Journal of Veterinary Internal Medicine ACVIM

American College of Veterinary Internal Medicine

STANDARD ARTICLE

Effect of prespecified therapy escalation on plasma NT-proBNP concentrations in dogs with stable congestive heart failure due to myxomatous mitral valve disease

Melanie J. Hezzell[1] | Chloë L. Block[1] | Danielle S. Laughlin[1] | Mark A. Oyama[1]

[1]Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

Correspondence
Melanie J. Hezzell, Bristol Veterinary School, University of Bristol, Langford House, Langford, Bristol, BS40 5DU, United Kingdom.
Email: mh16511@bristol.ac.uk

Present address
Chloë L. Block, Friendship Hospital for Animals, Washington, D.C.
Danielle S. Laughlin, Blue Pearl Veterinary Specialists, Atlanta, GA

Funding information
University of Pennsylvania Companion Animal Research Fund

Background: Treatment targeted to achieve reduction in N-terminal pro-B-type natriuretic peptide (NT-proBNP) improves outcomes in human congestive heart failure (CHF) patients.

Hypothesis: A pre-specified therapeutic algorithm that increased diuretic or pimobendan usage will reduce plasma NT-proBNP concentrations in dogs with CHF secondary to myxomatous mitral valve disease (MMVD).

Animals: Twenty-six dogs with clinically stable CHF secondary to MMVD.

Methods: Prospective, controlled before-and-after study. Dogs were examined up to 3 times over 21 days. Treatment was prescribed based on NT-proBNP as follows: <1500 pmol/L at baseline, no treatment adjustment at any point during the study (group 1); ≥1500 pmol/L and creatinine ≤3.0 mg/dL at baseline or SC visits, treatment escalated according to the algorithm (group 2); ≥1500 pmol/L at baseline, no treatment adjustment (group 3).

Results: N-terminal pro-B-type natriuretic peptide decreased significantly in group 2 (mean change = −1736 pmol/L (95% CI, −804 to −2668), $P < .001$) but not in groups 1 or 3 (623 pmol/L [−631 to 1877 pmol/L], $P = .14$ and 685 pmol/L [−304 to 1068 pmol/L], $P = .46$, respectively). Serum BUN and creatinine did not change significantly between visit 0 and visit 2 in group 1 (median = 23 mg/dL [range 13-32] versus 19 mg/dL [12-38], $P = .72$ and 1.15 mg/dL [0.70-1.40] versus 0.95 mg/dL [0.70-1.10], $P = .10$, respectively) or group 2 (28 mg/dL [18-87] versus 43.5 mg/dL [21-160], $P = .092$ and 1.10 mg/dL [0.90-2.50] versus 1.55 mg/dL [0.90-3.30], $P = .062$, respectively).

Conclusions and Clinical Importance: Use of this treatment escalation algorithm allows effective targeting of treatment for CHF in dogs against an objective criterion.

KEYWORDS
biomarker, canine, endocardiosis, treatment

1 | INTRODUCTION

Myxomatous mitral valve disease (MMVD) is the commonest cause of acquired heart disease in dogs[1,2] often resulting in congestive heart failure (CHF) and eventual cardiac-related death.[3,4] Median survival times after the onset of CHF range widely based on treatment, but are typically less than 1 year.[5–7] Standardized medical treatment recommendations for dogs with CHF are based on clinical trial results and consensus opinion[7–9]; however, decisions regarding dosing and dose adjustments, especially of diuretic medications, in individual dogs are made on a case-by-case basis and often rely on subjective criteria and the clinical acumen of the clinician. It is possible that the application of more objective criteria would allow for optimization of medical treatment in a greater proportional of dogs, potentially improving survival times.

Abbreviations: ACE, angiotensin converting enzyme; BUN, blood urea nitrogen; CHF, congestive heart failure; CI, confidence interval; ECG, electrocardiography; LA:Ao, ratio of left atrial to aortic root diameter; LVIDd, left ventricular internal dimension in diastole; LVIDDN, left ventricular internal dimension in diastole, normalized for body weight; LVIDs, left ventricular internal dimension in systole; LVIDSN, left ventricular internal dimension in systole, normalized for body weight; MMVD, myxomatous mitral valve disease; NT-proBNP, N-terminal pro-B-type natriuretic peptide; VHS, vertebral heart size

Examples of objective criteria that might be of value in monitoring the effectiveness of medical treatment for CHF include circulating biomarkers such as N-terminal proB-type natriuretic peptide (NT-proBNP). The hypothesis is that CHF treatment administered primarily to decrease NT-proBNP to a specified level results in decreased morbidity and case fatality. This hypothesis is appealing but controversial. Recent meta-analyses involving many thousands of human patients indicated a benefit in morbidity[10] and case fatality,[11,12] however important questions involving the optimal NT-proBNP level, nature of augmented treatment, cost effectiveness, and widespread use of biomarker-guided treatment remain.[13] In dogs with CHF secondary to MMVD, an observational study indicated that dogs with plasma NT-proBNP concentrations <965 pmol/L 7–30 days after initial diagnosis of CHF had significantly longer cardiac survival times than those in which plasma NT-proBNP concentrations were ≥ 965 pmol/L,[14] suggesting the possibility that treatment to specifically lower NT-proBNP could improve survival. Despite previous trials in humans and observational data in dogs, whether NT-proBNP concentration can be reduced by escalation of medical treatment in dogs with CHF has not been previously reported. The aim of our study was to determine whether a prespecified therapeutic escalation algorithm would result in reductions in plasma NT-proBNP concentrations in dogs with stable CHF secondary to MMVD.

2 | MATERIALS AND METHODS

Our study was approved by the University of Pennsylvania Animal Use and Care Committee (#805143, #803099) and the University of Bristol Animal Welfare and Ethical Review Body (VIN/16/055) and informed owner consent was obtained. Dogs presenting to a teaching hospital (Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania) or referral practice (HeartVets, Gloucestershire, England) with CHF secondary to MMVD were prospectively recruited. The inclusion criteria for the study were that a 1st episode of CHF had been diagnosed on the basis of radiographic evidence of pulmonary edema within 21 days before enrollment, clinical signs of CHF were controlled and the dog was receiving a minimum of furosemide (2 mg/kg/day), enalapril (0.5 mg/kg BID) and pimobendan (0.25 mg/kg BID). If the original diagnosis of CHF was made at another hospital thoracic radiographs taken at the time of diagnosis were made available for review. The exclusion criteria were more than 1 episode of CHF, treatment with diuretics before the onset of CHF, systemic hypertension (systolic blood pressure >170 mm Hg) and the presence of atrial fibrillation or supraventricular or ventricular tachycardia. Dogs with any other cardiac disease or clinically relevant organ-related or systemic disease were not eligible. Dogs requiring adjustments in treatment during the study period due to a deterioration in clinical status (eg, increasing sleeping respiratory rate, etc.) were withdrawn from the study. Pulmonary hypertension was not an exclusion criterion. Treatment with an angiotensin converting enzyme (ACE) inhibitor or pimobendan before the onset of CHF was not an exclusion criterion.

Dogs were examined at baseline (visit 0, ie, up to 21 days after the initial diagnosis of CHF, at which time dogs were receiving medications and were clinically stable), and at 2 SC visits, the 1st between 7 and 10 days (visit 1) and the 2nd between 14 and 20 days (visit 2) after visit 0 for a maximum of 3 visits (visits 0–2). Clinical stability was defined as absence of tachypnea or dyspnea and no evidence of pulmonary edema on thoracic radiographs. At visit 0, a medical history was taken and physical examination, blood sampling, blood pressure measurement, thoracic radiography, and echocardiography were performed. Systolic blood pressure was measured using Doppler sphygmomanometry using a thoracic limb and the inflatable cuff closest to 40% of the antebrachial circumference was chosen (Ultrasonic Doppler Flow Detector, Parks Medical, Aloha, Oregon). Approximately 3 mL of blood was collected by jugular or cephalic venipuncture and divided between serum and K-EDTA-treated tubes. Serum biochemistry was performed by the Veterinary Hospital of the University of Pennsylvania clinical laboratory. K-EDTA samples were separated by centrifugation at 1000*g* for 15 minutes and the resultant plasma transported at ambient temperature to a reference laboratory (IDEXX Laboratories Inc, Westbrook, Maine) for analysis of NT-proBNP concentration using a 2nd generation ELISA assay (Cardiopet Canine proBNP, IDEXX Laboratories Inc, Westbrook, Maine) that has been previously validated in dogs.[15] The lower and upper limits of detection of the NT-proBNP assay are 77 and 10 000 pmol/L, respectively.[15] Left and right lateral and dorsoventral thoracic radiographic projections were obtained. Vertebral heart size (VHS) was measured as previously described by a single observer (MJH).[16]

Echocardiographic examinations were performed either by a board-certified cardiologist or a cardiology resident-in-training under the direct supervision of a board-certified cardiologist. The echocardiographic examination was performed using an ultrasound unit (iE33, Phillips, Andover, Massachusetts) equipped with 3–8 and 1–5 MHz phased array transducers and electrocardiographic (ECG) monitoring. Standard imaging planes were digitally stored. Diagnosis of MMVD was on the basis of characteristic abnormalities of the valve leaflets (thickening, prolapse or both) and evidence of regurgitant flow across the valve detected by color flow Doppler. The left atrial to aortic root ratio (LA : Ao) was measured from the right parasternal short axis view.[17] Left ventricular internal dimension in systole (LVIDs) and diastole (LVIDd) were measured from 2D right parasternal short axis views. Left ventricular internal dimension in systole was normalized for body weight (LVIDSN) using the formula: LVIDs/(body weight [kg])$^{0.315}$. Left ventricular internal dimension in diastole was normalized for body weight (LVIDDN) using the formula: LVIDd/(body weight [kg])$^{0.294}$.[18] Dogs were grouped based on whether their NT-proBNP concentration at visit 0 was < or ≥1500 pmol/L. At visit 0, if the plasma NT-proBNP concentration was <1500 pmol/L, dogs were included in group 1 and no dose adjustments were made to the current therapeutic regimen and only a physical examination and measurement of circulating markers was performed at the SC visits. If at visit 0 the plasma NT-proBNP concentration was ≥1500 pmol/L, the dog was included in group 2. Dogs in group 2 were eligible for prespecified medical treatment escalation on visit 0 or visit 1 if the serum creatinine concentration was <3.0 mg/dL. If creatinine was ≥3.0 mg/dL, data from the dog would be included in the analysis, but the dog would be ineligible for further treatment escalation. Subsequent to recruitment of groups 1 and 2, a third cohort of dogs with

baseline NT-proBNP ≥1500 pmol/L that received no adjustment in treatment over a 21 day period was recruited as a control (group 3).

Treatment was escalated according to a prespecified plan as follows; if the current furosemide dose was <6 mg/kg/day, this dose was increased by 50%; if the current furosemide dose was ≥6 mg/kg/day, a combination of hydrochlorothiazide (1 mg/kg q24h) and spironolactone (1 mg/kg q24h; Aldactazide, Pfizer, New York) was added; if the current dose of furosemide was ≥6 mg/kg/day and the dog was already receiving hydrochlorothiazide and spironolactone the daily pimobendan dose was increased by 50%-100%. For dogs assigned to group 2 at visit 0 or visit 1, all diagnostic tests were repeated at the next visit. If plasma NT-proBNP measurements were <1500 pmol/L at visit 1 or 2 the endpoint was reached and the dog did not undergo any SC evaluation or visits. For dogs in group 3, no dose adjustments were made to the current therapeutic regimen and plasma NT-proBNP was measured at visit 0 and again 14–21 days later (equivalent to the timing of visit 2 for groups 1 and 2). Basic demographic information was collected and radiography and echocardiography were performed for all dogs in group 3 at visit 0. Only a physical examination and measurement of NT-proBNP was performed at the SC visit.

3 | STATISTICAL ANALYSIS

Statistical analysis and sample size calculation was performed using commercially available software (SPSS 22; IBM, Armonk New York; PSS: Vanderbilt University, Nashville, Tennessee; GraphPad Prism 7.00, GraphPad Software Inc, La Jolla, California). A value of $P < .05$ was considered significant. Data were assessed graphically for normality and by use of the Shapiro Wilk test. Means (± standard deviation) or medians (ranges) were used to provide descriptive statistics for normally and non-normally distributed continuous variables, respectively. Comparisons of continuous variables between groups were performed using Student's t-tests, Mann-Whitney U tests, one-way ANOVAs with Tukey's post-hoc tests for multiple comparisons or Kruskal-Wallis tests with Dunn's post-hoc tests for multiple comparisons, as appropriate. Comparison of proportions between groups was performed using Chi squared tests.

Repeated measures linear mixed models were used to study the effect of treatment group (groups 1–3), time and the interaction of treatment group and time on plasma NT-proBNP concentration and other variables measured during the study period. The interaction term (group × time) provides information regarding the significance of any change over time in the dependent variable for each group. A compound symmetry covariance structure was assumed between residuals of the same subject (dog) in the model. The assumption of normality of model residuals was assessed graphically and by use of the Shapiro Wilk test.

4 | RESULTS

Twenty-six dogs with CHF secondary to MMVD with a mean age of 11.1 ±2.1 years and a mean body weight of 7.31 ±3.07 kg were included in the study. Eleven male and 15 female dogs were recruited. Mixed breeds were most frequently represented (n = 6), followed by cavalier King Charles spaniels (n = 5), 2 each of Chihuahuas and Toy Poodles and 1 each of Beagle, Bichon Frisé, Bolognese, Cocker Spaniel, English Toy Spaniel, Havanese, Maltese terrier, Pomeranian, Shetland sheepdog, Shih Tzu, and Whippet. All measurements of plasma NT-proBNP were between the lower and upper limits of detection of the assay. The owner of 1 dog in group 2 was not compliant with the study protocol and the dog was removed from the study; no data from this dog are presented. The owners of 4 dogs in group 1 elected not to return for follow up, 2 after visit 0 and 2 after visit 1; no data from the 2 dogs that did not return for visit 1 are presented, whereas data from visits 0 and 1 are presented for the 2 dogs that did not return for visit 2. The reason given for electing not to return was client inconvenience in all cases and all dogs were reported to be clinically stable. Summary statistics at visit 0 are shown in Table 1. An overall difference between groups 1 (1170.8 ± 323.7 pmol/L), 2 (3641.0 ± 1505.2 pmol/L), and 3 (2591.0 ± 844.6) was detected at visit 0 for NT-proBNP ($P < .001$). Post-hoc analysis demonstrated that NT-proBNP concentrations were lower in group 1 compared with both groups 2 and 3 ($P < .001$ and $P = .040$, respectively) but did not differ between groups 2 and 3 ($P = .81$). An overall difference between groups 1 (10.8 ± 1.2), 2 (12.1 ± 0.9), and 3 (11.9 ± 0.8) was detected at visit 0 for VHS ($P = .049$). Post hoc analysis demonstrated that VHS measurements were lower in group 1 compared with group 2 ($P = .042$) but did not differ between groups 1 and 3 or groups 2 and 3 ($P = .18$ and $P = .87$, respectively). An overall difference between groups 1 (1.9 ± 0.3), 2 (2.3 ± 0.4), and 3 (2.6 ± 0.4) was detected at visit 0 for LA : Ao ($P = .011$). Post hoc analysis demonstrated that LA : Ao measurements were lower in group 1 compared with group 3 ($P = .008$) but did not differ between groups 1 and 2 or groups 2 and 3 ($P = .19$ and $P = .13$, respectively). An overall difference between groups 1 (142.7 ± 3.3 mmol/L), 2 (144.5 ± 2.7 mmol/L), and 3 (148.3 ± 4.3 mmol/L) was detected at visit 0 for Na^+ ($P = .023$). Post hoc analysis demonstrated that Na^+ measurements were lower in group 1 compared with group 3 ($P = .021$) but did not differ between groups 1 and 2 or groups 2 and 3 ($P = .52$ and $P = .088$, respectively).

A flow diagram summarizing the evolution of assignments to groups 1–3 during the study is depicted in Figure 1. No dog in any of the 3 groups required adjustments in treatment other than those that made were in accordance with the study algorithm. At visit 0, 8 dogs were assigned to group 1 and 12 dogs assigned to group 2. The NT-proBNP measurement was not reported for 1 dog in group 1 at visit 1 due to an error at the commercial laboratory (the NT-proBNP measurement at visit 2 was available from this dog). Treatment intensification at visit 0 consisted of a 50% increase in furosemide dose (10 dogs) or the addition of hydrochlorothiazide and spironolactone treatment (1 dog). After intensification of treatment at visit 0, 3 dogs in group 2 had NT-proBNP concentrations <1500 pmol/L at visit 1. No further treatment intensification was performed in these dogs and data from visit 2 did not contribute to the statistical analyses. Treatment intensification at visit 1 consisted of a 50% increase in furosemide dose (5 dogs), the addition of hydrochlorothiazide and spironolactone treatment (2 dogs), or a 50% increase in pimobendan

TABLE 1 Baseline signalment, basic echocardiographic data, treatments and serum and plasma biochemistry measurements of 23 dogs that had at least 1 recheck visit

Variable	Group 1 (n = 6)	Group 2 (n = 11)	Group 3 (n = 6)	P value (between groups)
Age (years)	11.2 ± 0.6	11.0 ± 2.3	8.9 ± 2.8	.75
Sex (no. male/no. female)	3/3	6/5	2/4	.70
Body weight (kg)	6.5 ± 1.1	8.1 ± 3.8	7.8 ± 2.7	.60
Time from onset of CHF (days)	10 (6–23)	10 (7–19)	8 (1–23)	.80
Murmur intensity	5 (4–5)	4 (3–5)	4 (4–5)	.098
Heart rate (bpm)	141.0 ± 22.7	141.5 ± 26.0	144.3 ± 23.7	.97
Systolic blood pressure (mm Hg)	132.3 ± 30.7	131.0 ± 16.9	123.2 ± 26.2	.76
Furosemide (mg/kg/day)	3.7 (3.3–4.9)	3.3 (1.9 – 11.0)	3.5 (3.3–4.1)	.56
ACE inhibitor (mg/kg/day)	1.1 ± 0.2	1.0 ± 0.3	0.7 ± 0.4	.058
Pimobendan (mg/kg/day)	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	.14
VHS	10.8 ± 1.2	12.1 ± 0.9	11.9 ± 0.8	.049
				Group 1 versus 2, P = .042
LA : Ao	1.9 ± 0.3	2.3 ± 0.4	2.6 ± 0.4	.011
				Group 1 versus 3, P = .008
LVIDDN (cm/kg$^{0.294}$)	1.90 (1.72-2.14)	2.15 (1.60-2.92)	2.25 (1.70-2.41)	.21
LVIDSN (cm/kg$^{0.315}$)	0.84 (0.59-1.10)	1.03 (0.61-2.12)	1.20 (0.69-1.43)	.16
Fractional shortening (%)	55.3 ± 7.1	48.9 ± 11.0	44.7 ± 8.3	.17
E wave velocity (m/s)	1.22 (1.12-1.85)	1.33 (1.11-2.00)	1.42 (0.79-1.62)	.57
Tricuspid regurgitation velocity (m/s)	3.1 ± 0.5	3.2 ± 0.4	2.6 ± 1.6	.62
NT-proBNP (pmol/L)	1170.8 ± 323.7	3641.0 ± 1505.2	2591.0 ± 844.6	<.001
				Group 1 versus 2, P < .001
				Group 1 versus 3, P = .040
BUN (mg/dL)	28 (13–32)	28 (16–87)	28.5 (23–94)	.58
Creatinine (mg/dL)	1.0 (0.5-1.4)	1.0 (0.9-2.5)	1.2 (0.9-1.7)	.70
Na$^+$ (mmol/L)	142.7 ± 3.3	144.5 ± 2.7	148.3 ± 4.3	.023
				Group 1 versus 3, P = .021
K$^+$ (mmol/L)	4.5 ± 0.5	4.7 ± 0.8	4.6 ± 0.6	.91

Normally distributed data are displayed as mean ± standard deviation. Non-normally distributed data are displayed as median (range).
Abbreviations: ACE, angiotensin converting enzyme; BUN, blood urea nitrogen; K$^+$, potassium; LA : Ao, left atrial to aortic ratio; LVIDDN, left ventricular internal dimension in diastole, normalized for body weight; LVISDN, left ventricular internal dimension in systole, normalized for body weight; NT-proBNP, N-terminal pro-B-type natriuretic peptide; Na$^+$, sodium.

dose (1 dog). After intensification of treatment at visit 1, 1 of the remaining 8 dogs in group 2 had NT-proBNP concentrations <1500 pmol/L at visit 2; data from all visits contributed to the statistical analyses in this dog. One dog in group 2 at visit 2 experienced serum creatinine ≥3.0 mg/dL. This dog was inappetant and appeared nauseous at the time of visit 2, but improved clinically after temporary suspension of heart failure medications and cautious fluid treatment. All data from this dog were included in the statistical analysis. All other dogs remained clinically stable throughout the study period and no other adverse events were reported. Subsequently an additional 6 dogs were recruited to group 3, all of which were examined twice, 14–21 days apart.

A significant difference in the mean change in plasma NT-proBNP concentrations over the 14–21 days study period was detected between group 2 (mean change = −1736 pmol/L [95% CI, −804 to −2,668 pmol/L]), group 1 (mean change = 623 pmol/L, 95% CI, −631 to 1877 pmol/L), and group 3 (mean change = 685 pmol/L, 95% CI, −304 to 1068 pmol/L; P = .001). Post hoc analysis demonstrated that the mean change in plasma NT-proBNP concentrations was lower in

groups 1 and 3 compared with group 2 (P = .005 and P = .004, respectively) but did not differ between groups 1 and 3 (P = .97). Plasma NT-proBNP decreased significantly over time within group 2 (P < .001) but did not significantly change within group 1 or group 3 (P = .14 and .46, respectively).

No significant change in serum BUN concentrations was detected between visit 0 (median = 23 mg/dL, range 13–32) and visit 2 (median = 19 mg/dL, range 12–38) for group 1 (P = .72). No significant change in serum BUN concentrations was detected between visit 0 (median = 28 mg/dL, range 18–87) and visit 2 (median = 43.5 mg/dL, range 21–160) for group 2 (P = .092). No significant change in serum creatinine concentrations was detected between visit 0 (median = 1.15 mg/dL, range 0.70-1.40) and visit 2 (median = 0.95 mg/dL, range 0.70-1.10) for group 1 (P = .10). No significant change in serum creatinine concentrations was detected between visit 0 (median = 1.10 mg/dL, range 0.90-2.50) and visit 2 (median = 1.55 mg/dL, range 0.90-3.30) for group 2 (P = .062). Plots of individual dogs' plasma NT-proBNP and serum BUN and creatinine concentrations throughout the study period are shown in Figures 2–4, respectively.
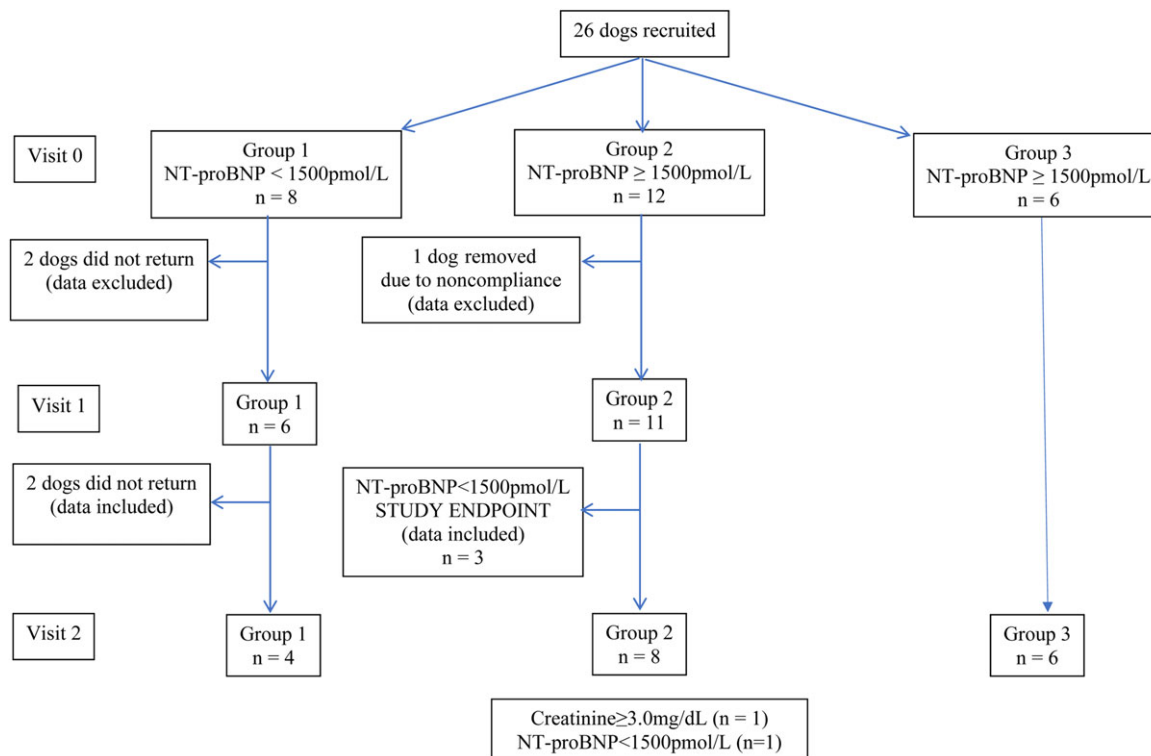
FIGURE 1 Flow diagram of the progress through the phases of the study for groups 1–3. Dogs presenting with plasma NT-proBNP ≥ 1500 pmol/L at the time of initial recruitment were assigned to group 2; an additional 6 dogs with plasma NT-proBNP ≥ 1500 pmol/L were subsequently recruited to group 3

5 | DISCUSSION

Our study demonstrates that application of a prespecified treatment escalation algorithm in dogs with recent history and treatment of 1st time CHF secondary to MMVD results in a decrease in plasma NT-proBNP concentrations. An important feature of our study is that dogs in all 3 groups were clinically stable after routine treatment of their recent CHF. Thus, the decision to escalate treatment was made on their NT-proBNP concentration rather than on the presence (or absence) of clinical signs. In this way, NT-proBNP or any biomarker directed treatment represents a treatment strategy different than what is typically performed.

The plasma NT-proBNP threshold of 1500 pmol/L was chosen on the basis of the results from a previous study, which reported that dogs with MMVD in which NT-proBNP was < 965 pmol/L after treatment for CHF survived significantly longer than those in which NT-proBNP remained ≥ 965 pmol/L.[14] In this earlier study, NT-proBNP was measured using the 1st-generation version of the ELISA, whereas measurements in our study were obtained using the 2nd-generation version of the assay, which is known to yield higher values compared with the 1st generation assay.[19] A scaling factor of ~1.5 to achieve the threshold of 1500 pmol/L was derived by comparing cut-offs for differentiating cardiac from noncardiac causes of respiratory distress using the 1st- and 2nd-generation versions of the assay.[19,20] In the aforementioned previous study, NT-proBNP concentration at time of active CHF was not found to be predictive of survival, suggesting that the NT-proBNP concentration achieved

after resolution of CHF signs and during SC maintenance treatment was important. It remains unknown whether the achieved decrease in plasma NT-proBNP concentrations in our study would improve survival or reduce risk of SC episodes of CHF, and future randomized study of the effects of NT-proBNP lowering on morbidity and case fatality is appealing.

Studies in human patients have used a variety of treatment escalation strategies including introduction or uptitration of loop and thiazide diuretics, ACE inhibitors, spironolactone, digoxin, beta-blockers, and inodilators. In some studies, a prespecified treatment escalation algorithm was followed[21,22] while in other studies the attending clinician was instructed to escalate treatment according to their individual clinical judgment.[23–25] Our study used an empirical prespecified treatment escalation strategy that, included increasing existing doses of furosemide followed by introduction of thiazide diuretics and spironolactone, similar to the algorithms described in the aforementioned human studies. If uptitration of diuretics did not result in a reduction of the plasma NT-proBNP concentration, an increased dose of pimobendan was given. While these treatment options are amongst those available for dogs that require more aggressive treatment,[8] the relative effect of alternative treatment strategies, such as those involving ACE inhibitors, digoxin, etc, requires further study.

Serum BUN and creatinine concentrations did not significantly increase with treatment escalation in our study. However, one dog did become clinically azotemic and so, as with any change in diuretic administration, careful monitoring of renal values is important during escalation of treatment.
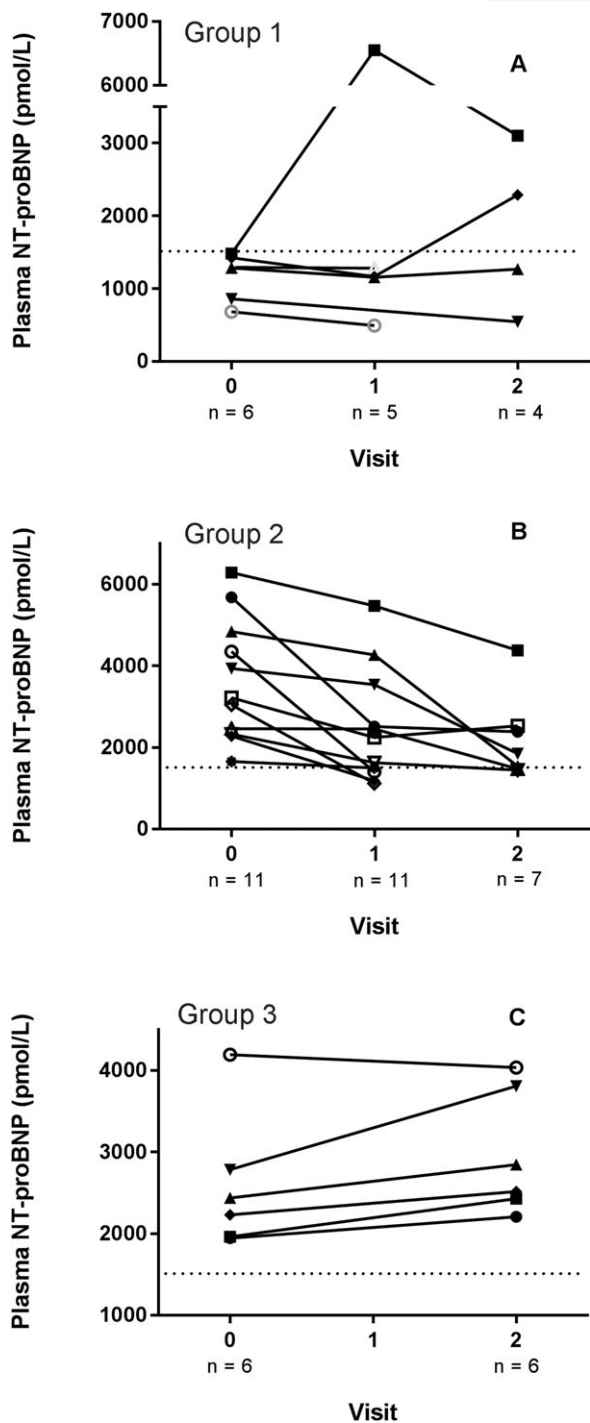
FIGURE 2 Plots of individual dogs' plasma NT-proBNP concentrations throughout the study period for (A) group 1, (B) group 2, and (C) group 3. The dotted line in each case represents the 1500 pmol/L cut-off

different concentrations remains unknown. The patient sample was small and restricted to dogs with recent onset and treatment of CHF, which limits the generalizability of the study findings. The decision to escalate treatment in dogs with NT-proBNP ≥1500 pmol/L was not made on the basis of a randomization protocol, but according to the time of presentation, as dogs presenting at the time of initial recruitment were all assigned to group 2 and dogs in group 3 was subsequently recruited as a convenience sample. Consequently, the dogs in group 3 were recruited at a different time point to dogs in groups 1 and 2 which might have influenced the results. Drop-out was relatively high as some owners found it difficult to comply with the frequent study rechecks, and the need for additional rechecks in an apparently stable dog could decrease the practicality of biomarker-guided treatment. All dogs were receiving diuretic treatment, and so renal concentrating ability could not be assessed; renal function was therefore estimated on the basis of BUN and creatinine concentrations alone and these elevations might reflect renal or prerenal causes. Finally, dogs of a variety of breeds were recruited and the groups were not balanced according to breed; this might have influenced the results of the study, as breed-specific differences in circulating NT-proBNP concentrations in healthy dogs have been reported.[26]

In conclusion, a prespecified treatment escalation algorithm reduces plasma NT-proBNP concentrations in dogs with clinically
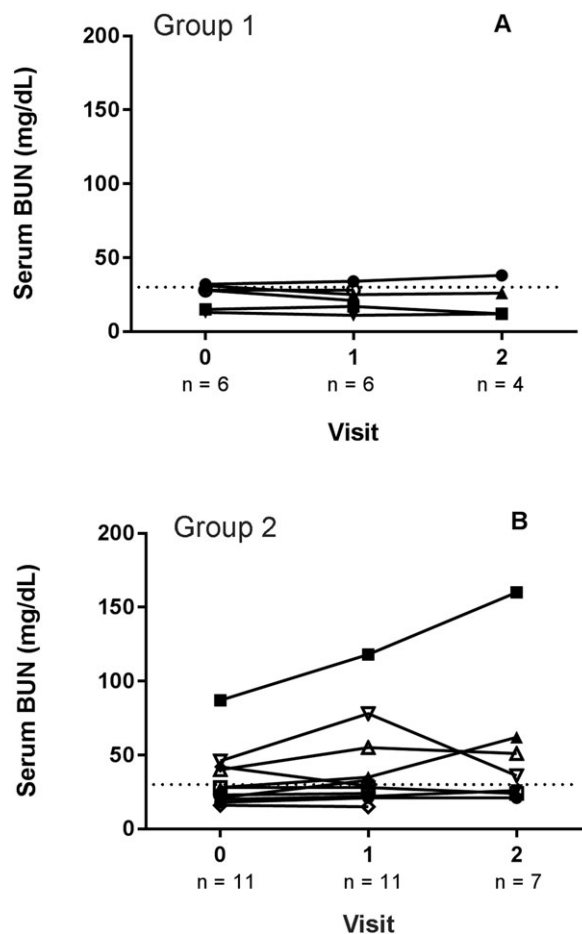

FIGURE 3 Plots of individual dogs' serum BUN concentrations throughout the study period for (A) group 1 and (B) group 2. The dotted line in each case represents the upper limit of the laboratory reference interval (30 mg/dL)
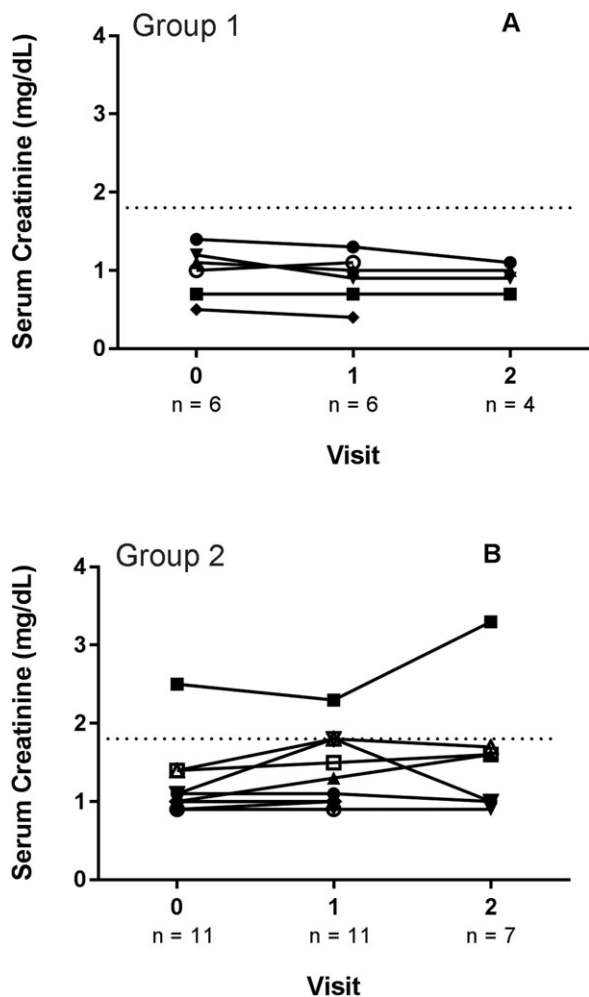
The study has a number of limitations. At visit 0, dogs in group 2 had higher VHS versus dogs in group 1, and dogs in group 3 had higher LA : Ao vs. dogs in group 1, as might be expected for dogs with higher plasma NT-proBNP concentrations, however, indices of heart size were not significantly different between the 2 groups with high NT-proBNP concentrations at baseline (ie, group 2 versus 3). Treatment was only escalated in dogs with NT-proBNP concentrations ≥1500 pmol/L and the effect of escalation of treatment in dogs with

FIGURE 4 Plots of individual dogs' serum creatinine concentrations throughout the study period for (A) group 1 and (B) group 2. The dotted line in each case represents the upper limit of the laboratory reference interval (1.8 mg/dL)

stable CHF secondary to MMVD. Careful monitoring of renal function is recommended during escalation of treatment for CHF. Further randomized studies involving larger numbers of dogs are warranted to assess the effect of lowering NT-proBNP on morbidity and case fatality.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST DECLARATION

Drs Hezzell and Oyama have previously received research funding from IDEXX Laboratories, Inc. Dr Laughlin is an independent consultant for IDEXX Laboratories, Inc.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

The study was approved by the University of Pennsylvania animal use and care committee (#805143, #803099) and the University of Bristol Animal Welfare and Ethical Review Body (VIN/16/055) and informed owner consent was obtained.

ORCID

Melanie J. Hezzell http://orcid.org/0000-0003-1890-6161
Mark A. Oyama http://orcid.org/0000-0002-5218-9226

REFERENCES

1. Detweiler DK, Patterson DF. The prevalence and types of cardiovascular disease in dogs. *Ann N Y Acad Sci.* 1965;127:481–516.
2. Buchanan JW. Chronic valvular disease (endocardiosis) in dogs. *Adv Vet Sci Comp Med.* 1977;21:75–106.
3. Borgarelli M, Savarino P, Crosara S, et al. Survival characteristics and prognostic variables of dogs with mitral regurgitation attributable to myxomatous valve disease. *J Vet Intern Med.* 2008;22:120–128.
4. Hezzell MJ, Boswood A, Chang YM, Moonarmart W, Souttar K, Elliott J. The combined prognostic potential of serum high-sensitivity cardiac troponin I and N-terminal pro-B-type natriuretic peptide concentrations in dogs with degenerative mitral valve disease. *J Vet Intern Med.* 2012;26:302–311.
5. Ettinger SJ, Benitz AM, Ericsson GF, et al. Effects of enalapril maleate on survival of dogs with naturally acquired heart failure. The Long-Term Investigation of Veterinary Enalapril (LIVE) Study Group. *J Am Vet Med Assoc.* 1998;213(11):1573–1577.
6. BENCH (BENazepril in Canine Heart disease) Study Group, Pouchelon JL, King J, et al. Long-term tolerability of benazepril in dogs with congestive heart failure. *J Vet Cardiol.* 2004;6:7–13.
7. Haggstrom J, Boswood A, O'Grady M, et al. Effect of pimobendan or benazepril hydrochloride on survival times in dogs with congestive heart failure caused by naturally occurring myxomatous mitral valve disease: The QUEST study. *J Vet Intern Med.* 2008;22:1124–1135.
8. Atkins C, Bonagura J, Ettinger S, et al. Guidelines for the diagnosis and treatment of canine chronic valvular heart disease. *J Vet Intern Med.* 2009;23:1142–1150.
9. Bernay F, Bland JM, Haggstrom J, et al. Efficacy of spironolactone on survival in dogs with naturally occurring mitral regurgitation caused by myxomatous mitral valve disease. *J Vet Intern Med.* 2010;24:331–341.
10. Savarese G, Musella F, D'Amore C, et al. Changes of natriuretic peptides predict hospital admissions in patients with chronic heart failure: A meta-analysis. *JACC Heart Fail.* 2014;2:148–158.
11. Felker GM, Hasselblad V, Hernandez AF, O'Connor CM. Biomarker-guided therapy in chronic heart failure: A meta-analysis of randomized controlled trials. *Am Heart J.* 2009;158:422–430.
12. Savarese G, Trimarco B, Dellegrottaglie S, et al. Natriuretic peptide-guided therapy in chronic heart failure: A meta-analysis of 2,686 patients in 12 randomized trials. *PLoS One.* 2013;8:e58287.
13. Chow SL, Maisel AS, Anand I, et al. Role of biomarkers for the prevention, assessment, and management of heart failure: A scientific statement from the American Heart Association. *Circulation.* 2017;135: e1054–e1091.
14. Wolf J, Gerlach N, Weber K, Klima A, Wess G. Lowered N-terminal pro-B-type natriuretic peptide levels in response to treatment predict survival in dogs with symptomatic mitral valve disease. *J Vet Cardiol.* 2012;14:399–408.
15. Cahill RJ, Pigeon K, Strong-Townsend MI, et al. Analytical validation of a second-generation immunoassay for the quantification of

N-terminal pro-B-type natriuretic peptide in canine blood. *J Vet Diagn Invest.* 2015;27:61–67.

16. Buchanan JW, Bucheler J. Vertebral scale system to measure canine heart size in radiographs. *J Am Vet Med Assoc.* 1995;206:194–199.

17. Haggstrom J, Hansson K, Karlberg BE, Kvart C, Olsson K. Plasma concentration of atrial natriuretic peptide in relation to severity of mitral regurgitation in Cavalier King Charles Spaniels. *Am J Vet Res.* 1994;55:698–703.

18. Cornell CC, Kittleson MD, Della Torre P, et al. Allometric scaling of M-mode cardiac measurements in normal adult dogs. *J Vet Intern Med.* 2004;18:311–321.

19. Fox PR, Oyama MA, Hezzell MJ, et al. Relationship of plasma N-terminal pro-brain natriuretic peptide concentrations to heart failure classification and cause of respiratory distress in dogs using a 2nd generation ELISA assay. *J Vet Intern Med.* 2015;29:171–179.

20. Oyama MA, Fox PR, Rush JE, Rozanski EA, Lesser M. Clinical utility of serum N-terminal pro-B-type natriuretic peptide concentration for identifying cardiac disease in dogs and assessing disease severity. *J Am Vet Med Assoc.* 2008;232:1496–1503.

21. Januzzi JL Jr, Rehman SU, Mohammed AA, et al. Use of amino-terminal pro-B-type natriuretic peptide to guide outpatient therapy of patients with chronic left ventricular systolic dysfunction. *J Am Coll Cardiol.* 2011;58:1881–1889.

22. Berger R, Moertl D, Peter S, et al. N-terminal pro-B-type natriuretic peptide-guided, intensive patient management in addition to multidisciplinary care in chronic heart failure a 3-arm, prospective, randomized pilot study. *J Am Coll Cardiol.* 2010;55:645–653.

23. Brunner-La Rocca HP, Buser PT, Schindler R, et al. Management of elderly patients with congestive heart failure–design of the Trial of Intensified versus standard Medical therapy in Elderly patients with Congestive Heart Failure (TIME-CHF). *Am Heart J.* 2006;151:949–955.

24. Felker GM, Ahmad T, Anstrom KJ, et al. Rationale and design of the GUIDE-IT study: Guiding Evidence Based Therapy Using Biomarker Intensified Treatment in Heart Failure. *JACC Heart Fail.* 2014;2:457–465.

25. Eurlings LW, van Pol PE, Kok WE, et al. Management of chronic heart failure guided by individual N-terminal pro-B-type natriuretic peptide targets: Results of the PRIMA (Can PRo-brain-natriuretic peptide guided therapy of chronic heart failure IMprove heart fAilure morbidity and mortality?) study. *J Am Coll Cardiol.* 2010;56:2090–2100.

26. Sjostrand K, Wess G, Ljungvall I, et al. Breed differences in natriuretic peptides in healthy dogs. *J Vet Intern Med.* 2014;28:451–457.