHF (MMVD stages C and D) from dogs without CHF (MMVD stage B). When comparing the AUC of NT-proBNP and cTnI for differentiating dogs with CHF, NT-proBNP was superior to cTnI according to the AUC.

To the best of our knowledge, this is the first study to compare the abilities of the rapid tests for cardiac circulating biomarkers in dogs with MMVD. We found that the NT-proBNP rapid test was superior to the cTnI rapid test in discriminating symptomatic dogs with MMVD from asymptomatic dogs with MMVD and controls. This finding is similar to that of the previous study that used enzyme-linked immunosorbent assay (ELISA) (Ogawa *et al.*, 2021). It appears that the rapid test is comparable with ELISA but provides quicker results within one hour and is suitable for point-of-care testing.

The plasma NT-proBNP test was considered as a diagnostic method in situations where conventional radiography may not provide sufficient sensitivity to allow an accurate diagnosis or when echocardiography is not readily available. In this study, the suggested cutoff value of the NT-proBNP level for diagnosing symptomatic MMVD was >787.65 pmol/l (sensitivity, 78.28%; specificity, 72.549%; and AUC, 0.792).

Several studies were conducted using various approaches to determine whether high NT-proBNP values afford accurate diagnosis. The suggested cutoff values to discriminate heart failure (HF) in dogs in previous studies were 201, 1,207 (Wolf *et al.*, 2013), and 1,772 pmol/l (Ogawa *et al.*, 2021). Our NT-proBNP cutoff value was fairly different compared with those in the previous studies. The variation in these cutoff values could be attributed to differences in assays and methods used in each study. Therefore, it is not appropriate to compare the cutoff values among different tests.

BNP is considered species-specific; therefore, it is necessary to validate ELISA to determine NT-proBNP in dogs (Lima and Ferreira, 2017). Moreover, ELISAs require a large sample volume, multiple steps, and incubation time that cannot be performed in clinical practice. Most importantly, it is necessary to prevent degradation during collection and transportation to a laboratory (Lima and Ferreira, 2017). For this reason, point-of-care immunoassays can be advantageous in reducing the time between sample collection and analysis and in minimizing inter-assay variation. In the recent study, the serum NT-proBNP level when measured by the canine immunoassay test from Bionote® had a cutoff value of 772 pmol/l with sensitivity rates of 90% and 81.8% in discriminating dogs with CHF (MMVD stage C) from dogs with evidence of MMVD and cardiomegaly but without CHF (MMVD stage B2) and normal healthy dogs (Riengvirodkij and Sakcamduang, 2021). This cutoff value was similar to a cutoff value from the present study, possibly due to the use of a similar NT-proBNP test. It indicated that the NT-proBNP level measured by the rapid immunoassay test was reasonably consistent and had less variation.

Various studies have been conducted and have shown that the circulating NT-proBNP level increases with increasing severity of MMVD (Häggström *et al.*, 2000; Bettencourt *et al.*, 2004; Oyama *et al.*, 2009; Moonarmart *et al.*, 2010; Lima and Ferreira, 2017; Ogawa *et al.*, 2021; Riengvirodkij and Sakcamduang, 2021). A 100-pmol/l increase in the NT-proBNP level has been associated with an increased risk of all-cause mortality in a population of dogs with both preclinical and clinical MMVD (Moonarmart *et al.*, 2010). The NT-proBNP levels of >1,500 pmol/l have been associated with an increased risk of HF or cardiac

death (Borgarelli *et al.*, 2021). This indicates that NT-proBNP can be used for monitoring and prognosis in dogs with MMVD. Consistent with previous studies, the present study demonstrates that patients with CHF have notably increased NT-proBNP levels compared with both the healthy control group and asymptomatic dogs with MMVD (Häggström *et al.*, 2000; Oyama *et al.*, 2009; Wolf *et al.*, 2013; Ogawa *et al.*, 2021).

In our study, cTnI can be used to differentiate healthy dogs from symptomatic dogs with MMVD. However, the discriminatory ability of cTnI in this study was inferior to NT-proBNP. The suggested cutoff value was 0.115 ng/ml. Similar to previous studies, higher cTnI levels are associated with severe MMVD (Spratt *et al.*, 2005; Ljungvall *et al.*, 2010; Polizopoulou *et al.*, 2014). One study has evaluated the use of cTnI for diagnosing heart disease in dogs. The median cTnI level in the control group was 0.05 ng/ml, which is close to the 0.06 ng/ml found in our study. However, in the previous study, the cTnI level for dogs with MMVD was 0.34 ng/ml, which is higher than the 0.17 ng/ml observed for symptomatic dogs with MMVD in our study (Spratt *et al.*, 2005). It is possible that the results primarily reflect a population of symptomatic dogs with MMVD in ACVIM stage C, as opposed to International Small Animal Cardiac Health Council class IIIA from the previous study, indicating more severe cardiomyocyte in-jury and cardiac remodeling (Spratt *et al.*, 2005). In the other way, in the present study, most symptomatic dogs with MMVD previously received diuretic drugs, which led to lower cTnI levels than those in the previous study. Another potential factor contributing to the difference could be the use of different assays. The previous study used an IMMUNE Troponin I immunometric chemiluminescence system, whereas this study employed the V-check system, which has not been validated for use in dogs. Currently, several assays and different cutoff values are used for diagnosing dogs with MMVD. Further study is required to compare the accuracy and correlation of cTnI between different assays.

The serum ANP levels did not significantly differ between dogs with MMVD and control dogs. Its discriminatory ability to differentiate dogs with MMVD from control dogs was low (AUC, 0.451). This finding is in contrast with those of the previous studies where the plasma ANP level measured by chemiluminescent enzyme immunoassay was significantly higher in dogs with MMVD than in control dogs (Ogawa *et al.*, 2021). The difference in results may be attributed to the use of different assays to measure the ANP levels. In this study, we used the ANP enzyme immunoassay kit (Detect X®), and both ANP assays had not been validated in dogs. Further study is required to compare the accuracy and correlation of ANP between different assays. In another way from previous studies, its fragment has significantly longer half-lives than ANP (McDowell *et al.*, 2002). The proANP fragment can be used to detect HF in dogs (Boswood *et al.*, 2003). Likewise, NT-proANP has demonstrated greater sensitivity in detecting mild increases in atrial filling pressure (Habibullah *et al.*, 1995). This suggested that NT-proANP is of interest in developing an immune assay kit to discriminate MMVD in dogs.

In this study, the median BUN level in the symptomatic group was higher than the normal reference range (7–27 mg/dl). However, the creatinine level was within the normal reference range and International Renal Interest Society chronic kidney disease (CKD) stage 1. Serum urea and creatinine are the most common markers used to assess kidney function. Their increase may result from prerenal azotemia. Multiple pathophysiological mechanisms are involved in cardiorenal syndrome (CRS) including the inability of the heart to generate forward flow, thus resulting in prerenal hypoperfusion. Inadequate renal afferent flow activates the renin–angiotensin–aldosterone system axis, sympathetic nervous system, and vasopressin secretion, leading to fluid retention, increased preload, and worsening pump failure. Increased central venous pressure results in renal venous hypertension, increased renal resistance, and impaired intrarenal blood flow (Ettinger *et al.*, 2017; Rangaswami *et al.*, 2019). Several studies have shown that the prevalence of CKD and anemia in dogs with MMVD is significantly higher than that in the general population in dogs and the medical management of HF affects the prevalence of CKD (Martinelli *et al.*, 2016). Another study confirmed that the more advanced the stage of CHF in dogs affected with chronic valvular disease, the higher the degree of azotemia (Nicolle *et al.*, 2007).

The increase in the BUN level and BUN-to-creatinine ratio in the symptomatic MMVD group suggests that elevated BUN-to-creatinine ratios may be associated with a greater likelihood of disease progression in dogs receiving diuretics. These may result from CRS or the previous use of diuretic drugs. According to the previous study, hospitalized patients with CHF and MMVD had significantly higher urea and creatinine levels (Brložnik *et al.*, 2023). In addition, the serum urea level was significantly negatively associated with survival (Brložnik *et al.*, 2023). It appears that the BUN and creatinine levels increase with the severity of CHF and could be used for monitoring along with drug prescriptions and mortality prediction.

Despite the fact that echocardiography is the gold standard for assessing cardiac structure and function, challenges related to expertise and resources can limit its availability in some clinical practice settings. In contrast to radiography, ECG and cardiac biomarkers are widely available in clinical practice. From the multivariate logistic regression results, when echocardiographic variables were excluded from the analysis, both the VHS and NT-proBNP levels demonstrated the ability to accurately diagnose dogs with HF.

From the ROC analysis, the suggested cutoff value of the VHS for discriminating symptomatic dogs with

MMVD was 11.4, with a sensitivity of 64.8% and specificity of 82.3%. This correlates with a previous study that suggested a cutoff point of >11.7 in the VHS to predict echocardiographic left heart enlargement (Poad *et al.*, 2020). Both values were approximately in line with the VHS of >11.5, which can be used to substitute for echocardiographic measurements in the diagnosis of MMVD stage B2 from canine MMVD guidelines (Keene *et al.*, 2019).

From univariate logistic regression, the OR of the VHS was 1.137, indicating a 13.7% higher likelihood of HF in dogs for every 0.1 increment in the VHS from 11.4. Our result was lower than that in the previous study that revealed that a 0.25 increase in the VHS was associated with a 7.9% increase in the OR of the presence of echocardiographic left heart enlargement and a VHS of >11.7 indicated left heart enlargement (Poad *et al.*, 2020). To the best of our knowledge, this is the first study to determine that the OR of the VHS indicates a higher likelihood of HF in dogs with MMVD.

VHS measurement is a radiographic technique used to assess the heart size in dogs. However, there are several factors that can affect the accuracy and interpretation of VHS measurements, such as cardiac cycles, respiratory phases, vertebral shape, and animal position (Brown *et al.*, 2020; Greco *et al.*, 2008; Olive *et al.*, 2015). One notable difference between the present and previous studies is the predominant breeds. In the present study, Chihuahua was the predominant breed in all groups. In contrast, in the previous study, the predominant breeds were mixed breeds and Cavalier King Charles Spaniel (Poad *et al.*, 2020). Previous studies have highlighted interbreed VHS differences (Lamb *et al.*, 2001; Jepsen-Grant *et al.*, 2013). In the previous study, the VHS value in healthy adult Chihuahua dogs was 10.0 ± 0.6, which was significantly higher than the canine reference value of 9.7 ± 0.5 (Puccinelli *et al.*, 2021). Another study suggested a VHS of >11.1 as a diagnostic criterion for MMVD stage B2 in Chihuahua with MMVD when echocardiography is not available (Ito, 2022).

In the multivariate logistic regression analysis of echocardiography parameters, both *E* value and LA/Ao ratio were found to be significantly associated with the OR of clinical presenting signs. A possible explanation of this finding is the association between increased E peak velocities and increased LA pressure. An increased LA/Ao ratio reflects LA enlargement, which typically results from prolonged increased LA pressure. The development of cardiogenic pulmonary edema is largely predicted by the magnitude of volume overload and the resulting increase in LV filling pressure (Schober *et al.*, 2010). The E peak velocity can be considered as an indirect indicator of the disease severity and negative prognostic factor (Borgarelli *et al.*, 2012; Hezzell *et al.*, 2012). Another study reported that the best predictor model for identifying dogs with an increased risk of reaching cardiac death or first occurrence of HF event was a composite of NT-proBNP, LA/Ao ratio, and E peak velocity (Borgarelli *et al.*, 2021).

This study has some limitations. First, the major limitation is the relatively small sample size in MMVD stages B1 and D, which may result in less significant diagnostic power for differentiating dogs between the groups. Second, the present study did not consider the effects of drugs used in dogs with MMVD stage B2 and higher. All treated dogs received pimobendan (0.25–0.3 mg/kg every 12 hours); however, the use of diuretics and angiotensin-converting enzyme inhibitors varied from one individual to another. Previous studies have found significant reductions in heart size based on radiographic and echocardiographic measurements in dogs receiving pimobendan (Häggström *et al.*, 2013; Boswood *et al.*, 2018). However, a longitudinal study for monitoring changes in cardiac biomarkers along with treatment and progression of MMVD requires further investigation.

In conclusion, the NT-proBNP and cTnI levels were significantly higher in symptomatic dogs with MMVD. Both cardiac biomarkers can be considered as point-of-care tests for patients, particularly with dyspnea in clinical practice. Interestingly, the serum BUN level and BUN-to-creatinine ratio increased in symptomatic dogs with MMVD, which may be useful in monitoring, and prognosis.

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***Authors' contributions***

Conceptualization, research design, data curation, data analysis, interpretation and discussion the results, K.C., N.R., P.C., P.K., W.B., Y.J., R.P., M.T. and W.S.; investigation, K.C., P.C., N.R., P.K., W.B., R.P., Y.J. and M.T.; writing—original draft preparation, K.C., N.R., M.T. and W.S.; writing—review and editing, K.C., N.R., P.C., P.K., W.B., Y.J., R.P., M.T. and W.S.; supervision, W.S.; project administration, W.S.; funding acquisition, W.S. All authors have read and agreed to the published version of the manuscript.

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***Conflict of interest***
The authors declare no conflict of interest. While this study received funding from the Faculty of Veterinary Science Mahidol University (Thailand), Scarecrow Inc (Japan), T.J. Animal Health Co., Ltd. (Thailand), and Pharmalink International Ltd. (Hong Kong), these entities had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

***Data availability***
The data used to support the findings of this study are available from the corresponding author upon request.

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