

Brain Natriuretic Peptide Concentration in Dogs with Heart Disease and Congestive Heart Failure

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Plasma brain natriuretic peptide concentration ([BNP]) is high in humans with cardiac disease and is further increased with congestive heart failure (CHF). The hypotheses of this study were that dogs with moderate to severe mitral regurgitation due to myxomatous mitral valve disease (MVD) would have increased plasma [BNP] compared to normal dogs, that plasma [BNP] would be higher in dogs with CHF, and that plasma [BNP] would predict premature death from cardiovascular disease. The study population consisted of 34 dogs: 9 normal dogs and 25 dogs with MVD. Patients were divided into 4 groups: group I—10 dogs with moderate to severe MVD and no radiographic evidence of CHF; group II—6 dogs with severe MVD and mild CHF; group III—7 dogs with severe MVD and moderate CHF; and group IV—2 dogs with severe MVD and severe CHF. Diagnostic tests included thoracic radiographs, an echocardiogram, a serum chemistry profile, and the measurement of plasma [BNP] by a canine-specific radioimmunoassay. There was a significant positive correlation between the plasma [BNP] and heart disease/failure groups ($P = .0036$). Plasma [BNP] increased with progressively increasing severity of MVD and CHF. Group I dogs had higher plasma [BNP] than did control dogs ($P < .0001$), and plasma [BNP] was higher in dogs with CHF (groups II-IV versus group I; $P = .012$). Plasma [BNP] was also weakly positively correlated with left atrial size ($r = 0.43$, $P = .04$). For every 10-pg/mL increase in plasma [BNP], the mortality rate over 4 months' time increased approximately 44%.

Key words: Diagnostic test; Mortality; Myxomatous mitral valve disease.

Plasma brain natriuretic peptide concentration ([BNP]) is high in human patients with cardiac disease, is higher in patients with congestive heart failure (CHF), and is a good prognostic indicator of premature death from cardiovascular disease.¹⁻¹⁰ There is minimal information regarding plasma [BNP] in normal dogs or in dogs with heart disease.¹¹⁻¹³

BNP is a hormone that is synthesized in the cardiac atria and ventricles.¹⁴ The induction of BNP synthesis is mostly due to increased ventricular wall stress, but local and circulating endothelin-1 (ET-1) inductions also increase BNP synthesis. Both increased systolic and increased diastolic wall stresses induce BNP synthesis.^{15,16} BNP's biologic effects include primary natriuresis and the inhibition of renin and ET-1 release, resulting in secondary vasodilation and natriuresis.¹⁷

Plasma [BNP] is high in many different cardiac diseases in several species.^{6,11,12,18-20} Plasma [BNP] is high in humans with dilated cardiomyopathy as well as in dogs and pigs with pacing-induced myocardial failure.^{6-9,11,21} Plasma [BNP] is also high with naturally occurring myxomatous mitral valve disease (MVD) in humans and dogs as well as with experimentally created mitral regurgitation in dogs.^{12,13,22} Diastolic dysfunction, hypertrophic cardiomyopathy, and systemic hypertension are other diseases associated with high plasma [BNP] in humans.^{2,3,20,23-26} Because

of its accuracy, plasma [BNP] is rapidly becoming a standard test for detecting the presence of left ventricular dysfunction and CHF in human medicine.^{4,5,27,28} The measurement of plasma [BNP] is also becoming an important prognostic indicator of cardiovascular mortality in humans.⁹

The hypotheses of this study were that dogs with moderate to severe MVD would have increased plasma [BNP] compared to normal dogs and that plasma [BNP] would increase further when CHF is present. We also hypothesized that plasma [BNP] would predict early cardiovascular mortality.

Materials and Methods

The study population consisted of canine patients with mitral regurgitation due to MVD that were presented to the University of California at Davis Veterinary Medical Teaching Hospital (VMTH) Cardiology Service from August 2000 to November 2000 and normal dogs owned by students, faculty, and staff of the VMTH. A physical examination and an echocardiogram were performed on each normal dog to rule out the presence of cardiac disease. Clients signed a consent form for the inclusion of their dog in the study. Cardiac patients were placed in 1 of 4 groups on the basis of radiographic and echocardiographic assessment of disease severity and radiographic assessment of the presence and severity of CHF at the time of the study. The investigators blindly placed the dogs in the heart disease groups before obtaining the plasma [BNP] results. CHF was diagnosed on the basis of radiographic evidence of caudodorsally distributed interstitial to alveolar pulmonary infiltrates and left atrial enlargement. Mild CHF was defined as radiographic evidence of mild perihilar to caudodorsal interstitial pulmonary infiltrates.²⁹ Moderate CHF was characterized by a moderate density of caudodorsal interstitial pulmonary infiltrates on thoracic radiographs.²⁹ Severe CHF was defined as radiographic evidence of alveolar pulmonary infiltrates.²⁹ Dogs with moderate to severe mitral regurgitation and no CHF were placed in group I, dogs with severe mitral regurgitation and mild CHF were placed in group II, dogs with severe mitral regurgitation and moderate CHF were placed in group III, and dogs with severe mitral regurgitation and severe CHF comprised group IV. Many of the patients in group I had been in CHF previously and were on medications for CHF at the time of the study. Patients were excluded from the study if there was evidence of clinically relevant systemic disease other than heart failure, as determined by physical examination, CBC, serum chemistries, urinalysis, and thoracic radiographs. Because renal failure causes increas-

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Table 1. Current heart failure medication administered to patients in each heart disease group.

Medication	Group I (n = 10)	Group II (n = 6)	Group III (n = 7)	Group IV (n = 2)
Furosemide	2	0	0	1
ACE inhibitor	0	0	1	0
Digoxin	0	0	0	0
Spirinolactone	0	0	0	0
Hydrocodone	1	0	0	0
Combination treatment:				
Lasix plus ACE inhibitor	1	1	3	1
Lasix, ACE inhibitor, digoxin		2	1	
Lasix, ACE inhibitor, hydrocodone	1		1	
Lasix, ACE inhibitor, digoxin, spironolactone			1	
% Dogs in each group on medication	50%	50%	100%	100%

ACE, angiotensin-converting enzyme.

es in plasma [BNP], dogs with a serum urea nitrogen concentration >50 mg/dL, a serum creatinine concentration >3 mg/dL, or both were excluded from the study.

Echocardiography was performed with an Agilent Sonos 5500 ultrasound machine.^a Measurements and calculations included left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), shortening fraction, left atrial : aortic diameter ratio obtained from 2-dimensional echocardiography in the right parasternal short-axis view (LA : Ao), color flow Doppler echocardiography, and continuous wave Doppler echocardiography-derived mitral regurgitant velocity. LVEDD was indexed (LVEDDI) to 1.44 kg^{0.32}, and LVESD was indexed (LVESDI) to 0.69 kg^{0.41}.^{29,30} Radiographic heart size was semiquantified by calculating a vertebral heart scale from the lateral view of thoracic radiographs.³¹ Plasma [BNP] was measured with a commercial, competitive radioimmunoassay kit specific for canine BNP-32.^b All materials and buffers were supplied with the kit and used in strict accordance with the kit guidelines. Radioimmunoassay measurement of plasma canine BNP-32 has been validated in a previous study.¹³ Blood samples were collected in polypropylene tubes containing EDTA and aprotinin. Samples were immediately centrifuged at 0°C, plasma was frozen at -70°C, and samples were batched for analysis. All plasma samples were extracted before the assay according to the manufacturers' instructions.

Owners of the dogs were verbally contacted to determine short-term patient survival data at 4 months after the initial diagnosis and long-term patient survival data at 18 months after the initial diagnosis. Deaths were classified as cardiac or noncardiac in origin.

Statistical analyses were performed by Statview 5^c and StatXact.^d The Jonckheere-Terpstra test was used to compare the trend in BNP concentration across the 4 groups of dogs with MVD and progressively increasing severity of CHF. The Mann-Whitney *U*-test was used to compare the plasma [BNP] of the control group with group I heart disease as well as to compare the plasma [BNP] of groups II-IV (dogs with severe MVD and CHF) with the control group and group I. Simple linear regression was used to evaluate the following independent variables with regard to the dependent variable of plasma [BNP]: LVEDDI, LVESDI, LA : Ao, and vertebral heart score. Pearson's product-moment correlation coefficients were calculated. Statistical significance was defined as $P \leq .05$. A Cox proportional hazards regression model was used to evaluate the relationship between plasma [BNP] at

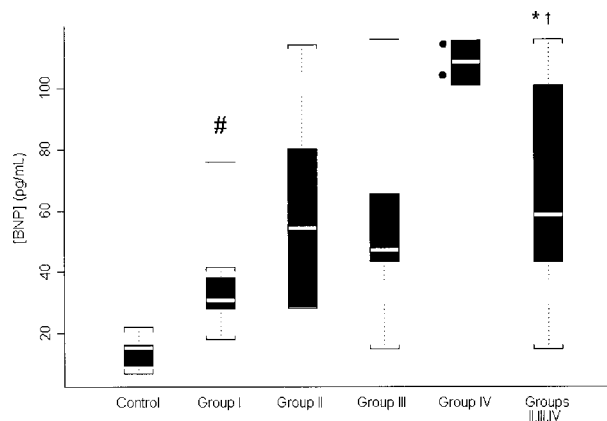


Fig 1. Distribution of plasma brain natriuretic peptide concentration ([BNP]) in control dogs and dogs with heart disease. Boxes show (from bottom to top) the 25th, 50th, and 75th percentiles. Whiskers extending from boxes capture approximately 95% of the data. Extreme observations are represented by lines. # Significant difference between group I and control; $P = .0001$. * Significant difference between groups II-IV and group I; $P = .012$. † Significant difference between groups II-IV and control; $P < .0001$.

the time of diagnosis as well as 4 and 18 months after the diagnosis. The proportionality and linearity assumptions of the model were assessed by likelihood ratio tests. Results of this model are presented as mortality rate ratios (MRRs) and 95% confidence intervals (95% CI).

Results

There were 25 dogs with MVD in the study and 9 normal control dogs. Ages of control dogs ranged from 4 to 10 years (mean, 6.9 years), and weights ranged from 7 to 55 kg (mean, 31.3 kg). Patient ages ranged from 18 months to 14 years (mean, 10.3 years), and weights ranged from 3.5 to 38 kg (mean, 16.6 kg). There were 10 dogs in group I, 6 dogs in group II, 7 dogs in group III, and 2 dogs in group IV. Table 1 depicts the current medications administered to patients in each heart disease group.

There was a significant ordinal correlation between the plasma [BNP] and heart disease groups ($P = .0036$) (Fig 1). Plasma [BNP] was significantly high in the patients with MVD and no CHF (group I) when compared to the control dogs ($P < .0001$). Additionally, plasma [BNP] was greater in dogs with MVD and CHF (groups II-IV) than in control dogs ($P < .0001$) as well as in dogs with MVD only (group I) ($P = .012$) (Fig 2).

There was minimal overlap in the plasma [BNP] of normal dogs and group I patients. A plasma [BNP] cutoff of 23 pg/mL provided the best sensitivity (86%; 95% CI, 65-97%) and specificity (100%; 95% CI, 72-100%). Similarly, a plasma [BNP] cutoff of 35 pg/mL provided the best sensitivity (86%; 95% CI, 57-98%) and specificity (70%; 95% CI, 35-93%) for distinguishing between dogs with heart failure due to MVD and dogs with MVD but without heart failure.

Four-month survival data were available for 21 of the 25 dogs. One dog was censored from the short-term survival analysis because it had undergone surgical repair of the mitral valve. Nine of the 20 remaining dogs with MVD died or were euthanized because of worsening CHF early, within

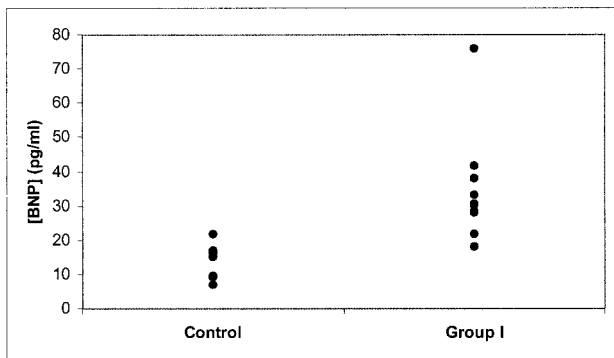


Fig 2. Scattergram of plasma brain natriuretic peptide concentration (BNP) in control dogs and group I heart disease dogs with moderate to severe mitral valve disease (MVD) but no heart failure.

4 months of the initial evaluation. A dose-response relationship between plasma [BNP] and the rate of death within 4 months of diagnosis (ie, short-term survival) was found: for every 10-pg/mL increase in plasma [BNP], the mortality rate increased approximately 44% (MRR = 1.4; 95% CI, 1.1–1.9). For example, a dog with a plasma [BNP] of 100 pg/mL had a 12.6-fold greater rate of mortality over 4 months' time than a dog with a plasma [BNP] of 30 pg/mL (MRR = 12.6; 95% CI, 6.8–82.3). Only 1 dog died of a noncardiac cause at 6 months after the initial diagnosis and was eliminated from the long-term survival analysis. Fifteen of the remaining 19 dogs (79%) died or were euthanized because of worsening CHF by 18 months after initial diagnosis. There was no relationship between plasma [BNP] and long-term patient survival at 18 months after the initial diagnosis.

Simple linear regression showed a statistically significant but clinically weak correlation between plasma [BNP] and LA: Ao ($r = 0.43$; $P = .04$). There was no significant correlation between [BNP] and the other measured variables.

Discussion

This study showed that plasma [BNP] was high in dogs with moderate to severe MVD and was further increased in dogs with CHF due to severe MVD. There are only 2 published veterinary studies that have evaluated plasma [BNP] in dogs with naturally occurring cardiac disease. One study measured plasma [BNP] in 19 dogs with CHF and MVD ($n = 17$) or dilated cardiomyopathy ($n = 2$).¹³ Another study measured plasma [BNP] in 76 Cavalier King Charles Spaniels with MVD of varying severity.¹² Both studies found that plasma [BNP] was high only in dogs with moderate and severe CHF. In contrast, the present study found that plasma [BNP] was high in dogs with moderate to severe myxomatous MVD in the absence of CHF and that plasma [BNP] was further increased with CHF due to severe MVD. Plasma [BNP] increased as CHF progressively worsened.

Similar to our study, a study in humans found that plasma [BNP] was high with moderate to severe MVD and that plasma [BNP] increased with worsening signs and heart failure class.²² That study reported that a plasma [BNP] >36 pg/mL was predictive of patients who later decompensated and required surgery for valve replacement.

In the current study, a plasma [BNP] >35 pg/mL was moderately sensitive and specific for differentiating dogs that were not in heart failure from dogs with CHF.

The present study showed that, over a 4-month evaluation period, as plasma [BNP] at the time of diagnosis increased, the rate of death increased. There are no other veterinary studies that have evaluated plasma [BNP] as a prognostic indicator of death from cardiovascular disease. However, plasma [BNP] is a good prognostic indicator of cardiovascular mortality in humans with CHF due to dilated cardiomyopathy as well as ischemic heart disease.^{9,10,32} One study measured plasma [BNP], plasma atrial natriuretic peptide concentration, and plasma norepinephrine concentration in persons with CHF due to dilated cardiomyopathy or ischemic heart disease. Only plasma [BNP] was an independent predictor of risk of death.⁹ Plasma [BNP] also provided prognostic information independent of other hemodynamic variables such as left ventricular ejection fraction.⁹ Another study surveyed 1,640 people randomly chosen from the general population and found that plasma [BNP] independently predicted death due to left ventricular dysfunction.³³

The measurement of plasma [BNP] has been used in human medicine as a screening test to detect asymptomatic left ventricular systolic dysfunction. One study evaluated 1,252 randomly selected people and found that a plasma [BNP] >18 pg/mL was moderately sensitive (77%) and specific (87%) for detecting asymptomatic left ventricular systolic dysfunction.²⁷ This value is very similar to the value of 23 pg/mL found in the current study for detecting heart disease. Another study measured plasma [BNP] in 200 patients referred for echocardiography and found that a high plasma [BNP] was highly specific (98%) for detecting left ventricular systolic dysfunction.²⁸

Increased diastolic wall stress also causes increased synthesis and release of BNP from ventricular myocardium.¹⁵ Plasma [BNP] is high in humans with diastolic dysfunction.^{20,23} The measurement of plasma [BNP] has been used as a screening test for the presence of asymptomatic diastolic dysfunction and left ventricular hypertrophy.²⁴ A plasma [BNP] >25 pg/mL was useful for detecting left ventricular diastolic dysfunction and hypertrophy in that study. Again, this value is very similar to the value of 23 pg/mL found in the current study for detecting heart disease.

Recently, a rapid bedside plasma [BNP] test became available for humans, and it has been used in the emergency room setting as a screening tool for detecting CHF.⁵ Two studies have evaluated plasma [BNP] in dyspneic patients who were presented to urgent care hospitals.^{4,5} Both studies found that plasma [BNP] was higher in patients with CHF than in patients without CHF. In one of the studies, 30 of 250 dyspneic patients were misdiagnosed clinically. When a plasma [BNP] measurement was used, there was only 1 misdiagnosis.⁵ In addition, the measurement of plasma [BNP] was highly specific for detecting the presence or absence of CHF, and the diagnostic accuracy was not improved by obtaining thoracic radiographs.⁵ To our knowledge, no veterinary studies have measured plasma [BNP] as a screening test for CHF. A future study should evaluate

the measurement of plasma [BNP] as a test to discriminate between primary respiratory disease and CHF in dogs presented for dyspnea.

Plasma [BNP] has also been used to predict a human patient's response to treatment.³² In one study, serial plasma [BNP] measurements of hospitalized CHF patients predicted treatment outcome and mortality. Patients whose plasma [BNP] declined during hospitalization were much less likely to be rehospitalized or die than patients whose plasma [BNP] increased during hospitalization. Future veterinary studies should evaluate plasma [BNP] during the treatment of CHF and assess whether the measurement of plasma [BNP] can be used to help tailor the medical treatment of CHF.

Variables such as age, posture, and time of day could theoretically change plasma [BNP]. The effect of aging on plasma [BNP] has been studied in humans and dogs. Although plasma [BNP] has been shown to mildly increase with age in humans, there is no correlation between plasma [BNP] and age in dogs.^{24,34} BNP is not affected by postural changes, and there is no circadian rhythm of secretion.^{35,36} Acute (ie, minutes) volume overload does not affect plasma [BNP].³⁷ However, within 1 hour of producing an increase in ventricular wall stretch, BNP gene expression is activated in the left ventricle.¹⁹

The primary direct biologic effect of BNP is the production of natriuresis in the proximal and distal renal tubules.³⁸ BNP also inhibits endothelin-I and renin secretion and therefore causes secondary decreases in angiotensin II (AT-II) and aldosterone concentrations.^{39–41} Consequently, it produces secondary biologic effects that include vasodilation and natriuresis.⁴² Natriuretic peptides also possess antiproliferative properties in the mesangial cells of the kidney, astrocytes, endothelial cells, cardiac fibroblasts, vascular smooth muscle cells, and cardiomyocytes.^{43–45} BNP inhibits collagen synthesis, possibly by ET-I inhibition.^{46–48} BNP is degraded by neutral endopeptidases in the myocardium, lungs, and kidneys.⁴⁹

High plasma [BNP] in human patients with moderate to severe cardiac disease is likely due to increased left ventricular synthesis.^{8,11} However, BNP synthesis and secretion are different, depending on the presence or absence of cardiac disease. BNP is synthesized as a prohormone, and most of the active hormone is released into circulation without being stored in granules.^{50,51} In the absence of cardiac disease, the tissue concentration of BNP is greatest in the atria.¹⁴ Given the large mass of the ventricles, the total cardiac content of BNP is greater in the ventricles. In fact, 50–60% of circulating BNP is synthesized in the ventricles in normal individuals.¹⁴ There appears to be a different regulation of atrial and ventricular BNP synthesis.^{11,14,52,53} BNP is secreted from ventricular myocytes quickly after synthesis via a constitutive pathway, but in the atria, it is stored in granules and released by a regulatory pathway.^{17,38,50,51,54} ET-I causes ventricular but not atrial synthesis of BNP in deoxycorticosterone acetate–salt hypertensive rats.⁵⁵ During early left ventricular dysfunction, plasma [BNP] is increased, and atrial tissue contents of BNP and BNP messenger RNA (mRNA) are also increased.¹¹ Once CHF is present, plasma [BNP] and left ventricular BNP mRNA are further increased.¹¹ The activation of BNP transcription is

one of the earliest and most reliable markers of ventricular cardiomyocyte hypertrophy.⁵⁶ Ventricular BNP secretion also increases with increasing severity of left ventricular dysfunction.⁸

The major determinant of BNP synthesis and secretion is wall stress.¹⁴ Both mechanical stretch and pressure overload induce BNP gene expression and increase BNP secretion.^{19,57} However, BNP synthesis can be induced by both load-dependent and load-independent mechanisms.⁵² There is debate regarding whether wall stress directly causes BNP synthesis and release or whether increased wall stress causes local autocrine or paracrine factors to induce BNP synthesis. Possible local factors may include the renin-angiotensin system and ET-1. ET-1 is synthesized and secreted from endothelial cells and cardiomyocytes and is released during endothelial stretch or pressure overload.⁵⁸ ET-1 has been shown to induce ventricular BNP synthesis and cause an increase in plasma [BNP].^{52,53} Although cardiomyocyte stretch causes acute AT-II release, one study showed that AT-II is not required for stretch to trigger increased BNP gene expression in the atria and ventricles.⁵⁹ Experiments with cultured neonatal cardiomyocytes have shown that mechanical stretch in the absence of neurohumoral control increases gene expression and secretion of BNP.⁵⁷ The same experiment showed that an AT-II receptor blocker, an ET-1 receptor blocker, or the combination of both AT-II and ET-1 receptor blockers reduce mechanical strain–induced BNP gene transcription by 50%.⁶⁰ Therefore, both mechanical strain and neurohumoral stimulation appear to be important inducers of BNP production. In humans with dilated cardiomyopathy, BNP-expressing cardiomyocytes have been found in the subendocardial layer, fibrous areas, and perivascular regions.⁶¹ These findings show that, in addition to global wall stress, there may be regional factors that induce BNP synthesis in the left ventricle.

Although plasma [BNP] was increased in the patients of our study with MVD and CHF, plasma [BNP] was not markedly related to radiographic heart size or echocardiographic-derived chamber sizes, including LVEDDI and LVESDI. By simple linear regression, there was a very weak relationship between left atrial size and plasma [BNP]. There is minimal clinical relevance with such a low correlation coefficient. The echocardiographic variables of left ventricular size are not good approximations of left ventricular wall stress. Because wall stress is the major stimulus for the synthesis and release of BNP, the calculation of ventricular wall stress would have been more appropriate than the measurement of left ventricular echocardiographic dimensions. However, left ventricular wall stress is difficult to calculate and requires invasive procedures.

The major limitation of this study is the small number of control dogs and patients included. Although there were statistically significant results, further studies should be carried out in larger populations of dogs to verify and refine these findings. Other potential areas of BNP research include the measurement of plasma [BNP] as a screening test for differentiating dyspneic patients with CHF from patients with primary respiratory disease, the measurement of plasma [BNP] in other cardiac diseases such as hypertrophic cardiomyopathy and dilated cardiomyopathy, and the measurement of plasma [BNP] for the therapeutic monitoring

of patients with CHF. The veterinary profession would benefit from having a simple diagnostic test that would help identify patients with heart disease and patients with CHF as well as help distinguish patients with respiratory disease from patients with CHF.

In conclusion, this study suggests that plasma [BNP] is high in dogs with moderate to severe MVD alone and is higher in dogs with severe MVD and CHF. This study also suggests that severe increases in plasma [BNP] may be predictive of premature death due to cardiovascular disease, much like in human medicine.

Footnotes

- ^a Agilent Sonos 5500 ultrasound machine, Philips Medical Systems, Best, The Netherlands
^b Canine BNP-32 radioimmunoassay, Peninsula Laboratories Inc, Belmont, CA
^c StatView, SAS Institute, Cary, NC
^d StatXact, Cytel Software Corp, Cambridge, MA
^e Triage BNP Test, Biosite Diagnostics, San Diego, CA
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