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<Original Article>

Evaluation of plasma N-terminal pro-brain natriuretic peptide and troponin I concentrations in dogs with congenital ventricular outflow tract stenosis

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Abstract: This study evaluated the levels of cardiac biomarkers in dogs with either pulmonic stenosis or aortic stenosis and the correlation between biomarkers and the severity of stenosis assessed by the echocardiography. To achieve this study goal, 38 dogs (10 healthy control dogs, 15 dogs with pulmonic stenosis and 13 dogs with aortic stenosis) were examined. The jet velocity and pressure gradient in this study population were measured by echocardiographic estimation, after which the study group was subdivided by the severity of stenosis. The plasma cardiac troponin I (cTnI) and N-terminal pro-brain natriuretic peptide (NT-proBNP) were measured in this study group. The median concentrations of cTnI and NT-proBNP of the disease group were significantly higher than those of the control group, and these increased gradually as stenosis worsened. The severity of stenosis and the concentrations of cTnI and NT-porBNP were also found to be significantly correlated. Finally, the plasma cTnI and NT-proBNP tests were found to beneficial for differentiating clinical patients, predicting the progression of disease, and monitoring the outcome of interventional therapy for stenosis.

Keywords: aortic valve stenosis, pressure gradient, pro-brain natriuretic peptide (1-76), pulmonary valve stenosis, troponin I

Introduction

Congenital ventricular outflow tract valve stenosis (CVOTS) is the most common congenital heart defects in dogs and characterized by ventricular hypertrophy due to increased ventricular afterload and wall stress [19]. The CVOTS is largely divided into aortic stenosis (AS; left ventricular outflow tract stenosis) and pulmonic stenosis (PS; right ventricular outflow tract stenosis) and is further divided into subvalvular, valvular and supravalvular types, depending on the location of stenotic lesion in the outflow tract.

In these diseases, the heart is gradually hypertrophied, if the pressure overload exceeds the adaptive mechanism, causing ventricular filling defect by marked ventricular hypertrophy and increased diastolic stiffness [15]. Furthermore, marked increased wall stress results in marked reduction of ejection fraction, subsequently causing heart failure. The degree of valvular stenosis can be estimated by echocardiography and cardiac biomarker assays [5, 6, 13, 21]. The jet velocity, mean transvalvular gradient and relative wall thickness are the most common echocardiographic method for estimation of the severity in valvular stenosis [3, 22], while the N-terminal pro-brain natriuretic peptide (NT-pro BNP) and cardiac troponin I (cTnI) are the common cardiac biomarker assays for estimation of the cardiac remodeling by valvular stenosis [3, 22]. Those methods were well described in human with congenital valvular stenosis [7]. However, only few studies have been conducted for assessing those methods in dogs with valvular stenosis. Therefore, in this study, we evaluated changes in level of cardiac biomarkers in dogs with CVOTS and the correlation between levels of biomarkers and the severity assessed by the echocardiography.

Materials and Methods

Animals

Dogs with either AS (n = 13) or PS (n = 15) were further divided into subgroups based on the pressure gradient of jet, that is, mild < 60 mmHg (G1), moderate 60–100 mmHg (G2), and severe > 100 mmHg (G3). Ten normal healthy dogs were also enrolled in this study. Table 1 summarized demographic features of this study population. Only dogs diagnosed as congenital isolated type of PS or AS were included in this study. Any dogs with secondary and compound type of PS or AS were excluded (KIACUC-16-0094).

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Table 1. Demographic distribution and biomarker concentration in this study population

Group	Sub-group	Age	Body weight	PS-peak	PS- pressure gradient	AS-peak	AS- pressure gradient	Troponin I (cTnI)	N-terminal pro-brain natriuretic peptide (NT-ProBNP)
Normal (n = 10)		8 (6–13)	3.4 (2.3–9.8)	0.69 (0.5–1.32)	1.9 (1–7)	0.93 (0.69–1.26)	3.4 (1.9–6.4)	0.01 (0.01–0.03)	395 (125–760)
Pulmonic stenosis (PS) (n = 15)	G1	1 (1–9)	5.6 (3.4–12)	3.08* (2.61–3.45)	37.9* (27.2–52.1)	– –	– –	0.08* (0.03–0.12)	1000* (575–1780)
	G2	1 (0.8–10)	4.3 (2.6–14)	4.44*,† (4.33–4.67)	78.9*,† (75–87)	– –	– –	0.12*,† (0.08–0.15)	1405*,† (650–3100)
	G3	3 (0.4–5)	2.6 (2.1–5.6)	5.6*,† (5.17–6.71)	125.4*,† (106–180)	– –	– –	0.15*,† (0.08–0.98)	2800*,† (2200–3500)
Aortic stenosis (AS) (n = 13)	G1	3.5 (2–6)	3 (2.5–5)	– –	– –	2.96* (2.72–3.81)	35* (30–58)	0.11* (0.03–0.58)	900* (620–1800)
	G2	0.4 (0.3–0.4)	12.8 (3.5–34)	– –	– –	4.45*,† (4.17–4.80)	79*,† (70–92)	0.16*,† (0.07–1.25)	1639*,† (850–2505)
	G3	1 (0.5–3)	15 (3.5–34)	– –	– –	5.54*,† (5.11–6.12)	123*,† (104–160)	1.25*,† (0.31–3.4)	3550*,† (1250–8500)

G1, mild; G2, moderate; G3, severe. *$p < 0.05$ normal *vs*. disease group. †$p < 0.05$ mild *vs*. severe group. Median (range).

Echocardiographic estimation of valvular stenosis

Echocardiographic measurements were performed with an ultrasound machine (X-300; Simens, Germany) with a 3–9 MHz phase-array transducer. Aortic jet velocity and mean transaortic gradient were measured at aortic valve level of left apical 5 chamber long axis plane, using continuous wave Doppler echocardiography, while pulmonic jet velocity and mean transpulmonic gradient were measured at pulmonic valve level of the right parasternal short axis plane, using continuous wave Doppler echocardiography.

Cardiac biomarker assays

Blood samples were collected from either jugular or cephalic vein in blood tubes and were centrifuged within 30 min after collection and the plasma was separated. Plasma cTnI concentration was determined using a human Access AccuTnI assay (Beckman Coulter, USA), which has been validated previously in dogs [21]. Plasma NT-proBNP concentrations were determined using an enzyme immunoassay for canine NT-proBNP (Cardiopet proBNP; IDEXX Laboratories, USA), which has been validated previously in dogs [10].

Statistical analysis

All values are expressed as median (min-max). A Kruskal-Wallis test was used to analyze distributed data among groups (age, body weight, echocardiographic variables, plasma NT-proBNP and cTnI concentrations) followed by Dunn's test for multiple comparisons. The Spearman method was used to test for correlations between cardiac biomarkers and body weight, age, and echocardiographic variables. A value of $p < 0.05$ was considered to indicate statistical significance.

Results

The PS groups consisted of 5 mild, 5 moderate and 5 severe PS dogs, while the AS groups consisted of 4 mild, 4 moderate and 5 severe AS dogs (Table 1). Age of the control group (11.9 ± 3.1 yrs) was much older than the PS group (3.3 ± 2.5 yrs) and the AS group (1.8 ± 1.0 yrs) (Table 1). Maltese and Shih Tzu were predominant in the PS group, whereas toy Poodle was predominant in the AS group.

The median peak velocities (pressure gradient) of PS in mild, moderate and severe groups were 3.08 m/sec (38 mmHg), 4.44 (79 mmHg) m/sec and 5.60 (125 mmHg) m/sec, respectively, while that of normal group was 0.69 m/sec (1.9 mmHg). The median peak velocities (pressure gradient) of AS in mild, moderate and severe groups were 2.96 m/sec (35 mmHg), 4.45 (79 mmHg) m/sec and 5.54 (123 mmHg) m/sec, respectively, while that of normal group was 0.93 m/sec (3.4 mmHg; Table 1).

The median concentration of cTnI of PS in mild, moderate and severe groups were 0.08 (range, 0.03–0.12), 0.12 (range, 0.08–0.15) and 0.15 (range, 0.08–0.98) ng/mL, respectively, and were significantly higher than normal group 0.01 (range, 0.01–0.03) ng/mL ($p < 0.05$; Table 1, Fig. 1). The median concentration of cTnI of AS in mild, moderate and severe groups were 0.11 (range, 0.03–0.58), 0.16 (range, 0.07–1.25) and 1.25 (range, 0.31–3.4) ng/mL, respectively and were also significantly higher than normal group ($p < 0.05$; Table 1, Fig. 1). The cTnI concentrations were not correlated to age ($r = -0.331$) and body weight ($r = 0.396$), while those in the PS ($r = 0.700$) and AS groups ($r = 0.763$) were well correlated to the severity of stenosis (Fig. 2).

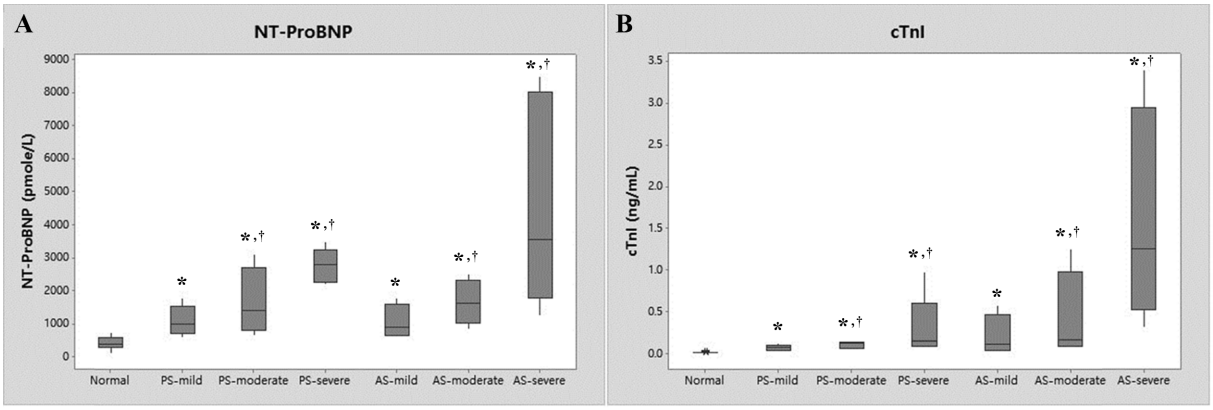

Fig. 1. The concentrations of NT-proBNP (A) and cTnI (B) in this study population. *$p < 0.05$ normal *vs*. disease group. †$p < 0.05$ mild *vs*. severe group.

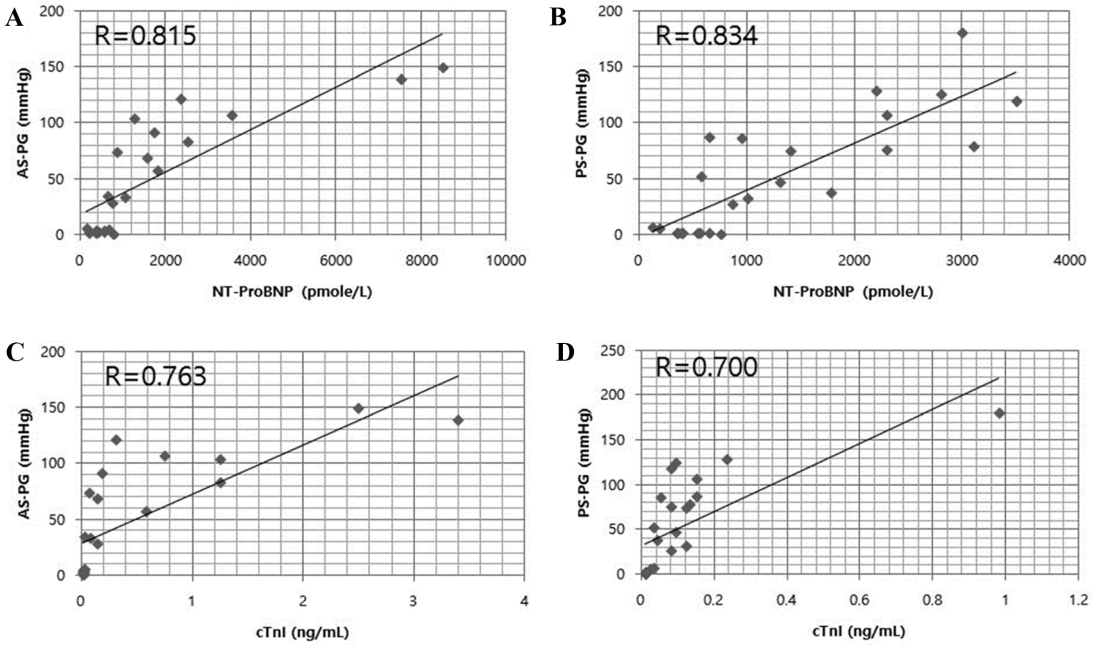

Fig. 2. The correlation assessment between the severity of stenosis and concentration of biomarkers in this study population. A, AS *vs*. NT-proBNP; B, PS *vs*. NT-proBNP; C, AS *vs*. cTnI; D, PS *vs*. cTnI.

The median concentration of NT-proBNP of PS in mild, moderate and severe groups were 1000 (range, 575–1780), 1405 (range, 650–3100) and 2800 (range, 2200–3500) pmole/L, respectively, and were significantly higher than normal group 395 (range, 125–760) pmole/L ($p < 0.05$; Table 1, Fig 1). The median concentration of NT-proBNP of AS in mild, moderate and severe groups were 900 (range, 620–1800), 1639 (range, 850–2505) and 3550 (range, 1250–8500) pmole/L, respectively, and were also higher than normal group (Table 1, Fig 1). The NT-proBNP concentrations were not correlated to age ($r = -0.369$) and body weight ($r = 0.280$), while those in the PS ($r = 0.834$) and AS groups ($r = 0.815$) were well correlated to the severity of stenosis (Fig. 2).

Discussion

Continuous wave (CW) Doppler echocardiography is the easiest and most reliable diagnostic method for grading the severity of valvular stenosis. The pressure gradient (PG) across the stenotic valve has been found to be well correlated to the severity of stenosis [22, 25]. Therefore, in this study, we sub-grouped our study population, based on the PG across the stenotic valve. According to human guideline [3], the severity of AS was graded into < 30 mmHg in mild, 30–50 mmHg in moderate, and > 50 mmHg in severe, while that of PS was < 36 mmHg in mild, 36–64 mmHg in moderate, and > 64 mmHg in severe. Although dogs with either AS or PS with PG < 60 mmHg are mostly clinically asymptomatic,

this study population was divided by different categorization. Recent studies found that Doppler-derived PG > 60 mmHg was associated with poor prognosis [11]. Since our categorization was more focused on clinical status, the levels of cardiac biomarker (*e.g.*, cTnI and NT-proBNP) were more clearly reflected the severity of stenosis in this study.

Cardiac biomarker assays are rapidly replacing to conventional diagnostic imaging for predicting the progression of heart diseases in human. Because this assay requires tiny amount blood samples and no training, it is most useful for large scale screening and cardiac emergency. The cTnI and NT-proBNP are the most commonly performed cardiac biomarker assays in human and are all validated in dogs with heart diseases [5, 8, 21].

There are 3 types of cardiac troponin complex in mammalian heart (cTnI, cTnT, and cTnC). The cardiac troponins are involved in the regulation of excitation-contraction coupling of the sarcomeric proteins and are easily released into blood stream in response to sarcomeric injury [9]. Increased levels of circulating cTnI or cTnT are a diagnostic indicator for myocardial injury in heart diseases in human [1, 17]. Since the canine cTnI has close homology to human cTnI, the cTnI concentration can be measured using human immunoassays for cTnI. In this study, we used human immunoassay kit (previously validated in dogs; 20) and found this test kit could produce reliable and reproducible results in dogs. Recent study for evaluating diagnostic value of cTnI in dogs with chronic mitral valvular insufficiency (CMVI) found the cTnI was significantly higher in the CMVI dogs than healthy dogs and was gradually higher as the disease was progressed [14]. Another recent study evaluating cTnI in dogs with various heart diseases also found a good diagnostic value for detecting heart disease. In that study, the median concentrations of cTnI were 0.14 ng/mL (range, 0.03–1.88 ng/mL) in cardiomyopathy, 0.11 ng/mL (range, 0.01–9.53 ng/mL) in CMVI, and 0.08 ng/mL (range, 0.01–0.94 ng/mL) in subaortic stenosis, while that of cTnI in most healthy dogs was < 0.03 ng/mL [20]. Our study results were well agreed to the previous studies [20]. The median value of cTnI in the mild PS and mild AS dogs was 8–11 times higher than healthy dogs, suggesting this test is discriminative asymptomatic AS or PS dogs from healthy dogs. Furthermore, the cTnI concentration was gradually higher, as the stenosis was worsened, suggesting this test also has a good prognostic value in clinical PS or AS dogs. Although our study revealed the PG across the stenotic valve was closely correlated to the level of cTnI, other study only found modest correlation with the ventricular wall thickness [20]. Since our study population consisted of only AS, instead of subaortic stenosis, the trend on cardiac remodeling by pressure overload might be different and thus the degree of myocardial injuries might be much severe in the AS. Therefore, in our study, the PG was more well-correlated with the cTnI concentration. One study found circulating TnI concentrations were positively correlated with age [14]. However, our study could not detect any influence on the cTnI concentration from age, although our healthy control dogs were much older than the PS and AS dogs. The number of healthy control was insufficient to conclude that there was no influence from the age of study groups.

Circulating NT-proBNP is a byproduct from cleavage of BNP, which is released from brain and ventricle in response to increased myocardial wall stress from increased cardiac loading conditions [12]. Therefore, the plasma concentrations of both BNP and NT-proBNP are generally elevated in patients with congestive heart failure from any causes [2, 18]. Those tests are currently used for large scale screening in large population, for diagnosis of acute onset of CHF, and for the prediction of clinical outcome [4]. One veterinary study also found the NT-proBNP is independently predictive of survival in dogs with CMVI [16]. One recent study also found the plasma NT-proBNP levels were significantly higher in dogs with PS [13]. Right ventricular pressure overload by the PS is thought to be a cause of secretion of NT-proBNP [26]. One human study also revealed the right ventricular pressure overload could increase plasma BNP concentrations, even in patients with asymptomatic right ventricular heart diseases [24]. As noticed in other studies, we also found the levels of NT-proBNP were higher in dogs with PS. The levels of NT-ProBNP were closely associated with the PG across the stenotic valve in this study, suggesting a good prognostic marker for the progression of PS or for the outcome of interventional balloon valvuloplasty in dogs. To date, no study has been conducted to evaluate plasma NT-proBNP levels in dogs with AS, although the NT-proBNP could be higher in the AS, due to left ventricular pressure overload by the left ventricular obstruction. This study has firstly proved that the levels of NT-proBNP were closely correlated to the severity of AS in dogs.

There are several study limitations. Firstly, because our study population was limited by the small sample size, there would be the possibility for misinterpretation by gender, breed, age, and body weight. Due to small sample size, there might be type I error on interoperation of this study result. Secondly, some dogs with PS or AS were under cardiac medication (*e.g.*, β-blockers, calcium-channel blockers) which could affect significantly loading conditions. Therefore, there is possibility for under-estimation of cTnI and NT-proBNP levels in these dogs. Thirdly, because the echocardiographic measurement for PG often leads overstatement of the severity of the stenosis [23], more accurate assessment using invasive catheterization on the stenotic lesion might be necessary to obtain more precise estimation of severity of stenosis. Lastly, the increased cTnI and NT-proBNP levels in this study population might be also influenced by non-cardiac causes (*e.g.* renal diseases, infections, and dehydration; 5, 6), although we excluded any dogs having signs of other systemic diseases prior to study.

In conclusion, this study evaluated the diagnostic value of cardiac biomarkers in dogs with either PS or AS and the correlation between levels of biomarkers and the severity

assessed by the echocardiography, and found the plasma cTnI and NT-proBNP were discriminative for dogs with heart disease from healthy dogs and were well correlated to the severity of stenosis.

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