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Association of Plasma N-Terminal Pro-B-Type Natriuretic Peptide Concentration with Mitral Regurgitation Severity and Outcome in Dogs with Asymptomatic Degenerative Mitral Valve Disease

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Background: The clinical outcome of dogs affected by degenerative mitral valve disease (MVD) without overt clinical signs is still poorly defined, and criteria for identification of animals that are at a higher risk of early decompensation have not yet been determined.

Hypothesis: N-terminal pro-B-type natriuretic peptide plasma concentration (NT-proBNP) is correlated with mitral regurgitation (MR) severity and can predict disease progression in dogs with asymptomatic MVD.

Animals: Seventy-two dogs with asymptomatic MVD, with or without heart enlargement (International Small Animal Cardiac Health Council: ISACHC classes 1a and 1b), and a control group of 22 dogs were prospectively recruited.

Methods: Severity of MR was quantitatively assessed from the regurgitation fraction (RF) by the proximal isovelocity surface area method. Consequences of MR were evaluated from measurements of the left atrium/aorta ratio (LA/Ao), fractional shortening (FS), end-diastolic and end-systolic left ventricular volumes indexed to body surface area (EDVI and ESVI). The relevance of these echo-Doppler indices and NT-proBNP for prediction of outcome at 12 months was studied.

Results: A significant correlation was found between NT-proBNP and RF, LA/Ao, FS, and EDVI ($P < .05$). NT-proBNP was higher in dogs with MVD (ISACHC classes 1a and 1b) compared with the control group ($P = .025$ and $< .001$, respectively). The difference was not significant when only dogs from ISACHC class 1a with RF $< 30\%$ were considered. Lastly, NT-proBNP was higher in dogs that underwent MVD decompensation at 12 months ($P < .05$).

Conclusions and Clinical Importance: NT-proBNP is correlated with MVD severity and prognosis in dogs with asymptomatic MVD.

Key words: Canine; Echocardiography; PISA; Pulmonary hypertension; Regurgitation.

Degenerative mitral valve disease (MVD) is the most commonly acquired heart disease of dogs and results in systolic mitral regurgitation (MR) with potential complex neurohormonal and hemodynamic consequences.[1,2] Although most dogs with MVD remain asymptomatic for years and even for life (International Small Animal Cardiac Health Council [ISACHC] class 1),[3–7] severe complications can occur including exercise intolerance, cough, and dyspnea caused by the development of left-sided congestive heart failure (CHF), and ascitis or pleural effusion as signs of right-sided CHF secondary to pulmonary arterial hypertension (PAH). These complications can lead to death or euthanasia because of worsening or unresponsive clinical signs.[8–10]

Criteria for the identification of dogs in ISACHC class 1 that risk decompensation remain a major issue in practice. Various factors have been hypothesized to influence the time to onset of CHF. It is generally accepted that, as in humans,[11] MR severity is a major determinant of natural disease progression and the clinical outcome in asymptomatic dogs.[12] In 1 study dedicated to the quantification of MR in canine MVD by the proximal isovelocity surface area (PISA) method,[12] dogs without clinical signs exhibited a wide range of regurgitation fractions (RF), and approximately one third of them showed moderate to severe RF (ie, $> 50\%$). Such MR can in turn lead to cardiac remodeling and development of CHF. Several complications including PAH,[13] *chordae tendinae* rupture,[14] renal impairment,[15] and cardiac arrhythmias[a] can also contribute to accelerate cardiac decompensation of dogs with asymptomatic MVD. It might therefore be hypothesized that the detection of such complications, along with echo-Doppler markers of MR severity, could be used to identify dogs from ISACHC class 1 that are likely to progress to the symptomatic phase. Several biomarkers, especially natriuretic peptides, which are involved in volume homeostasis and cardiovascular remodeling,[16] might also be useful in detecting dogs at risk of CHF.

Abbreviation:

EDVI	end-diastolic left ventricular volume indexed to body surface area
ESVI	end-systolic left ventricular volume indexed to body surface area
FS	fractional shortening
ISACHC	International Small Animal Cardiac Health Council
LA/Ao	left atrium/aorta ratio
MR	mitral regurgitation
MVD	degenerative mitral valve disease
NT-proBNP	N-terminal pro-B-type natriuretic peptide plasma concentration
PAH	pulmonary arterial hypertension
PISA	proximal isovelocity surface area
RF	regurgitation fraction

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To date, B-type natriuretic peptides, including both brain natriuretic peptide (BNP) and its inactive aminoterminal portion (N-terminal pro-B-type natriuretic peptide [NT-proBNP]), are considered as among the most reliable neurohormonal markers of human heart diseases.[17] BNP is produced and stored as pro-BNP in response to wall stretch, mainly in ventricular cardiomyocytes, and to a lesser extent, in atrial cardiomyocytes.[16,17] Inactive pro-BNP is then cleaved into the circulating biologically active BNP and the inactive NT-proBNP.[16,17] NT-proBNP is a more stable biomarker than BNP, making its measurement more practical in clinical settings.[16]

Studies on BNP and NT-proBNP in the dog have mostly focused on their accuracy to discriminate between CHF and primary respiratory diseases[18,19] or to assess disease severity,[20,21,b] but not on their prognostic ability, particularly in compensated heart diseases. The potential interest of BNP for the diagnosis of occult heart disease was described in 1 report using a canine model of dilated cardiomyopathy,[22] but its ability to identify asymptomatic dogs at risk for decompensation was not studied. Moreover, in dogs with ISACHC class 1a MVD, correlation between NT-proBNP and MR regurgitation severity or MR progression remains unclear,[c] although NT-proBNP has been recently shown to be increased in Cavalier King Charles Spaniels (CKC) with preclinical MVD and severe MR compared with both controls and CKC with no, minimal, or moderate MR assessed by color flow mapping.[21] Therefore, to the best of our knowledge, evidence is still lacking regarding the usefulness of NT-proBNP as a prognostic biomarker of asymptomatic MVD, and more specifically its ability to identify dogs that are at high risk for MVD complications and cardiac decompensation.

The aims of this prospective study were therefore (1) to determine the correlation between NT-proBNP and various echo-Doppler variables reflecting MR severity in a large population of dogs with asymptomatic MVD (ISACHC classes 1a and 1b), and (2) to evaluate the comparative ability of all these markers to predict outcome at 12 months in the same population of dogs.

Material and Methods

Study Population

The study population consisted of client-owned dogs that prospectively underwent a complete echocardiographic and Doppler examination at the Cardiology Unit of Alfort (National Veterinary School of Alfort, France) from April 2007 to June 2008. In order to limit the influence of age and weight on the studied factors (eg, systolic function and biochemical markers), only adult ($\geq$2 years old) small-breed ($<$20 kg) dogs were included in the study. The owner's consent was obtained for each animal, before its enrollment in the study. Dogs were assigned to either Group C (control group) or Group 1. Group C consisted of healthy control dogs referred for a cardiovascular check-up and characterized by both normal physical and echo-Doppler examinations, whereas Group 1 included dogs with asymptomatic MVD (ISACHC class 1) referred during the same period for exploration of a left apical systolic heart murmur.

Diagnosis of MVD was based on the following criteria: (1) left systolic apical heart murmur detected after the age of 1 year, (2) no history of infectious disease, and (3) echocardiographic and Doppler signs of MVD including irregularly thickened mitral valve leaflets (observed on the right parasternal 4-chamber view) and a color-flow jet of systolic mitral insufficiency in the left atrium (observed on the left parasternal 4-chamber view). Dogs with MVD were included in the study only if the color-flow jet of systolic mitral insufficiency was adequate for color and continuous Doppler mode examination and subsequent assessment of MR by the PISA method. They were further assigned to subgroups 1-a and 1-b (corresponding to ISACHC classes 1a and 1b, respectively), according to the absence or presence of cardiac enlargement (left atrial [LA] dilation with or without left ventricular [LV] dilation).

Echocardiography and Standard Doppler Examination

Conventional echocardiographic and Doppler examinations were performed by experienced cardiologists (Dipl. ECVIM, resident, or practitioner with at least 3 years experience of echocardiography at our unit: V.C., F.S., V.G., C.C.S.), in awake dogs gently restrained in standing position, using continuous ECG monitoring with ultrasound units[d] equipped with 7.5–10, 5–7.5, and 2–5 MHz phased-array transducers as previously described and validated at our unit.[23]

LA and Ventricular Dimensions. Measurements of the aortic (Ao) and LA diameters were obtained by a 2D method using the right parasternal transaortic short axis view as previously described, and the LA/Ao ratio was calculated.[24] LV measurements were obtained from the right parasternal location during 2D-guided M-mode echocardiography according to recommendations of the American Society of Echocardiography.[25] Left ventricular systolic and diastolic diameters were then used to calculate the LV fractional shortening (FS).

LV end-systolic and end-diastolic volumes (ESV and EDV, respectively) were assessed by applying the Simpson's derived planimetric method,[e] as previously described and validated.[26] These volumes were then used to calculate the LV ejection fraction (EF) according to the following formula:

$$EF = 100 \times (EDV - ESV)/EDV$$

ESV and EDV were then indexed to body surface area, which was derived from body weight using the described equation.[27]

Assessment of MR Severity and Associated Complications. MR was quantitatively assessed in all dogs with MVD by the PISA method as previously described and validated at our unit.[12] The studied PISA variable was RF. In all cases the left apical 4-chamber view was used for color-flow Doppler examination of the tricuspid valve. When tricuspid regurgitation (TR) was identified, the peak systolic TR velocity was quantitatively assessed by continuous-wave Doppler mode, and the systolic pulmonary arterial pressure (SPAP) was calculated by applying Bernouilli's equation and adding the estimated right atrial pressure to the systolic right ventricle to right atrium pressure gradient, as previously described.[13,28] An SPAP exceeding 30 mmHg was considered as suggestive of systolic PAH.[13] Maximal diastolic mitral flow velocities (E and A waves) were also calculated. Lastly, the presence of ruptured chordae tendinae was assessed according to the previously published diagnostic criteria.[14]

Blood Sample Collection and Processing (NT-proBNP and Biochemical Analysis)

Venous blood samples were obtained from 12-hour fasted dogs on the day of echocardiographic examination. Four milliliters of blood were collected from each dog. Two milliliters were placed into a tube containing potassium-EDTA and 2 mL were placed in another tube containing lithium heparin as anticoagulant. Samples were centrifuged at 4°C within 60 minutes after collection, and the supernatant

separated from the cells, and stored at −70°C. Samples were then batched for analysis and submitted frozen on dry ice to the appropriate assay laboratories. The heparinized plasma was subjected to biochemical analysis, which included measurement of urea and creatinine concentrations. The ratio of urea to creatinine was then calculated. The plasma NT-proBNP concentration was measured with EDTA-potassium samples and a commercially available canine specific assay.[f] This sandwich ELISA assay has been previously used and validated for diagnostic purposes in the dog.[g,19] Operators performing the biochemical analyses and NT-proBNP assays were blinded to the dog's diagnosis. Moreover, owners were not informed about the NT-proBNP values of their dogs at the time of diagnosis. Indeed, NT-proBNP was not implemented in clinical routine at our unit for the management of preclinical heart disease at that time.

Follow-up of Dogs with MVD (Group 1)

The medical treatment of all previously treated dogs remained unchanged, and no previously untreated dog received medical treatment for heart disease until the development of CHF. Clinical or telephonic follow-up was performed 12 months after initial presentation for all dogs. Dogs were classified as stable (S) if they were still asymptomatic or decompensated (D) if they had either died from or presented clinical signs attributed to CHF. The diagnosis of CHF was accepted only when the data concerning the case history, physical examination, and thoracic radiographs were available. Dogs lost at follow-up or that died of noncardiac diseases were censored for the statistical analysis.

Statistical Analysis

Data are expressed as mean ± SD. Statistical analyses were performed by computer software.[h] Descriptive statistic analysis was used for age, heart rate, sex, and body weight. Normality of the data was tested by a Kolmogorov-Smirnov analysis. NT-proBNP values were normally distributed in Group C, but not in subgroups 1-a and 1-b. Therefore, NT-proBNP values and NT-proBNP values indexed to plasma creatinine concentrations were compared between groups by a nonparametric Kruskall-Wallis test. Differences in other continuous variables between groups were evaluated by a one-way analysis of variance (ANOVA), followed if necessary by Student's *t*-test with Bonferroni correction. Correlations between markers of MR severity (LA/Ao, FS, RF, mitral E/A, ESVI, EDVI, EF, and SPAP) and NT-proBNP plasma concentrations were examined by the nonparametric Spearman's coefficient of correlation. Receiver operating characteristic (ROC) analyses were performed to determine optimal cut-off values for NT-proBNP concentrations in prediction of the 12-month evolution (ie, stable or decompensated state). ROC curves were drawn by plotting all the sensitivity values against their corresponding (1−specificity) values. The area under the ROC curve was calculated. Similar ROC curves were drawn for other variables in order to calculate the area under curve as well as sensitivity and specificity for the optimal cut-off values. For all comparisons, $P < .05$ was considered significant.

Results

Seventy-two dogs with asymptomatic MVD and MR quantified by the PISA method (Group 1) were prospectively recruited from April 2007 to June 2008. These dogs were further assigned to either subgroups 1-a (n = 53) or 1-b (n = 19), corresponding to ISACHC classes 1a and 1b, respectively. Twenty-two healthy control dogs were also recruited during the same period (Group C).

Epidemiologic Characteristics of the Study Population

Group 1 was mostly composed of males, aged adult small-breed dogs (Table 1). The healthy control

Table 1. Demographic characteristics of the study population including 22 dogs from the control group (Group C) and 72 dogs with asymptomatic MVD (Group 1), without and with heart enlargement (subgroups 1-a and 1-b, respectively)

		Group 1: Dogs with Asymptomatic MVD		
Demographic Characteristics	Group C: Control Group (n = 22)	Whole MVD Population (n = 72)	Without Heart Enlargement (Subgroup 1-a, n = 53)	With Heart Enlargement (Subgroup 1-b, n = 19)
Sex				
Male	41% (9/22)	75% (54/72)	74% (39/53)	79% (15/19)
Female	59% (13/22)	25% (18/72)	26% (14/53)	21% (4/19)
Age (years) (mean ± SD, [range])	9.1 ± 1.6 [6.0–12.0]	10.0± 3.3 [2.2–17.0]	9.6 ± 3.5 [2.2–17.0]	11.2 ± 2.7* [6.8–15.0]
Body weight (kg) (mean ± SD, [range])	6.8 ± 3.6 [1.8–17.0]	9.7 ± 4.2* [1.4–19.6]	10.0 ± 4.1* [3.6–19.6]	9.1 ± 4.2 [1.4–17.8]
Breed				
King Charles and Cavalier King Charles Spaniels	5% (1/22)	31% (22/72)	34% (18/53)	21% (4/19)
Bichon	18% (4/22)	8% (6/72)	8% (4/53)	10% (2/19)
Yorkshire Terrier	32% (7/22)	3% (2/72)	2% (1/53)	5% (1/19)
Cross breeds	0% (0/22)	21% (15/72)	17% (9/53)	32% (6/19)
Other breeds	45% (10/22)	37% (27/72)	40% (21/53)	32% (6/19)

*$P < .05$ versus Group C.

MVD, degenerative mitral valve disease.

dogs (Group C) and dogs with MVD (Group 1) were of comparable ages. However, the dogs in subgroup 1-b were significantly older than those in Group C ($P = .003$). English Toy Spaniels (KC and CKC), Yorkshire Terriers, Bichons, and cross breed dogs were overrepresented in both groups. However, Yorkshire Terrier was the most common breed in Group C, whereas English Toy Spaniels were predominant in Group 1.

None of the dogs in Group C received any treatment whereas 30/72 dogs (42%) from Group 1, ie, 21/53 (40%) dogs from subgroup 1-a and 9/19 (47%) dogs from subgroup 1-b, were receiving 1 treatment or more at the time of diagnosis prescribed by referral veterinarians despite the lack of clinical signs of CHF (cough, dyspnea, and exercise intolerance). Treatment included angiotensin-converting enzyme inhibitors such as benazepril (0.19–0.51 mg/kg), imidapril (0.25 mg/kg), and ramipril (0.09–0.15 mg/kg) in all treated dogs (n = 30/72, 42%), associated with spironolactone in 5/72 dogs (7%, 0.9–2.7 mg/kg) and furosemide in 3/72 dogs (4%, 1–2.7 mg/kg).

Echocardiographic and Doppler Findings

RF was 33.6±16.4% (range: 4.1–66.8%) in all dogs from Group 1 (Table 2). TR adequate for indirect assessment of SPAP was present in all but 22 dogs, ie, 14 dogs from Group C, and 8 from Group 1 (7 from subgroup 1-a and 1 from subgroup 1-b). Systolic PAH, based on the SPAP value, was diagnosed in 35 out of the 64 (55%) dogs with MVD in which SPAP was assessed (25/46 from subgroup 1-a and 10/18 from subgroup 1-b).

According to RF, MR was considered as moderate to severe (ie, with an RF > 30%) in 39 out of the 72 (54%) dogs with MVD, ie, 21/53 from subgroup 1-a and 18/19 from subgroup 1-b.

Table 2. Standard echo-Doppler variables assessed in 22 dogs from the control group (Group C) and 72 dogs with asymptomatic MVD (Group 1), without and with heart enlargement (subgroups 1-a and 1-b, respectively)

		Group 1: Dogs with Asymptomatic MVD		
Echo-Doppler Variables	Group C: Control Group (n = 22)	Whole MVD Population (n = 72)	Without Heart Enlargement (Subgroup 1-a, n = 53)	With Heart Enlargement (Subgroup 1-b, n = 19)
Left atrium to aorta ratio				
Mean ± SD	0.98±0.08	1.09±0.30	0.95±0.14	1.48±0.29*‡
[range]	[0.76–1.10]	[0.65–2.01]	[0.65–1.12]	[1.15–2.01]
Regurgitant fraction (%)				
Mean ± SD	NA	33.6±16.4	27.8±13.1	49.9±13.4‡
[range]		[4.1–66.8]	[4.1–62.6]	[18.7–66.8]
SPAP (mmHg)				
Mean ± SD	25.1±7.9	39.0±15.0*	36.7±13.8*	45.0±16.7*‡
[range]	[10–35]	[10–76]	[10–76]	[20–75]
(assessed in n dogs)	(n = 8)	(n = 64)	(n = 46)	(n = 18)
Mitral E wave assessed by pulsed wave Doppler (m/s)				
Mean ± SD	0.80±0.12	0.98±0.31*	0.88±0.22	1.24±0.36*‡
[range]	[0.56–1.00]	[0.60–1.88]	[0.60–1.43]	[0.65–1.88]
(assessed in n dogs)	(n = 18)	(n = 65)	(n = 48)	(n = 17)
Fractional shortening (%)				
Mean ± SD	40.2±5.7	43.1±7.9	41.8±7.6	47.0±7.7*‡
[range]	[31.3–50.5]	[31.2–61.0]	[31.2–61.0]	[32.0–57.7]
Simpson's derived left ventricular volumes (assessed in n dogs)				
EDVI (mL/m^2)	47.4±13.0	64.4±19.0*	58.9±15.3*	79.6±20.6*‡
Mean ± SD	[27.2–88.5]	[32.8–118.9]	[32.8–94.6]	[48.5–118.9]
[range]	(n = 22)	(n = 69)	(n = 51)	(n = 18)
ESVI (mL/m^2)	15.9±4.0	18.2±6.5	17.3±7.3	20.8±7.7*‡
Mean ± SD	[10.5–26.2]	[7.7–40.5]	[7.7–33.9]	[10.0–40.5]
[range]	(n = 22)	(n = 69)	(n = 51)	(n = 18)
EF (%)	65.8±6.0	71.4±7.2*	70.4±7.3*	74.0±6.3*
Mean ± SD	[56.7–89.1]	[59.3–85.4]	[59.3–83.1]	[60.9–85.4]
[range]	(n = 22)	(n = 69)	(n = 51)	(n = 18)
Presence of *chordae tendinae* rupture	NA	11% (8/72)	6% (3/53)	26% (5/19)

*$P < .05$ versus Group C.

‡$P < .05$ versus subgroup 1-a.

EDVI, end-diastolic volume indexed to body surface area; EF, ejection fraction of the left ventricle; ESVI, end-systolic volume indexed to body surface area; MVD, degenerative mitral valve disease; n, number of dogs for which variables were available; NA, not applicable; SPAP, systolic pulmonary arterial pressure.

Several echocardiographic parameters, including SPAP, EDVI, and EF, were significantly higher in Group 1, and in both subgroups 1-a and 1-b compared with Group C (Table 2). Dogs from the subgroup 1-b also had a significantly higher LA/Ao ratio, RF, SPAP, mitral E wave velocity, FS, and LV volumes compared with dogs from subgroup 1-a ($P < .05$). As in a previous study, chordae tendinae rupture was observed in a small subset of dogs with asymptomatic MVD (8/72, 11%).[14]

Plasma NT-proBNP, Urea, and Creatinine Concentrations

Plasma NT-proBNP concentration was significantly higher in dogs from Group 1 ($P = .001$), and also subgroups 1-a ($P = .025$) and 1-b ($P < .001$) compared with the control group (Table 3 and Fig 1A). Dogs from subgroup 1-b had significantly higher plasma NT-proBNP concentrations than those from subgroup 1-a ($P < .001$). NT-proBNP values were not significantly different between dogs in Group 1 that received a medical treatment and those that did not. When NT-proBNP values were indexed to plasma creatinine concentration, all the differences remained statistically significant ($P < .05$).

In dogs from Group 1 (n = 72), a significant correlation ($P < .01$) was found between NT-proBNP and several echo-Doppler markers of MR severity (RF, LA/Ao, EDVI, and FS, Fig 2). The plasma NT-proBNP concentration (330±130 pmol/L) in dogs from subgroup 1-a with mild RF (≤30%) was not significantly different from that of control dogs ($P = .271$, Fig 1B). Conversely (Fig 1B), dogs from subgroup 1-a with moderate to severe MR (ie, RF > 30%) had significantly higher NT-proBNP values (506±249 pmol/L) than both dogs from the control group ($P = .002$) and dogs from subgroup 1-a with mild RF ($P = .011$).

Plasma urea, creatinine, and urea to creatinine ratio were not significantly different between groups (Table 3).

Follow-up of Dogs with Asymptomatic MVD

Follow-up at 12 months was available for 65/72 (90%) dogs with asymptomatic MVD. Ten dogs were censored for the statistical analysis owing to noncardiac-related death, ie, neoplasia (n = 5), neurological diseases (n = 4), and peritonitis (n = 1). Among the 55 dogs available for statistical analysis, MVD remained stable in 45 (82%) dogs (S group), whereas the other 10 dogs (18%) either died from acute pulmonary edema (n = 3) or developed clinical signs of CHF (n = 7) confirmed by examination at our unit. These 10 dogs, which were assigned to the decompensated (D) group, initially belonged either to subgroup 1-a (n = 4) or subgroup 1-b (n = 6). Regarding the S group, clinical and telephonic follow-up was performed in 26/45 (58%) and 19/45 (42%) dogs, respectively.

Dogs from the D group had significantly higher LA/Ao ($P < .001$), RF ($P = .003$), SPAP ($P = .019$) mitral E wave velocity ($P = .02$), FS ($P = .013$), EDVI ($P < .001$), ESVI ($P = .02$), and plasma urea ($P = .047$) at initial presentation than dogs from the S group (Table 4). Dogs from the D group also more commonly presented with chordae tendinae rupture ($P = .02$). Plasma creatinine and urea to creatinine ratio were not significantly different between groups (Table 4). Conversely, plasma NT-proBNP ($P = .02$) concentration was highly significantly

Table 3. Plasma urea, creatinine, and NT-proBNP concentrations assessed in 22 dogs from the control group (Group C) and 72 dogs with asymptomatic MVD (Group 1), without and with heart enlargement (sub-groups 1-a and 1-b, respectively)

		Group 1: Dogs with Asymptomatic MVD		
Blood Variables	Group C: Control Group (n = 22)	Whole MVD Population (n = 72)	Without Heart Enlargement (Subgroup 1-a, n = 53)	With Heart Enlargement (Subgroup 1-b, n = 19)
Plasma NT pro-BNP concentration (pmol/L)				
median	278	406*	378**	634#‡
[range]	[68–515]	[175–2007]	[175–1101]	[284–2007]
Plasma creatinine concentration (μmol/L)				
Mean ± SD	72±22	75±18	75±19	76±18
[range]	[42–130]	[43–125]	[45–125]	[43–111]
Plasma urea concentration (mmol/L)				
Mean ± SD	5.5±1.6	5.5±2.4	5.2±1.8	6.1±3.5
[range]	[2.7–7.8]	[1.8–16.7]	[1.8–9.6]	[2.4–16.7]
Plasma urea to creatinine ratio				
Mean ± SD	81±30	74±29	72±25	74±29
[range]	[49–140]	[30–207]	[30–129]	[37–207]

*$P = .001$ versus Group C.
**$P < .05$ versus Group C.
‡$P < .001$ versus Group C.
#$P < .001$ versus subgroup 1-a.
NT-proBNP, N-terminal pro-B-type natriuretic peptide.

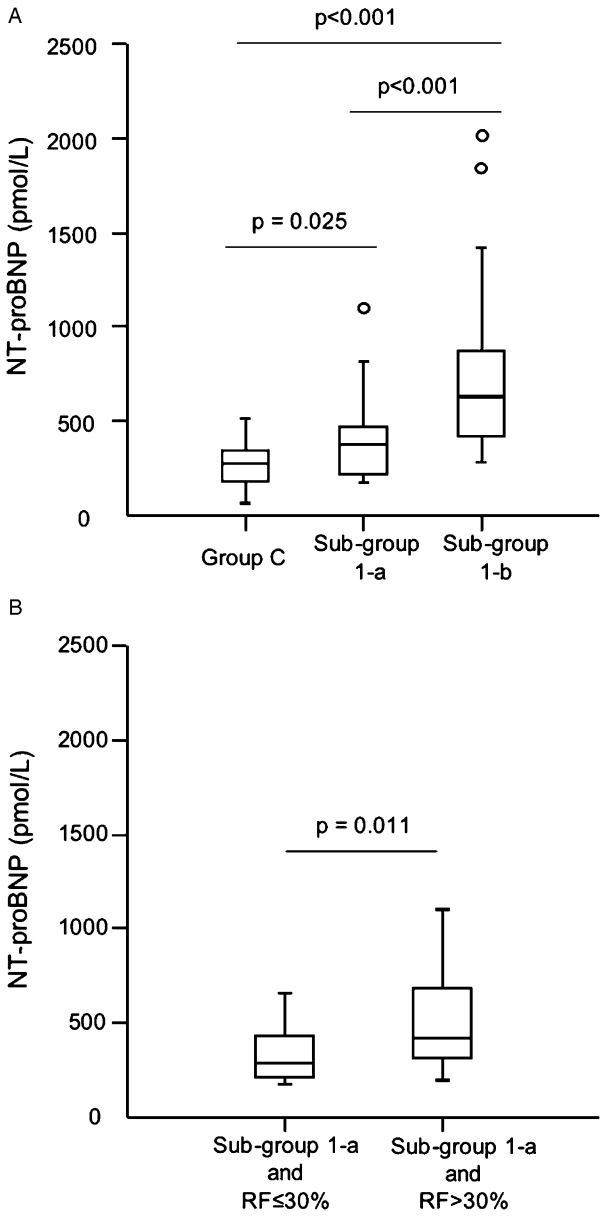


Fig. 1. (**A**) Box plots representing 10th, 25th, 50th, 75th, and 90th percentiles of N-terminal pro-B-type natriuretic peptide (NT-proBNP) plasma concentrations in the 3 considered groups, ie, Group C (control group, n = 22), and subgroups 1-a and 1-b (dogs with asymptomatic degenerative mitral valve disease without or with heart enlargement, respectively). Empty circles represent outliers. (B) Subgroup 1-a was further divided according to mitral regurgitation severity assessed by the regurgitation fraction (RF) (RF $\leq$ 30% or $>$ 30%, calculated by the proximal isovelocity surface area method).

higher in dogs from the D group than those from the S group (Fig 3).

The characteristics of ROC curves used to predict 12-month evolution according to various echo-Doppler parameters and NT-proBNP values were studied for the canine population for which 12-month follow-up was available. Results are presented in Table 5 and in Fig 4. The highest sensitivity and specificity (80 and 76%, respectively) were found for NT-proBNP with a threshold of 466 pmol/L (Fig 4). ROC curves were not significantly different among the investigated parameters.

Discussion

The clinical outcome of MVD-affected dogs without overt clinical signs is still poorly defined, and criteria for identification of dogs in ISACHC class I that are at a higher risk of early decompensation remain a major issue in veterinary cardiology. This report demonstrates the ability of NT-proBNP together with several imaging variables to help in predicting 12-month evolution of heart disease in dogs from various breeds with asymptomatic MVD.

The present study has several advantages. First, all the animals were prospectively investigated in the same hospital according to a well-standardized protocol by well-trained observers using the same echocardiographic and Doppler methods. Second, the number of recruited animals with asymptomatic MVD was high with a wide range of MR severity, and comparison with an age-matched control group was performed. Third, in the present report, MR severity was assessed by the PISA method with calculation of RF. Previous studies of NT-proBNP in canine MVD did not use the PISA method, but rather the color Doppler mapping technique with calculation of the area of regurgitant jet relative to the left atrial area (ARJ/LAA),[b,c,21] and ARJ/LAA, as a semiquantitative index, presents several limitations.[12]

In the current study, epidemiologic characteristics of the asymptomatic MVD population were in accordance with previous reports, with a predominance of male aged adult small-breed dogs. The overrepresentation of English Toy Spaniels in Group 1 is in accordance with previous results.[29,30] This unbalanced breed distribution between Groups C and 1, which represents a limitation of the present study, may somewhat explain the difference in body weight between the 2 groups, with a higher weight for dogs with MVD compared with healthy controls. Its potential impact on the present results is unknown, as the influence of breed on NT-proBNP plasma levels has not been studied in the dog as yet.

Numerous studies focusing on the diagnostic interest of BNP and NT-proBNP in humans have been published. NT-proBNP is now widely recognized as a powerful marker for diagnostic purpose, especially to distinguish patients with dyspnea of cardiac and noncardiac origin,[31] which has also been demonstrated in the dog.[18,19] More recent reports have emphasized the prognostic usefulness of B-type NP in both symptomatic and asymptomatic human patients. In a large cohort of asymptomatic persons, plasma BNP level was recently shown to predict a wide range of cardiovascular outcomes such as CHF, atrial fibrillation, and cardiac death after adjustment for traditional risk factors.[32] Little evidence exists on the prognostic value of such biomarkers in veterinary cardiology, and more particularly on their ability to predict worsening of an asymptomatic heart disease. In a recent study performed in 1 single canine breed (CKC), plasma concentrations of NT-proBNP at

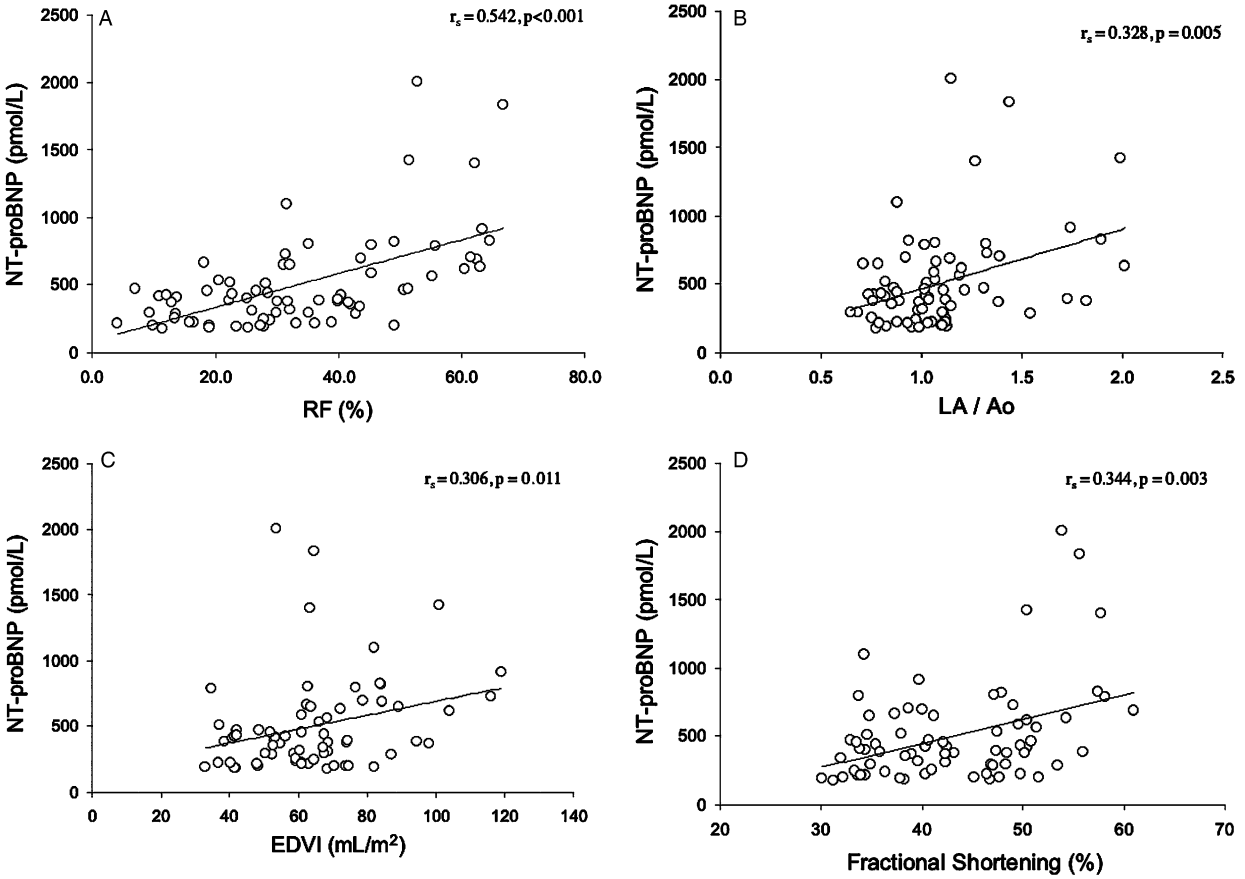


Fig. 2. Correlations between N-terminal pro-B-type natriuretic peptide (NT-proBNP) plasma concentrations and 4 echo-Doppler markers of degenerative mitral valve disease (MVD) severity assessed in dogs from Group 1 (dogs with asymptomatic MVD, n = 72): regurgitation fraction assessed by the proximal isovelocity surface area method (regurgitation fraction [RF], **A**), left atrium on aorta ratio (LA/Ao, **B**), left ventricular end-diastolic indexed volume (EDVI **C**), and fractional shortening (FS, **D**).

reexamination could predict progression in regurgitant jet size in dogs with asymptomatic MVD. However, the ability of NT-proBNP to predict clinical outcome could not be evaluated.[21]

The present study first demonstrates that plasma NT-proBNP concentration varies according to the clinical status. NT-proBNP, indexed or not to plasma creatinine concentration, was shown to be significantly higher in dogs with ISACHC class 1 MVD compared with healthy dogs. Additionally, dogs from ISACHC class 1b had significantly higher plasma NT-proBNP concentrations than those from ISACHC class 1a. Similarly, in a recent study,[20] NT-proBNP was shown to discriminate healthy dogs from dogs with MVD, and asymptomatic dogs with heart enlargement (corresponding to ISACHC class 1b) had significantly higher NT-proBNP concentration than dogs with heart disease but no heart enlargement (ISACHC class 1a); however, these groups were not compared with a healthy control group. Similar results were also obtained by Drourr and colleagues, who demonstrated a significant increase in NT-proBNP values in dogs with MVD from ISACHC class 1b compared with those from ISACHC class 1a.[b] In the present report and in comparison with the latter studies, a further distinction within the asymptomatic MVD stage was made with 2 new subsets of animals, ie, those without heart enlargement (ISACHC class 1a) and mild MR (RF $\leq$ 30%) and those without heart enlargement (ISACHC 1a) but moderate to severe MR (RF $>$ 30%). The latter subgroup had significantly higher NT-proBNP values than the former and than dogs from the control group, thus suggesting that MR severity is one of the major determinants of MVD severity. Similarly, in the present study, a significant correlation was found between plasma NT-proBNP concentration and various markers of MVD severity including RF, LA/Ao, FS, and EDVI. However, these correlations were weak (as shown by the low *r* values), thus suggesting that other factors were probably involved in elevating plasma NT-proBNP concentration.

The present report also demonstrates the prognostic value of NT-proBNP in the asymptomatic phase of MVD in dogs. Dogs from ISACHC class 1 that either died or developed clinical signs of CHF within the next 12 months after admission had significantly higher initial plasma NT-proBNP concentrations than the others. These dogs that underwent MVD progression and thus

Table 4. Standard echo-Doppler variables and plasma urea, creatinine, and NT-proBNP concentrations assessed in dogs with asymptomatic MVD (Group 1) for which follow-up at 12 months was available (n = 55)

Echo-Doppler and Biochemical Variables	Stable (S Group, n = 45)	Decompensated (D Group, n = 10)	*P*-Value S Group versus D Group
Left atrium to aorta ratio			
Mean ± SD	1.00±0.24	1.36±0.35	< .001
[range]	[0.65–2.01]	[0.94–1.90]	
Regurgitant fraction (%)			
Mean ± SD	29.5±14.4	46.1±18.5	.003
[range]	[7.0–63.0]	[18.3–66.8]	
SPAP (mmHg)			
Mean ± SD	36.0±11.4	47.7±20.9	.019
[range]	[10.0–63.0]	[11.0–76.0]	
(assessed in n dogs)	(n = 41)	(n = 10)	
Mitral E wave assessed by pulsed wave Doppler (m/s)			
Mean ± SD	0.90±0.27	1.15±0.37	.020
[range]	[0.60–1.86]	[0.73–1.88]	
(assessed in n dogs)	(n = 40)	(n = 10)	
Fractional shortening (%)			
Mean ± SD	41.9±6.9	48.2±7.7	.013
[range]	[31.2–56.0]	[37.3–57.7]	
Simpson's derived left ventricular volumes (assessed in n dogs)			
EDVI (mL/m^2)	59.4±15.1	83.2±21.2	< .001
Mean ± SD	[36.7–97.9]	[62.6–118.9]	
[range]	(n = 42)	(n = 9)	
ESVI (mL/m^2)	17.4±6.2	23.1±7.7	.020
Mean ± SD	[8.0–33.9]	[13.9–40.5]	
[range]	(n = 42)	(n = 9)	
EF (%)	70.5±7.9	72.1±6.2	NS
Mean ± SD	[49.8–85.4]	[60.6–78.6]	
[range]	(n = 42)	(n = 9)	
Presence of *chordae tendinae* rupture	7% (3/45)	40% (4/10)	.02
Plasma NT-proBNP concentration (pmol/L)			
median	368	773	.02
[range]	[175–2007]	[193–1837]	
Plasma creatinine concentration (μmol/L)			
Mean ± SD	76.3±18.6	81.8±18.7	NS
[range]	[46–125]	[43–111]	
Plasma urea concentration (mmol/L)			
Mean ± SD	5.2±1.8	6.6±2.8	.047
[range]	[1.8–9.6]	[2.4–13.2]	
Plasma urea to creatinine ratio			
Mean ± SD	70±27	80±17	NS
[range]	[30–149]	[43–111]	

Dogs were classified as being stable if they were still asymptomatic after 12 months (n = 45) or decompensated if they either died from or presented clinical signs of congestive heart failure during the 12-month period (n = 10).

EDVI, end-diastolic volume indexed to body surface area; EF, ejection fraction of the left ventricle; ESVI, end-systolic volume indexed to body surface area; MVD, degenerative mitral valve disease; n, number of dogs for which variables were available; NS, not significant; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SPAP, systolic pulmonary arterial pressure.

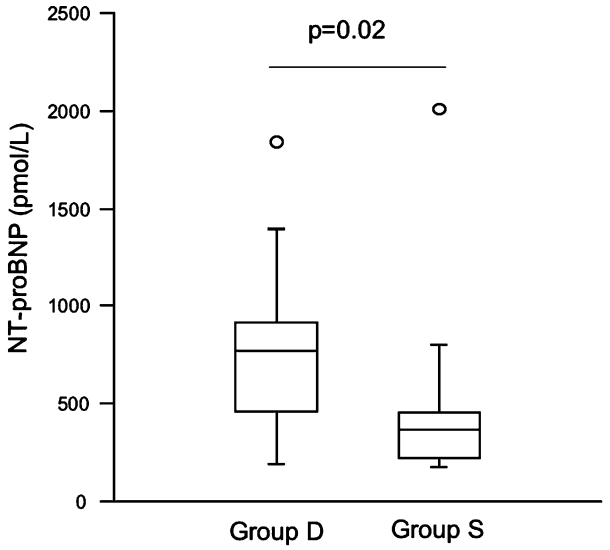


Fig. 3. Box plots representing 10th, 25th, 50th, 75th, and 90th percentiles of N-terminal pro-B-type natriuretic peptide plasma (NT-proBNP) concentrations according to the clinical progression (stable, S and decompensation, D) assessed in dogs with asymptomatic degenerative mitral valve disease and for which follow-up at 12 months was available (n = 55). The empty circles represent outliers.

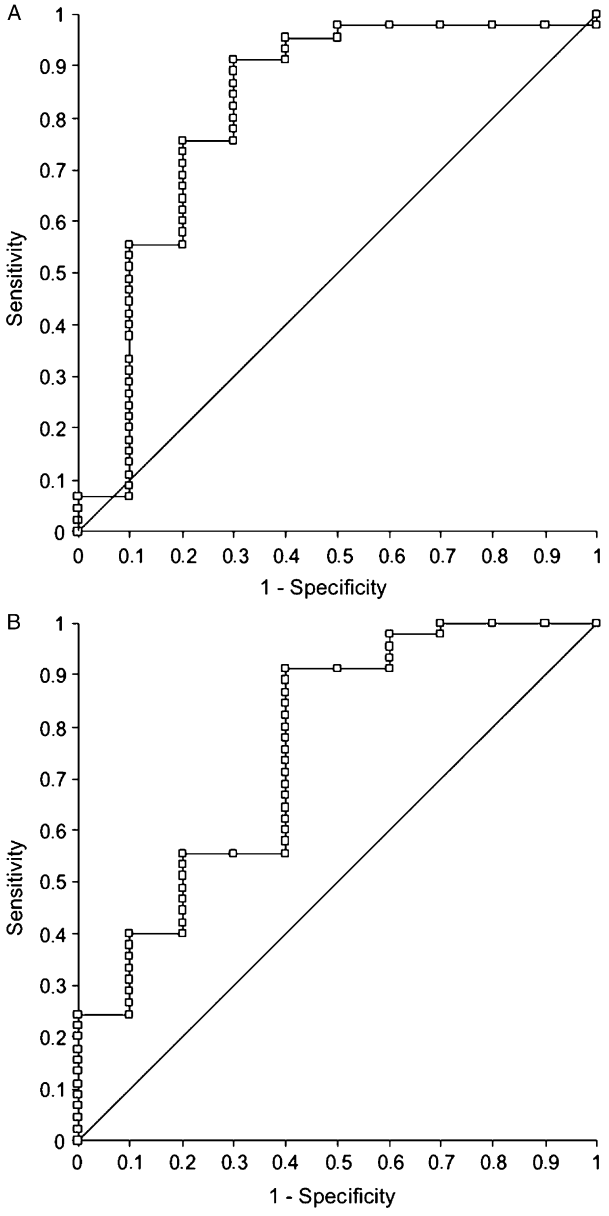
belonged to the D group were also characterized by significantly worse echocardiographic and Doppler alterations than dogs with a stable state belonging to the S group. These imaging alterations included higher MR, LA, and LV dilation, and apparent ventricular performance (as assessed by RF, LA/Ao, EDVI, ESVI, and FS, respectively), as well as higher mitral E wave and SPAP. These data suggest that activation of the natriuretic peptide system occurs in a subset of dogs with asymptomatic MVD concomitantly with morphological and functional cardiac changes owing to the increase in MR severity. We can hypothesize that these cardiac alterations (ie, LA and LV dilation) are responsible for an increased pro-BNP production by cardiomyocytes in response to wall stretch.[16,17] This could explain why, in the present study (Table 5), both NT-proBNP and several echo-Doppler indices were able to identify asymptomatic dogs at high risk for MVD worsening. Plasma NT-proBNP measurement may therefore be used to complement ultrasound-imaging techniques and help stratify and monitor MR severity during the asymptomatic phase of canine MVD. Detecting dogs with the most severe MR or that are at a higher risk of decompensation could have a therapeutic impact[5,6] that needs to be accurately assessed in further studies.

Lastly, the present study provides additional evidence that ISACHC class 1 is not a homogeneous stage but rather includes several degrees of MVD severity according to both echocardiographic and Doppler alterations and plasma NT-proBNP levels. These data are in accordance with previous reports published by our group, which have demonstrated that ISACHC class 1 MVD may include more severely affected animals than clinically suspected, with moderate or even severe MR,

Table 5. Sensitivity and specificity of echo-Doppler markers and plasma NT-proBNP and urea concentration to predict 12-month progression (cardiac death or congestive heart failure) in dogs with asymptomatic MVD and for which follow-up at 12 months was available (n = 55)

Variable	Area under ROC Curves	95% CI	Cut-Off Points	Se	Sp	*P* Value
Left atrium on aorta ratio	0.82	0.68–0.96	>1.13	0.82	0.70	**< .001**
Regurgitation fraction (%)	0.76	0.57–0.94	>49.2	0.91	0.60	**.004**
SPAP (mmHg)	0.70	0.46–0.93	>43	0.68	0.70	**.049**
Mitral E wave assessed by pulsed wave Doppler (m/s)	0.72	0.54–0.90	>1.01	0.75	0.60	**.009**
EDVI (mL/m^2)	0.83	0.70–0.97	>74.1	0.88	0.67	**< .001**
ESVI (mL/m^2)	0.74	0.57–0.90	>17.8	0.60	0.89	**.002**
Plasma urea concentration (mmol/L)	0.67	0.48–0.87	>5.5	0.57	0.80	**.040**
Plasma NT-proBNP concentration (pmol/L)	0.81	0.62–1.00	>466	0.80	0.76	**< .001**

CI, confidence interval; EDVI, end-diastolic volume indexed to body surface area; ESVI, end-systolic volume indexed to body surface area; MVD, degenerative mitral valve disease; NT-proBNP, N-terminal pro-B-type natriuretic peptide; ROC, receiver operating characteristic; Se, sensitivity; Sp, specificity; SPAP, systolic pulmonary arterial pressure.



chordae tendinae rupture, and PAH.[12,13,28] In the present report, respectively, 54, 11, and 55% of dogs from Group 1 showed moderate to severe MR, chordae tendinae rupture, and PAH. This dispersion of MVD severity, which is confirmed by and correlated with the heterogeneity of NT-proBNP values, provides a new approach to the MVD asymptomatic stage and underlines the usefulness of complementary examinations during this apparently silent phase of the disease.

This study presents several limitations. First, the present protocol only referred to 1 absolute NT-proBNP value at admission. Therefore, possible daily variations of plasma NT-proBNP concentrations were not taken into consideration. Another limitation regarding the present study is that the prognostic usefulness of NT-proBNP was studied through only a single sampling at the time of diagnosis, and not through its progression over time using serial measurements. NT-proBNP reduction percentage, rather than its absolute value at admission, has been demonstrated to be the best predictor of cardiovascular death during the follow-up period in human patients with acutely decompensated CHF.[33] The age difference between Group C and subgroup 1-b as well as the larger number of affected dogs compared with the control dogs are other limitations of the present study. Moreover, only 10 dogs reached the decompensation endpoint at 12 months. Nevertheless, this small number provided significant results in terms of outcome predictors. Splitting subgroup 1-a into dogs with moderate to severe MR and those with mild MR was a posthoc comparison, which is another limitation of the present study. Lastly, more than 40% of dogs with asymptomatic MVD were receiving one or more treatments at the time of

Fig. 4. Receiver operating characteristic curves of N-terminal pro-B-type natriuretic peptide plasma concentration (**A**) and regurgitant fraction (**B**) to predict clinical progression at 12 months in dogs with asymptomatic degenerative mitral valve disease and for which follow-up at 12 months was available (n = 55). Sensitivity is reported on the *Y*-axis and 1−specificity on the *X*-axis.

diagnosis, including at least an angiotensin-converting enzyme inhibitor, which could have influenced both the MVD outcome[5,6] and plasma NT-proBNP concentrations.

Footnotes

[a] O'Sullivan ML, O'Grady MR, Walker C. Frequency of ventricular ectopy in dogs with chronic mitral valve disease and congestive heart failure treated with pimobendan or benazepril. Presented at the 25th Annual ACVIM Forum Seattle, WA, June 6–9, 2007. J Vet Intern Med 2007;21:587 (abstract)

[b] Drourr LT, Gordon SG, Roland RM, et al. NT-Pro-BNP concentration in preclinical (ISACHC 1b & 1b) chronic degenerative atrioventricular valve disease. Presented at the 26th Annual ACVIM Forum San Antonio, TX, June 4–7, 2008. J Vet Intern Med 2008;22:758 (abstract)

[c] Tarnow I, Pedersen HD, Kvart C, et al. Natriuretic peptides are elevated in Cavalier King Charles Spaniels with congestive heart failure but not in dogs with clinically inapparent mitral valve disease. Presented at the 25th Annual ACVIM Forum Seattle, WA, June 6–9, 2007. J Vet Intern Med 2007;21:587 (abstract)

[d] Vivid 7, General Electric Medical System, Waukesha, WI

[e] Echopac Dimension, General Electric Medical System

[f] VETSIGN Canine CardioSCREEN Nt-proBNP, Guildhay Ltd, Guildford, UK

[g] Zieba M, Beardow A, Carpenter C, et al. Analytical validation of a commercially available canine N-terminal prohormone Brain natriuretic peptide elisa. Presented at the 26th Annual ACVIM Forum San Antonio, TX, June 4–7, 2008. J Vet Intern Med 2008;22:717 (abstract)

[h] Medcalc, Medcalc Software, Mariakerke, Belgium

Acknowledgments

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