Research Articles

Assessment of Two New Commercial Rapid Tests for Canine N-terminal Pro-B-type Natriuretic Peptide to Distinguish the Severity of Mitral Valve Disease in Dogs

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Abstract

N-terminal pro-B-type natriuretic peptide (NT-pro-BNP) is a potential cardiac biomarker released primarily from ventricular myocytes. Previous studies in dogs suggest that the NT-proBNP assay can be used to distinguish dogs with respiratory signs between congestive heart failure and primary respiratory illnesses. The objective of this study is to determine and assess the clinical sensitivity and specificity of the two new NT-pro-BNP immunoassay tests for distinguishing dogs affected by myxomatous mitral valve disease (MMVD) between with and without congestive heart failure (CHF), as well as between dogs with MMVD and normal dogs. Thirty-seven dogs were enrolled in this study. Thirty-two dogs were measured by the NT-proBNP immunoassay test (Bionote®), including 11 healthy dogs, 11 dogs with MMVD stage B2 (absence of CHF) and 10 dogs with MMVD stage C (presence of CHF). Twenty-eight dogs were measured by the NT-proBNP rapid detection kit (Dianotech®), including 6 healthy dogs, 7 dogs with MMVD stage B2 and 15 dogs with MMVD stage C.

The median NT-proBNP concentration in dogs with MMVD stage C was significantly higher than the median NT-proBNP concentration in dogs with MMVD stage B2 and normal healthy dogs (p-value <0.001). The Receiver operating characteristic (ROC) curve analysis showed that serum NT-proBNP concentration could differentiate dogs with CHF signs (MMVD stage C) from dogs without CHF signs (normal healthy dogs and MMVD stage B2. The area under the curve (AUC) was 0.932 and 0.928 for NT-proBNP Bionote® and Dianotech® test, respectively. It also could discriminate dogs affected by MMVD with CHF (MMVD stage C2) from dogs affected by MMVD without CHF (MMVD stage B2) with AUC of 0.818 and 0.867 for NT-proBNP Bionote® and Dianotech® tests, respectively. In conclusion, the results suggested that serum NT-proBNP concentrations tests from both companies could discriminate between dogs affected by CHF from those without CHF and normal healthy dogs with reasonable accuracy.

Keywords: N-terminal pro-B-type natriuretic peptide, NT-pro-BNP, mitral valve disease, dogs

การประเมินชุดตรวจ N-terminal pro-B-type natriuretic peptide ในการแยกระดับความรุนแรงของโรคลิ้นหัวใจเสื่อมในสุนัข

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บทคัดย่อ

N-terminal pro-B-type natriuretic peptide (NT-pro-BNP) เป็นตัวชี้วัดทางชีวภาพที่มีศักยภาพในการวินิจฉัยโรคหัวใจ NT-pro-BNP จะถูกหลั่งออกมาจากเซลล์กล้ามเนื้อหัวใจห้องล่างเป็นหลัก มีรายงานจากการศึกษาก่อนหน้าระบุว่าสามารถใช้ชุดตรวจ NT-pro-BNP ในการวินิจฉัยสุนัขที่มีปัญหาโรคลิ้นหัวใจเสื่อม และใช้ตรวจแยกสุนัขที่มีอาการหายใจลำบากที่มีสาเหตุมาจากโรคหัวใจออกจากรายที่มีสาเหตุมาจากโรคระบบทางเดินหายใจได้ การศึกษานี้มีเป้าหมายในการศึกษาประสิทธิภาพของชุดตรวจ NT-pro-BNP รุ่นใหม่จาก 2 บริษัท ในการใช้ตรวจวินิจฉัยโรคลิ้นหัวใจเสื่อมในสุนัข และ เพื่อประเมินความไวและความจำเพาะในการใช้ตรวจแยกสุนัขโรคลิ้นหัวใจเสื่อมที่เกิดภาวะหัวใจล้มเหลวออกจากรายที่ยังไม่เกิดภาวะหัวใจล้มเหลว รวมไปถึงการใช้ตรวจแยกสุนัขที่มีโรคลิ้นหัวใจเสื่อมออกจากสุนัขปกติ

การศึกษาทำโดยเก็บตัวอย่างจากสุนัขทั้งหมด 37 ตัว โดยตัวอย่างจากสุนัข 32 ตัวจะทำการตรวจวัดระดับ NT-pro-BNP ด้วยชุดตรวจจากบริษัท Bionote® โดยแบ่งเป็นสุนัขปกติ 11 ตัว สุนัขลิ้นหัวใจเสื่อมระยะ B2 (ยังไม่มีภาวะหัวใจล้มเหลว) 11 ตัว และสุนัขลิ้นหัวใจเสื่อมระยะ C (มีภาวะหัวใจล้มเหลว) 10 ตัว และตัวอย่างจากสุนัข 28 ตัวจะทำการตรวจวัดระดับ NT-pro-BNP ด้วยชุดตรวจจากบริษัท Dianotech® แบ่งเป็นสุนัขปกติ 6 ตัว สุนัขลิ้นหัวใจเสื่อมระยะ B2 7 ตัว และสุนัขลิ้นหัวใจเสื่อมระยะ C 15 ตัว

ผลการศึกษาพบว่าระดับ NT-pro-BNP ในสุนัขลิ้นหัวใจเสื่อมระยะ C มีค่าสูงกว่าระดับ NT-pro-BNP ในสุนัขลิ้นหัวใจเสื่อมระยะ B2 และสุนัขปกติอย่างมีนัยสำคัญ (p-value < 0.001) การวิเคราะห์ด้วยเส้นโค้ง Receiver operating characteristic (ROC) พบว่าระดับ NT-pro-BNP สามารถใช้ตรวจแยกสุนัขลิ้นหัวใจเสื่อมที่มีภาวะหัวใจล้มเหลว (ระยะ C ออกจากสุนัขที่ยังไม่มีภาวะหัวใจล้มเหลว (ระยะ B2 และสุนัขปกติ) ได้ โดยมีค่าพื้นที่ใต้เส้นโค้ง (area under the curve; AUC) ที่ 0.932 และ 0.928 สำหรับชุดตรวจจากบริษัท Bionote® และ Dianotech® ตามลำดับ การวิเคราะห์ด้วยเส้นโค้ง ROC ยังสามารถใช้ตรวจแยกสุนัขลิ้นหัวใจเสื่อมที่มีภาวะหัวใจล้มเหลว (ระยะ C) ออกจากสุนัขลิ้นหัวใจเสื่อมที่ยังไม่มีภาวะหัวใจล้มเหลว (ระยะ B2) ได้อีกด้วย โดยมีค่าพื้นที่ใต้เส้นโค้งอยู่ที่ 0.818 และ 0.867 สำหรับชุดตรวจจากบริษัท Bionote® และ Dianotech® ตามลำดับ โดยสรุปพบว่าชุดตรวจ NT-pro-BNP จากทั้ง 2 บริษัทมีประสิทธิภาพในระดับที่ยอมรับได้ในการใช้เป็นตัวช่วยในการตรวจวินิจฉัยโรคลิ้นหัวใจเสื่อมในสุนัข และใช้ตรวจแยกสุนัขโรคลิ้นหัวใจเสื่อมระยะที่เกิดภาวะหัวใจล้มเหลวออกจากรายที่ยังไม่เกิดภาวะหัวใจล้มเหลว

คำสำคัญ : เปปไทด์ เอ็นเทอร์มินอลโปรบีเอ็นพี โรคลิ้นหัวใจเสื่อม สุนัข

Introduction

Myxomatous mitral valve disease (MMVD) is the most common acquired heart disease in dogs (Borgarelli et al., 2012; Buchanan 1977). It often leads to congestive heart failure (CHF) and cardiac-related death, eventually (Borgarelli et al., 2012). Differentiation between dogs affected by MMVD with and without CHF is quite challenging. Diagnosis of mitral valve disease (MMVD) with different stages in dogs has conventionally been made on the basis of physical examination, cardiorespiratory auscultation, thoracic radiography and echocardiography. Unfortunately, clinical signs of MMVD are usually non-specific and could be similar to some respiratory tract diseases making it difficult to distinguish between those two diseases just from the physical examination. Detection of lung edema in the perihilar area from thoracic radiography could also be used for identifying dogs with CHF. However, in patients with severe respiratory distress, this technique is limited. For this reason, a biomarker that could be used to differentiate disease severity would be helpful in the diagnosis of dogs with MMVD clinically.

N-terminal pro-B-type natriuretic peptide (NT-pro-BNP) is a cardiac peptide released primarily from ventricular myocytes when stretching (Hosoda et al., 1991). It is synthesized as a prohormone and cleaved into 2 peptide fragments, NT-pro BNP and C-terminal BNP (C-BNP). They are increased in the blood circulation of dogs with heart disease (Boswood et al., 2008; Oyama et al., 2008). However, C-BNP is rapidly degraded and unstable in blood circulation (Thomas and Woods 2003), making it difficult to be used as a clinical biomarker. In contrast, NT-pro-BNP is more stable with a longer half-life than C-BNP (Mueller et al., 2004). In human medicine, the NT-pro-BNP test has been used to differentiate between patients with respiratory distress cause from cardiac and non-cardiac causes (Alibay et al., 2005; Januzzi et al., 2005). Evaluation of serum NT-pro-BNP concentration is currently also recommended as a screening test for patients suspected of heart failure (Swedberg et al., 2005). Previous studies in dogs indicate the use of NT-proBNP assays in differentiating the presence of congestive heart failure from primary respiratory diseases (Boswood et al., 2008; Fine et al., 2008; Fox et al., 2015; Oyama et al., 2008). Recently, two new NT-pro-BNP tests from Bionote® and Dianotech® companies have been introduced for clinical use in dogs with heart disease. Both tests are fluorescent immunoassay which can quantitatively measure the NT-pro BNP concentrations in dogs within minutes, allowing clinicians to immediately decide the appropriate treatment. A serum sample of 100 μl is recommended for the Bionote® test. This test can detect NT-pro BNP levels ranging from 500-10,000 pmol/L with a storage temperature of 2-8 oC. For the Dianotech® test, the detection range is 150-6,000 pmol/L. Either serum or plasma sample is acceptable for this test. Moreover, its storage temperature is broader than the Bionote® test, with a temperature range of 4-30 °C.

This study aims to determine and evaluate the clinical utility of these two new NT-pro-BNP tests from the Bionote® and Dianotech® companies for differentiating dogs affected by MMVD between with and without CHF and between dogs with MMVD versus normal dogs. We hypothesized that these two new NT-pro-BNP assays could be used as diagnostic tests for identifying dogs affected by MMVD with CHF from those without CHF and dogs with MMVD from normal dogs.

Meterials and Methods

Thirty-seven dogs visited at Prasu-Arthorn Small Animal Teaching Hospital, Faculty of Veterinary Science, Mahidol University, were enrolled in the study after their owners have signed consent forms. The protocol used in this study was approved by Mahidol University-Institute Animal Care and Use Committee of the Faculty of Veterinary Science, number MUVS-2017-12-57. All enrolled dogs underwent complete physical examination, thoracic radiography, echocardiography, and electrocardiography. Diagnosis of mitral valve disease (MMVD) was made based on echocardiographic findings of thickened or prolapsed mitral leaflets with evidence of color-flow mitral regurgitation. American College of Veterinary Internal Medicine (ACVIM) staging system was used to classify the severity of dogs with MMVD. Stage B2 was defined by detection of echocardiographic evidence of cardiomegaly, including the left atrial to the aorta (LA: Ao) ratio >1.6 and the normalized left ventricular internal diameter in diastole >1.7, but without clinical signs of CHF. Stage C was defined by the presence of CHF signs with evidence of cardiac enlargement as mentioned above in stage B2 (Keene et al., 2019). Animals were divided into three groups: 1. Normal healthy dogs as a control group, 2. MMVD stage B2 group and 3. MMVD stage C group. Three milliliters (ml) of blood were obtained from each patient. Two ml of blood were placed in the EDTA tube and Heparinized tube to measure complete blood count (CBC) and routine biochemistry. Dogs with abnormalities of hematological and/or biochemical parameters were excluded from the study. The remaining 1 ml was placed in a plain tube, and serum was separated by centrifugation and was kept at -80^{o}C until analyses. Canine NT-proBNP immunoassay test (Bionote®) and canine NT-proBNP rapid detection kit (Dianotech®) were used to determine serum NT-proBNP concentrations.

Measurements of both assays were according to the product instrustions (https://www.bionote.com/vcheck-canine-nt-probnp, and https://irp-cdn.multiscreensite.com/20fad1a2/files/uploaded/Dianotech%20Fluorescence%20Quatitative%Analyer.pdf for products of the Bionote® and Dianotech respectively).

The attending laboratory was blinded to the clinical classification of the cases to avoid bias in the analysis.

Statistical analysis

Normal distribution was tested by the Shapiro Wilk test. Data were present as mean and standard deviation (SD) if data were normally distributed or median and interquartile range if data were not normally distributed. ANOVA or Kruskal-Wallis test was used to determine the difference of age, body weight and NT-proBNP concentration among the groups. The chi-square test was used for the proportion between females and males among the groups. Receiver Operating Characteristic (ROC) curve was plotted to identify the sensitivity, specificity and area under the curve (AUC) and suggested cut-off values of NT-proBNP concentrations. P-value <0.05 was considered significant.

Results

Thirty-seven dogs were included in this study, 32 dogs were analyzed for the canine NT-proBNP immunoassay test (Bionote®) and 28 dogs were analyzed for the canine NT-proBNP rapid detection kit (Dianotech®). Of the 32 dogs with Bionote® test, 11 were considered normal healthy, 11 were considered MMVD stage B2 and 10 were considered MMVD stage C. Six from 11 dogs of the normal healthy group were Chihuahuas. Three were Poodles, 1 was Pug and 1 was Miniature Pincher. Dogs with MMVD stage B2 consisted of 4 Chihuahuas, 2

Pomeranian and 1 of each following breed: Poodle, Yorkshire Terrier, Shih Tzu, Dachshund and mixed-breed dog. The MMVD stage C group included 3 Chihuahuas, 3 Shih Tzus, 3 mixed-breed dogs and 1 Poodles. Of the 28 dogs with Dianotech® test, 6 were classified in the normal healthy group, which consisted of 2 Chihuahuas, 2 Poodles, 1 Pug and 1 Miniature Pincher. Seven were classified in MMVD stage B2 group, which comprised 3 Chihuahuas and 1 of each following breed: Pomeranian, Yorkshire Terrier, Poodle and mixed-breeding. Fifteen were considered MMVD stage C, which included 5 Chihuahuas, 4 mixed-breeding, 3 Poodles, and 3 Shih Tzus. All dogs enrolled in stage C were in the chronic stage of CHF. Demographic data and NT-proBNP concentrations of the 3 groups measured by the canine NT-proBNP immunoassay test (Bionote®) and the canine NT-proBNP rapid detection kit (Dianotech®) were present in Table 1 and Table 2, respectively. Data were reported as a median and interquartile range because the data were not normally distributed. The median age, the median body weight and the proportion of females versus males were not significantly different among the groups. From the 32 samples of the Bionote® test, there were two outliers of body weight in the MMVD stage B2 group (14.2 and 13 kg) and one outlier in the MMVD C group. (16.4 kg). From the 28 samples of the Dianotech® test, there were one outlier of body weight in the MMVD stage B2 group (14.2 kg) and two outliers in the MMVD C group (16.4 and 16 kg). For both NT-proBNP tests, the median NT-proBNP concentration in dogs with MMVD stage C was significantly higher than the median NT-proBNP concentration in dogs with MMVD stage B2 and normal healthy dogs (p-value <0.001) (Table 1, 2 and Figure 1). The NT-proBNP levels measured by the Bionote® test in the normal dogs and dogs with MMVD stage B2 were equal at 500 pmoL/L because of the testís detection limit.

Table 1. Demographic data and NT-proBNP concentrations of 32 dogs measured by the canine NT-proBNP immunoassay test (Bionote®). Data was present as median and interquartile range.

Variables	Normal dogs (n=11)	MMVD stage B2 (n=11)	MMVD stage C (n=10)	p-vaule
Age (years)	7 (5-10.5)	11 (9.25-13)	10.05 (8.98-13.03)	0.065
Body weight (kg)	4.5 (3.7-7.2)	5.4 (4.5-8.2)	5 (4.28-7.08)	0.606
Female (percent)	7/11 (63.6)	2/11 (18.2)	6/10 (60%)	0.062
NT-proBNP (pmoL/L)	500 (500-762)	500 (500-4817.5)	2189.9* (646.7-5198.7)	<0.001

*Significantly different (p<0.01) from the other groups.

Table 2. Demographic data and NT-proBNP concentrations of 28 dogs measured by the canine NT-proBNP rapid detection kit (Dianotech®). Data was present as median and interquartile range.

Variables	Normal dogs (n=6)	MMVD stage B2 (n=7)	MMVD stage C (n=15)	p-vaule
Age (years)	7.25 (4.8-10.88)	11 (6-13)	10.1 (8.9-13)	0.232
Body weight (kg)	5 (3.6-7.63)	5.1 (4.5-6.1)	4.8 (4.3-7.5)	0.86
Female (percent)	4/6 (66.7%)	2/7 (28.6%)	7/15 (46.7%)	0.389
NT-proBNP (pmoL/L)	172 (150-445)	353 (266-2,295)	1,341* (471-6,785)	<0.001

*Significantly different (p<0.01) from the other groups.

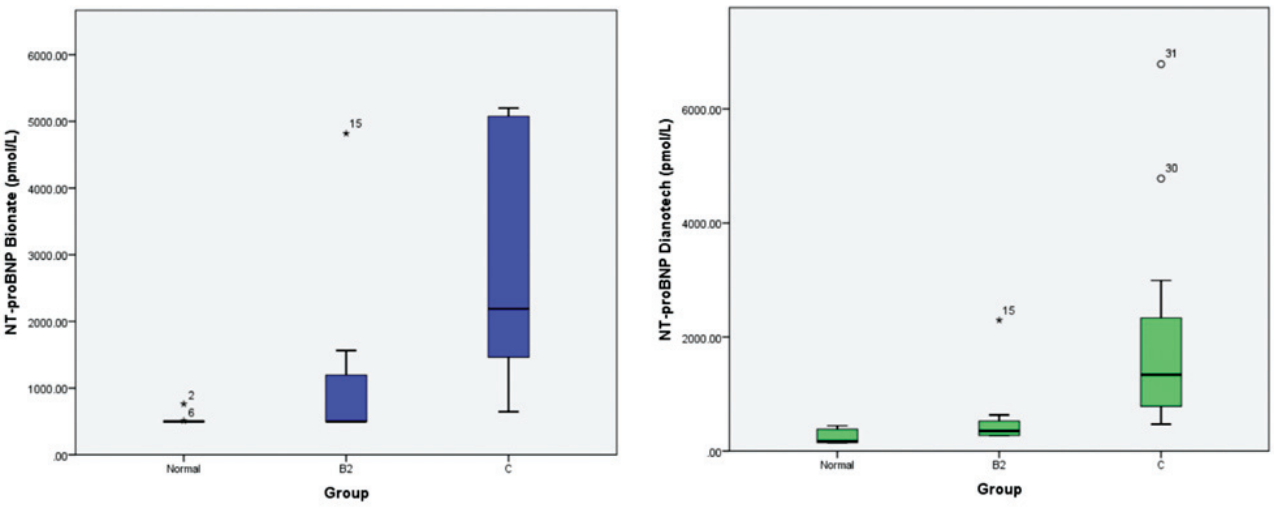

Figure 1. (A) A box plot demonstrates NT-proBNP concentrations measuring by canine NT-proBNP immunoassay test (Bionote®) (blue box) in 11 normal healthy dogs, 11 dogs with MMVD stage B2 and 10 dogs with MMVD stage C. (B) A box plot demonstrates NT-proBNP concentrations measuring by canine NT-proBNP rapid detection kit (Dianotech®) (green box) in 6 normal healthy dogs, 7 dogs with MMVD stage B2 and 15 dogs with MMVD stage C. The whiskers represent the range of values, the box represents the 25th and 75th percentiles and the line within the box represents the median of data. Outliers are shown by individual points.

ROC curve analysis showed that serum NT-proBNP concentration could differentiate dogs with CHF signs (MMVD stage C) from dogs without CHF signs (normal healthy dogs and MMVD stage B2). The AUC were 0.932 and 0.928 for NT-proBNP Bionote® and Dianotech® tests, respectively (Figure 2). Suggested cut-off values for differentiation of dogs with CHF signs from those without CHF signs were established in Table 2.

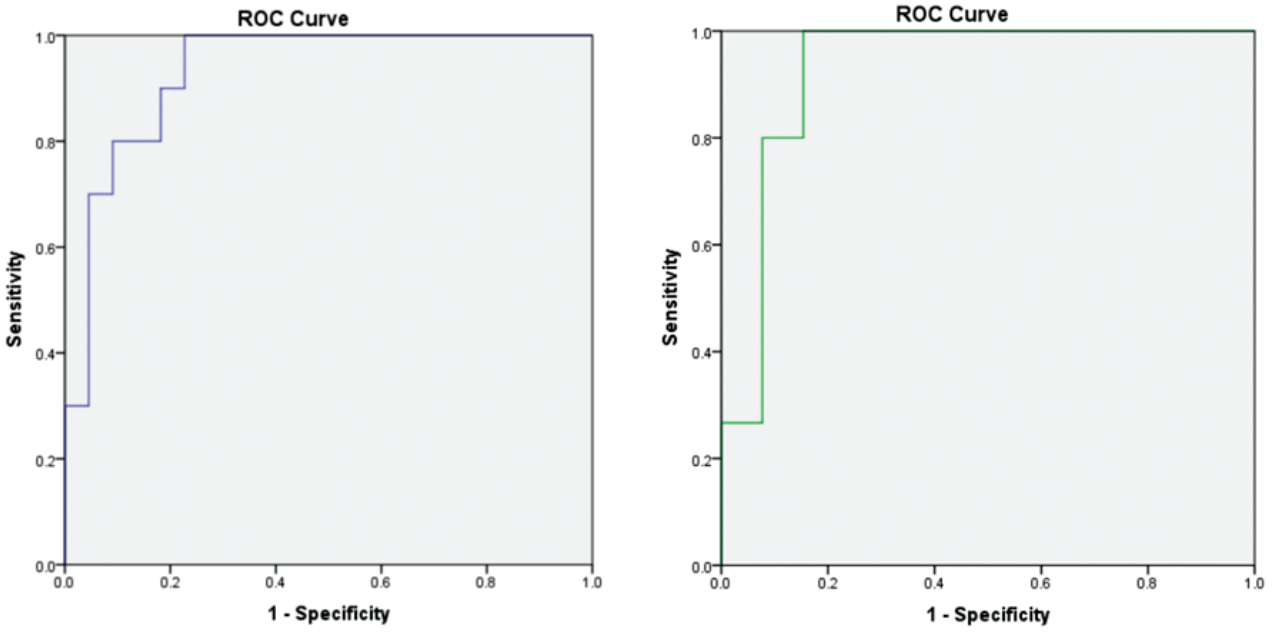

Figure 2. ROC curves discriminate dogs with CHF signs from dogs with no CHF signs. (A) AUC were 0.938 for NT-proBNP Bionote® test and (B) 0.923 for NT-proBNP Dianotech® test.

Table 3. Suggested cut-off values of NT-proBNP test with sensitivity and specificity for the differentiation of dogs with CHF and no CHF.

Assay	Suggested cut-off value	Sensitivity (%)	Specificity (%)
Canine NT-proBNP	633.7 pmol/L	100	77.3
immunoassay test	772 pmol/L	90	81.8
(Bionote®)	1,440 pmol/L	80	90.9
	1,646 pmol/L	70	95.5
Canine NT-proBNP rapid	458 pmol/L	100	84.6
detection kit (Dianotech®)	705 pmol/L	80	92.3
	2,378 pmol/L	26.7	100

ROC curve analysis also displayed that serum NT-proBNP concentration could discriminate dogs affected by MMVD with CHF (MMVD stage C2) from dogs affected MMVD without CHF (MMVD stage B2). The AUC were 0.818 and 0.867 for NT-proBNP Bionote® and Dianotech® tests, respectively (Figure 3). Suggested cut-off values for differentiation between these 2 stage of MMVD were established in Table 3.

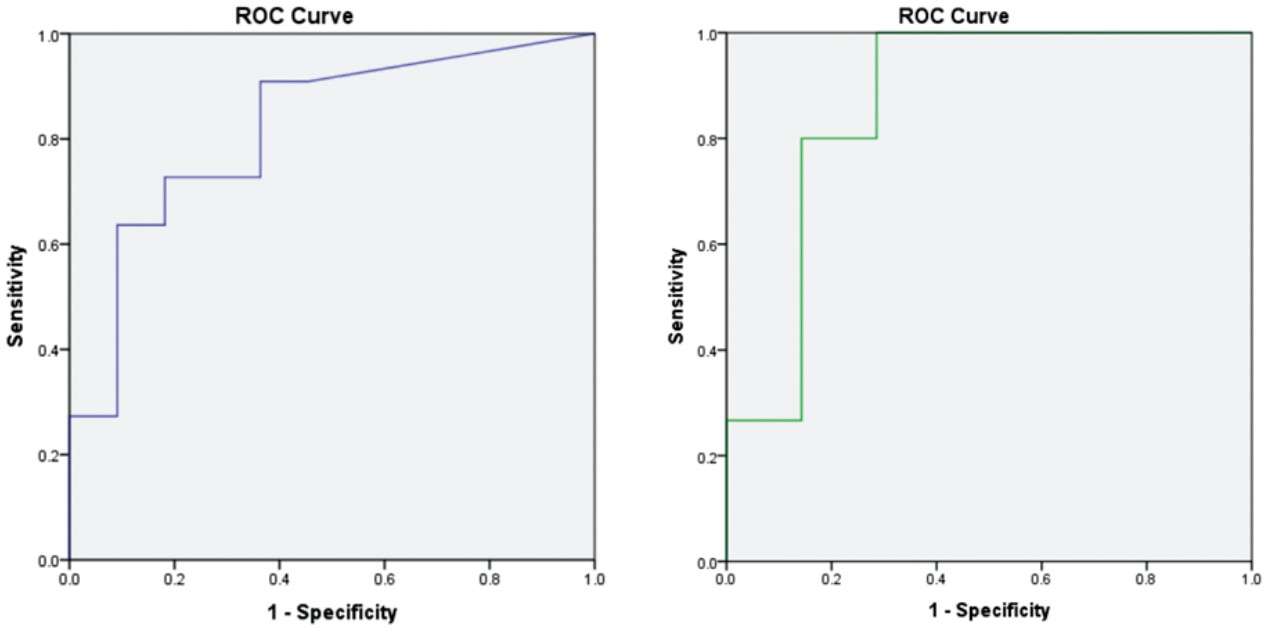

Figure 3. ROC curves discriminate dogs with MMVD stage C from dogs with MMVD stage B2. (A) AUC were 0.818 for NT-proBNP Bionote® test and (B) 0.867 for NT-proBNP Dianotech® test.

Table 4. Suggested cut-off values of NT-proBNP test with sensitivity and specificity for the differentiation of dogs with MMVD stage C and stage B2.

Assay	Suggested cut-off value	Sensitivity (%)	Specificity (%)
Canine NT-proBNP	633.7 pmol/L	90.9	63.6
immunoassay test	1,440.7 pmol/L	72.7	81.8
(Bionote®)	1,646.15 pmol/L	63.6	90.9
Canine NT-proBNP	444.5 pmol/L	100	71.4
detection kit (Dianotech®)	546 pmol/L	80	85.7
	2,250 pmol/L	26.7	100

Serum NT-proBNP concentration could also be used to discriminate dogs with MMVD (MMVD stage B2 and C) from normal healthy dogs (normal control). The AUC were 0.789 and 0.932 for NT-proBNP Bionote® and Dianotech® tests, respectively (Figure 4). Suggested cut-off value for differentiation of dogs with MMVD from those without MMVD signs was established in Table 4.

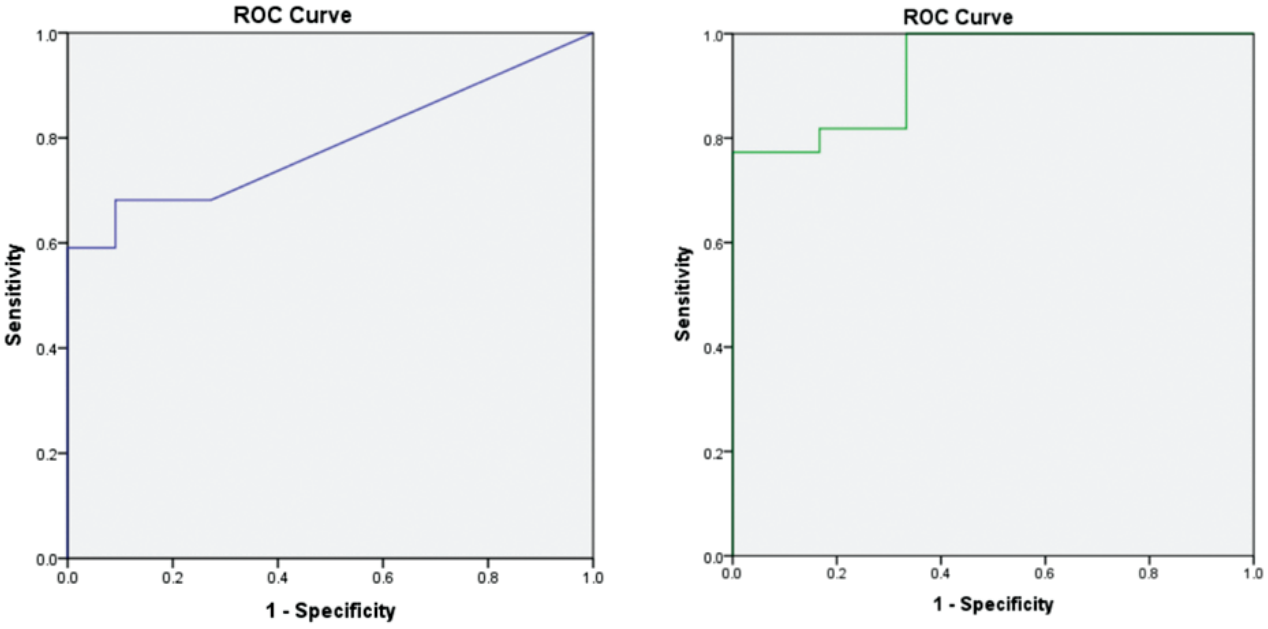

Figure 4. ROC curves discriminate dogs with MMVD stage B2 and C from normal healthy dogs. (A) AUC were 0.789 for NT-proBNP Bionote® test and (B) 0.932 for NT-proBNP Dianotech® test.

Table 5. Suggested cut-off value of NT-proBNP test with sensitivity and specificity for discriminating dogs with MMVD stage B2 and C from normal healthy dogs.

Assay	Suggested cut-off value	Sensitivity (%)	Specificity (%)
NT-proBNP V-check test (Bionote®)	565.8 pmol/L	68.2	90.9
Canine NT-proBNP detection kit (Dianotech®)	224.5 pmol/L	100	66.7
	400 pmol/L	81.8	83.3
	458 pmol/L	77.3	100

Discussion

The results of this study showed that serum NT-proBNP concentrations when measured by canine test from Bionote® and Dianotech® can discriminate between dogs with CHF signs from dogs without CHF signs, and between dogs with MMVD stage C from dogs with MMVD stage B2.

In the present study, serum NT-proBNP concentrations from both companies (Bionote® and Dianotech®) were significantly higher in dogs with CHF (MMVD stage C) than in dogs with evidence of MMVD and cardiomegaly but without CHF (MMVD stage B2) and normal healthy dogs. The ROC curve analysis suggested that serum NT-proBNP concentrations could be used to discriminate these groups. For the canine NT-proBNP immunoassay test (Bionote®), the authors recommended using the cut-off value of 772 pmol/L, which yielded a sensitivity of 90% and specificity of 81.2%. The high sensitivity (90%) indicated that most dogs with CHF have serum NT-proBNP concentrations higher than 772 pmol/L. Additionally, the high specificity (81.2%) indicated that most dogs with NT-proBNP concentrations higher than this cut-off would truly have CHF. Another suggestive cut-off value could be 633.7 pmol/L, which yielded a sensitivity of 100% and specificity of 77.3%. A 100% sensitivity indicated that all dogs with CHF have serum NT-proBNP concentrations higher than this cut-off value which meant dogs with serum NT-proBNP concentration lower than this cut-off value could truthfully rule out CHF. However, its specificity was lower than the first suggestive cut-off value. The moderate specificity (77.3%) indicated that some dogs with serum NT-proBNP concentrations higher than this value might falsely interpret for developing CHF, and other confirmative procedures such as thoracic radiography and echocardiography, are further suggested to identify CHF. For the canine NT-proBNP rapid detection kit (Dianotech®), only one suggested cut-off value of 458 pmol/L was recommended. This cut-off value had 100% sensitivity indicated that all dogs with serum NT-proBNP concentration lower than this cut-off can truly rule out CHF. It also had good specificity at 84.6%, indicating that only a small amount of dogs without CHF had serum NT-proBNP concentration higher than this level. Other suggested cut-off values were not recommended due to their inappropriate sensitivity and specificity.

In dogs with MMVD, ROC curve analysis revealed that serum NT-proBNP concentration could differentiate dogs with CHF (MMVD stage C2) from dogs without CHF (MMVD stage B2). For the canine NT-proBNP immunoassay test (Bionote®), the authors recommended using the cut-off value of 633.7 pmol/L, which yielded a sensitivity of 90.9% and specificity of

63.6%. The high sensitivity (90.9%) indicated that most dogs with MMVD stage C have serum NT-proBNP concentrations higher than 633.7 pmol/L. However, its specificity was too low (63.6%) to be used as a basis for clinical diagnosis. For the canine NT-proBNP rapid detection kit (Dianotech®), the authors recommended using the cut-off value of 444.5 pmol/L, which yielded a sensitivity of 100% and specificity of 71.4%. A 100% sensitivity indicated that all dogs with serum NT-proBNP concentration lower than this cut-off could truly rule out MMVD stage C. The moderate specificity at 71.4 % indicated that some dogs with MMVD stage B2 might have serum NT-proBNP concentration greater than this level. Echocardiography and thoracic radiography are further recommended to confirm MMVD stage C. Previous study from Oyama et al. (2008) has reported the high levels of NT-proBNP concentrations in dogs with heart diseases and its usefulness in distinguishing dogs affected by MMVD between with and without CHF. They reported that serum NT-proBNP was significantly higher in dogs with CHF than dogs with heart disease without CHF. The ROC curve with AUC 0.83 and a suggested cut-off value of serum NT-proBNP concentration >1,725 pmol/L demonstrated that serum NT-proBNP concentration could be used to discriminate dogs affected by MMVD with CHF from those without CHF with a sensitivity of 88.2% and specificity of 76.7% (Oyama et al., 2008). All suggestive cut-off values obtained from this study were lower than the value in the previous study. The difference of these cut-off values could be due to the difference of serum NT-proBNP tests (Canine Cardiocare NT-proBNP, Irvine, Calif vs. Canine NT-proBNP immunoassay test (Bionote®) and canine NT-proBNP rapid detection kit (Dianotech®)). The manufacturers of NT-proBNP tests from Bionote® and Dianotech® suggest that dogs with heart failure (MMVD stage C) will have serum NT-proBNP levels > 1,800 pmol/L and >800 pmol/L, respectively. These cut-offs are higher than the results in the present study. As a result, more research is needed to optimize the cut-off values of these two companies for clinical usage.

When using these 2 NT-proBNP tests to distinguish dogs affected by MMVD (MMVD stage B2 and C) from normal healthy dogs, the ROC curve analysis demonstrated several cut-off values to differentiate between these groups. For the canine NT-proBNP immunoassay test (Bionote®), the authors did not suggest any cut-off value due to its inappropriate sensitivity or specificity. At a cut-off value of 565.8 pmol/L, serum NT-proBNP level had a sensitivity of 68.2% and specificity of 90.9% for discriminating dogs with MMVD from normal dogs. The clinical utility of this cut-off value in this setting was limited by its low sensitivity. This indicated that some dogs with MMVD stage B2 and C might have serum NT-proBNP concentration lower than this cut-off (false negative). For the canine NT-proBNP rapid detection kit (Dianotech®), a suggested cut-off value of 224.5 pmol/L had 100% sensitivity indicated that all dogs with serum NT-proBNP concentration were lower than this cut-off could truly rule out MMVD stage B2 and C. However, the specificity was quite low (66.7%), indicating that several normal dogs would have serum NT-proBNP concentration higher than this cut-off (false positive). A suggested cut-off value of 458 pmol/L had 100% specificity indicated that all dogs with serum NT-proBNP concentration higher than this cut-off truly had MMVD stage B2 or C. The moderate sensitivity at 77.3 % indicated that some dogs with MMVD stage B2 or C might have serum NT-proBNP concentration lower than this level. These sensitivity and specificity were quite close to the previous

study by Oyama et al. (2008), which investigated serum NT-proBNP concentration in dogs with mitral valve disease (MMVD) and dilated cardiomyopathy (DCM) compared with healthy dogs. They reported that serum NT-proBNP was significantly higher in dogs with heart diseases than in normal control dogs. The ROC curve with AUC 0.92 and suggested cut-off value of serum NT-proBNP concentration > 445 pmol/L demonstrated that serum NT-proBNP concentration could be used to discriminate dogs with heart diseases (MMVD or DCM) from healthy normal dogs with a sensitivity of 83.2% and specificity of 90% (Oyama et al., 2008).

The suggestive cut-off values for differentiating dogs with CHF from those with no CHF of the Bionote® assay are generally higher than the Dianotech® assay. The sensitivity and specificity of the Dianotech® assay were slightly higher than the Bionote® assay. There are some advantages of the Dianotech® assay when compared to the Bionote® assay, including less sample volume for analysis (50 μL vs. 100 μL), less detection time (10 min vs. 15 min), broader storage temperature (4-30oc vs. 2-8oc). In addition, both serum and plasma samples can be used in the Dianotech® assay. This is contrast to the measurement of the Bionote® assay, which requires only a serum sample. However, significant differences do not exist between these two assays. According to this result, the authors recommended that these two assays can be used alternatively with different cut-off values as screening tests for identifying dogs affected by MMVD with CHF.

There were some limitations in this study. First, the relatively small sample size in each group might result in less significant diagnostic power for differentiation dogs between the groups. Another limitation was that the sample size of each group in Bionote® and Dianotech® NT-proBNP tests was not equal Lastly, the study was lack of positive and negative control to validate the tests. These limited a comparison of diagnostic accuracy in the differentiation of dogs with MMVD between these two tests. Despite these limitations of the sample size, these two tests remained satisfactory results in discriminating the CHF. This study was considered a preliminary study, and further investigations with more sample sizes in each group are expected to support our results.

In conclusion, the results of this study suggested that serum NT-proBNP concentrations from the new tests of both companies could be used to discriminate between dogs with CHF from those without CHF and normal healthy dogs with acceptable accuracy, but at different cut-off values. It has shown that the measurement of serum NT-proBNP concentrations by these tests would be helpful as an adjunctive clinical assessment for identifying dogs with CHF and evaluating the severity of MMVD in dogs.

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Conflict of Interest

This study was performed independently, with both BestAgro Companion Co, Ltd., and VetAnyMall Co, Ltd. having no influence over study design, data acquisition, analyses, results, manuscript preparation or scientific publication. None of the authors has any other financial or personal relationship that could inappropriate influence or bias the content of the manuscript.

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