Cardiac Troponin I and Amino-Terminal Pro B-Type Natriuretic Peptide in Dogs With Stable Chronic Kidney Disease

L. Pelander, J. Häggström, C.J. Ley, and I. Ljungvall

Background: Increased concentrations of N-terminal pro B-type natriuretic peptide (NT-proBNP) and cardiac troponin I (cTnI) in dogs with azotemia have been documented. Knowledge of mechanisms behind increased concentrations of cardiac biomarkers in dogs with azotemia is warranted for correct interpretation of test results.

Objectives: The aim of the article was to investigate possible associations between plasma concentrations of cTnI and NT-proBNP, respectively, and patient characteristics, glomerular filtration rate (GFR), a plasma volume factor (PVF) derived from scintigraphic examination (PVf), systolic blood pressure (SBP), selected hematologic and biochemical variables, and echocardiographic measurements in dogs with stable chronic kidney disease (CKD) and in healthy dogs.

Animals: Fifty student-, staff-, and client-owned dogs were included. Twenty-three of the dogs were healthy and 27 were diagnosed with CKD.

Methods: In this cross-sectional observational study, dogs with a previous diagnosis of CKD and healthy control dogs were included. At inclusion, all dogs were characterized by physical examination, repeated blood pressure measurements, complete urinalysis, hematology and biochemistry panel, echocardiography, abdominal ultrasound examination of the entire urinary tract, and scintigraphic examination for measurement of GFR.

Results: Plasma volume factor and PCV were independently associated with NT-proBNP (R²_adj = 0.42; P < .0001). Age, body weight (BW), and SBP were independently associated with cTnI (R²_adj = 0.50; P < .0001).

Conclusions and Clinical Importance: Neither NT-proBNP nor cTnI concentrations were independently associated with measured GFR. Thus, findings were not suggestive of passive accumulation of either marker, suggesting that increased circulating concentrations of cTnI and NT-proBNP can be interpreted similarly in dogs with stable CKD as in dogs without CKD.

Key words: Azotemia; Biomarker; Cardiovascular; Renal.

Several interactions exist between the kidneys and the cardiovascular system in health and disease. In human medicine, the term cardio-renal syndrome frequently is used.1,2 In veterinary medicine, the term “cardiovascular renal axis disorders” recently was proposed in a publication aimed to stimulate advancement in the understanding of this complex interplay.3 Cardiovascular biomarkers have been studied extensively and evaluated for clinical use in recent years.4-6 Many factors, technical as well as biological, may complicate interpretation of these markers.7-10 One example is kidney disease, which might influence biomarker concentrations by several mechanisms.11-20

B-type natriuretic peptide (BNP) is a neuroendocrine hormone synthesized in myocardial cells in response to volume expansion or pressure overload.21 Plasma concentration of amino-terminal pro B-type natriuretic peptide

Abbreviations:

ACE: angiotensin-converting enzyme
ALP: alkaline phosphatase
ALT: alanine aminotransferase
BNP: B-type natriuretic peptide
BUN: blood urea nitrogen
BW: body weight
Ca: total calcium
CHF: congestive heart failure
chol: cholesterol
CKD: chronic kidney disease
CI: chloride
CRP: C-reactive protein
cTnI: cardiac troponin I
eGFR: estimated glomerular filtration rate
IQR: interquartile range
IRIS: international renal interest society
K: potassium
LA/Ao: left atrial-to-aortic diameter ratio
LVIdd°5inc: increase in LVIdd compared to expected LVIdd
LVIdd: left ventricular end-diastolic internal diameter
LVId°5inc: increase in LVIDd compared to expected LVIDd
LVIDds: left ventricular end-systolic internal diameter
mGFR: measured glomerular filtration rate with scintigraphy, integral method
mGFR°r: measured glomerular filtration rate
mGFR°pv: measured glomerular filtration rate with scintigraphy, plasma volume method
MMVD: myxomatous mitral valve disease
Na: sodium
NT-proBNP: amino-terminal pro B-type natriuretic peptide
P: inorganic phosphate
PVf: plasma volume factor
SBP: systolic blood pressure
TP: total protein
UPC: urine protein-to-creatinine ratio
(NT-proBNP), an inactive amino-terminal fragment that is separated from proBNP to produce BNP, is used for diagnostic and prognostic purposes in human and veterinary cardiovascular medicine. 5,22–25 Increased concentrations of NT-proBNP have been documented in dogs and humans with azotemia. 17,26–28 Reasons for increased concentrations of NT-proBNP in human chronic kidney disease (CKD) patients have been investigated. Estimated glomerular filtration rate (GFR) (derived from calculations using circulating concentrations of creatinine, cystatin c, or both, estimated glomerular filtration rate [eGFR]) is an independent predictor of plasma NT-proBNP concentration. 20–31 Despite this, NT-proBNP is useful for the diagnosis of congestive heart failure (CHF) in humans with CKD and decreased eGFR. 30,32 Cardiac troponin I (cTnI) is a highly myocardial-specific protein used in humans, cats, and dogs for detection of myocardial cell injury. 33–35 Cardiac ischemia, toxicity, remodeling, trauma, or inflammation may contribute to myocardial cell injury and lead to release of cTnI from the myocardium. 35–41 Increased concentrations of cTnI have been documented in dogs and humans with azotemia. 19,20,42 It is unknown whether increased concentration of cTnI in human CKD patients is a result of true myocardial cell injury or if it occurs because of decreased renal clearance of cTnI, or both, but most studies indicate that the main reason is increased cardiac release of cTnI. 19,20,31,43,45 In most studies of NT-proBNP and cTnI concentrations in dogs with azotemia, groups were heterogeneous and the azotemia could have been perrenal or resulted from either acute or CKD. 19,20,26,27 A different response to a different degree of azotemia on the cardiovascular system might be expected depending on if the azotemia is acute or chronic, and pre- or postrenal. The aim of our study therefore was to investigate possible associations between NT-proBNP and cTnI, respectively, and patient characteristics, measured glomerular filtration rate (mGFR), a plasma volume factor (PVI) derived from scintigraphic examination (PVI), systolic blood pressure (SBP), selected hematologic and biochemical variables and echocardiographic measurements in stable canine CKD patients and in healthy dogs.

Materials and Methods

Study Population

This cross-sectional observational study was performed at the Swedish University of Agricultural Sciences, Uppsala, after approval by the local ethical committee. All owners approved and signed an informed consent form. Client-owned dogs of any breed and age with a previous diagnosis of CKD were prospectively included. This diagnosis had been made previously in each dog using standard methods (compatible clinical signs, results of urinalysis, hematologic and biochemical analyses, morphological renal abnormalities detected by urinary tract ultrasound examination, or some combination of these). Dogs with structural or functional abnormalities of 1 or both kidneys that had persisted for at least 3 months were included. Exclusion criteria were the presence of other systemic or organ-related diseases. Dogs chronically medicated with corticosteroids or nonsteroidal anti-inflammatory drugs were excluded. Medication with glycosaminoglycan supplements administered PO or sodium pentosan polysulfate injections was not an exclusion criterion. If the dog was receiving an angiotensin-converting enzyme (ACE) inhibitor, the drug was withdrawn for a week before inclusion in the study and reintroduced after study inclusion. Renal diets were allowed. Dogs with primary cardiac disease were excluded from the study, but dogs with mild myxomatous mitral valve disease (MMVD) were included because plasma cTnI and NT-proBNP concentrations are unchanged at this stage of the disease. 40,46–48 Healthy student-, client-, and staff-owned dogs of various breeds and ages were included as controls. At the day of enrollment into the study, all dogs (including all control dogs) underwent physical examination, repeated blood pressure measurements, collection of venous blood and urine, echocardiographic examination, abdominal ultrasound examination of the entire urinary tract, and a scintigraphic examination for calculation of individual kidney mGFR. Dogs with CKD were grouped according to the International Renal Interest Society (IRIS) classification system, based on stable serum creatinine concentration. 49

Blood Pressure Measurement

Indirect blood pressure measurements were performed by oscillometry in after rest and accommodation to the premises. The cuff was put on the base of the tail, and pressure was recorded with the dog standing, either on the floor or on the examination table, depending on where the dog seemed to be most comfortable. Measurements were performed with the veterinarian (LP), a veterinary student, or both in the room. A minimum of 5 measurements were recorded for each dog, more in case of presumably incorrect measurements (such as an obviously incorrect heart rate registration on the device, a >20% variation in SBP between measurements, or an obviously distressed animal).

Blood and Urine Examinations

Blood was drawn from the cephalic vein and transferred to the clinical chemistry laboratory at the university animal hospital for immediate hematology (CBC including manual differential cell count) and biochemical analyses (creatinine, blood urea nitrogen [BUN], alkaline phosphatase [ALP], alanine aminotransferase [ALT], total calcium [Ca], inorganic phosphate [P], sodium [Na], potassium [K], chloride [Cl], cholesterol [cholesterol]), C-reactive protein [CRP], total protein [TP], albumin, and fibrinogen). After analysis, serum and EDTA plasma were frozen (−20°C) in aliquots and subsequently (within 24 hours) transferred to storage at −70°C. For most dogs, urine was obtained by cystocentesis at the time of the abdominal ultrasound examination. If cystocentesis was not possible, fresh spontaneously voided urine was collected. Urine was aliquoted, and 5–10 mL (depending on the total volume of urine obtained) was immediately used for analysis (dipstick and sediment examinations, specific gravity, urine protein-to-creatinine ratio [UPC], and aerobic culture).

Abdominal Ultrasound Examination

Complete upper and lower urinary tract ultrasound examinations were conducted by experienced radiologists at the university animal hospital diagnostic imaging clinic. Examinations were performed according to a predefined protocol.

Echocardiography

Echocardiographic examinations of all dogs were performed to exclude primary heart disease. All examinations were performed...
by experienced ultrasonographers (IL, JH). Dogs were placed in right and then left lateral recumbency on an ultrasound examination table. The echocardiographic evaluation was conducted by use of an ultrasonographic unit equipped with a 5-1 matrix transducer and electrocardiographic (ECG) monitoring. The following cardiac measurements were obtained left atrial-to-aortic diameter ratio (LA/Ao), left ventricular end-diastolic internal diameter (LVIDd), and left ventricular end-systolic internal diameter (LVIDs). The left atrial-to-aortic root (LA/Ao) ratio was measured as previously described. Measurements were made on 3 consecutive cardiac cycles, and the mean value from each dog was used in the statistical analysis. The expected LVIDs was calculated using the formula 0.95BW0.315 and expected LVIDd using the formula 1.53BW0.294. The increase in LVIDs compared to the expected LVIDs (LVIDs %inc) was determined by use of the formula (LVIDs – LVIDs expected/LVIDs) × 100, and the increase in LVIDd compared to the expected LVIDd (LVIDd %inc) was determined by use of the formula ((LVIDd – LVIDd expected)/LVIDd) × 100.51

**Glomerular Filtration Rate and Plasma Volume Estimation by Scintigraphy**

Estimation of individual kidney GFR was performed by 2 different methods using renal scintigraphy, the integral method, and the plasma volume method as previously described, by an experienced radiologist.52 When using the plasma volume method, the rate of glomerular filtration is indexed to an estimation of plasma volume instead of to body weight in kg (BW), which is used in the integral method.52 In the univariate and multiple regression analyses, results from both methods were included, separately. The estimations of GFR derived using the plasma volume method were titled mGFRpv, and the estimations of GFR derived from the integral method were titled mGFRi. A total (left + right kidney) mGFRpv <30.8 mL/min/L and a total mGFRi <2.66 mL/min/kg, respectively, were considered subnormal.5354 There was good agreement between the integral and the plasma volume methods of GFR estimation, as indicated by a Spearman correlation coefficient of 0.91.

After GFR measurements by both methods in each dog, a PVF correlating with plasma volume in each dog could be calculated. This was accomplished by solving for “L” (liters) in the equation: mL/kg/min = mL/kg/L after including scintigraphy results from each dog and both methods into the equation. This factor then was indexed to BW (PVF/kg). The simplified equation for calculation of PVF/kg was mGFRi/mGFRpv.

**Amino-Terminal Pro B-Type Natriuretic Peptide Analysis**

Frozen EDTA plasma was analyzed in batch by a commercially available canine ELISA for quantification of NT-proBNP.9 The lower detection limit of the assay was 250 pmol/L. Each sample was run in duplicate, and the mean NT-proBNP concentration between the 2 runs was used for each dog in all statistical analyses. For the purpose of these calculations, all samples containing a cTnI concentration lower than the detection limit of the assay were assigned a concentration of 0.005 ng/mL.

**Statistical Analyses**

Statistical calculations were performed by a commercially available software program.6 Kruskal–Wallis/Wilcoxon rank-sum test and Wilcoxon’s nonparametric comparisons for each pair were used for continuous variables, and chi-square test for discrete variables, to compare patient characteristics and clinical variables between healthy dogs and dogs in different IRIS stages of CKD. A P-value of <.008 was considered significant in the group comparison analyses (Bonferroni correction).

For the regression analyses, log-transformation of cTnI and NT-proBNP was performed because of non-normal distributions. Simple linear regression was performed to check for associations between log NT-proBNP (or log cTnI, respectively) and the following variables: age, BW, sex, mGFR, SBP, LA/Ao, LVIDs inc %, LVIDd inc %, creatinine, erythrocyte volume fraction (PCV), P, albumin, UPC, PVF/kg, and storage time.

The multiple regression analyses were performed in a backward stepwise manner. All variables that were linearly correlated with log NT-proBNP (or log cTnI, respectively) were included in each step until all remaining variables were significant. For regression analyses, residuals were plotted and subjectively examined as well as tested for normality by Shapiro–Wilks test. A P-value of <.05 was considered significant.

**Results**

**Study Population**

A total of 50 dogs were included in the study. Clinical and laboratory characteristics of included dogs are presented in Table 1. The study population comprised 7 mixed breed dogs, 6 Labradors, 4 Golden Retrievers, and ≤3 individuals of 28 other breeds. Twenty-three were healthy control dogs. The 27 dogs with CKD were classified into IRIS stage I through III (Table 1). Median (interquartile range [IQR]) age of all included dogs was 6.0 (2.5–9.3) years. Median (IQR) BW was 19.5 (11.4–26.2) kg. There was no difference in age, sex, or BW among dogs in the 4 groups.

**Amino-Terminal Pro B-Type Natriuretic Peptide**

Results from comparisons of NT-proBNP concentrations between healthy dogs and dogs in different IRIS stages of CKD are presented in Table 1. In the univariate analyses, NT-proBNP increased with increasing PVF/kg, UPC, and creatinine concentration and with decreasing PCV, mGFR, and albumin concentration. The variables PCV, PVF/kg, mGFR, BW, UPC, albumin, and storage time were included in the multiple regression analysis. Creatinine was not included in the model, because of collinearity with GFR (r = −0.62 for mGFRi and r = −0.72 for mGFRpv). PCV and PVF/kg were the variables independently associated with NT-proBNP (R adj2 = 0.42; P < .0001). This result was identical despite replacing mGFRpv with mGFRi in the multiple regression analysis. Figure 1 illustrates the univariate regression analyses regarding the 2 variables that
were significant in the final model. Complete results from the univariate and multiple regression analyses regarding NT-proBNP are presented in Table 2.

Cardiac Troponin I

Results from comparisons of cTnI concentrations between healthy dogs and dogs in different IRIS stages of CKD are presented in Table 1. In the univariate analyses, cTnI increased with increasing age, SBP, PVf/kg, BW, and creatinine concentration. Age, UPC, SBP, BW, mGFR, PVf/kg, sex, and storage time were included in the multiple regression analysis. Again, creatinine was not included. Age, BW, and SBP were the variables independently associated with cTnI. Thus, these results were not indicative of passive accumulation of either of these biomarkers.

Dogs in IRIS stage III had higher plasma concentrations of NT-proBNP than did healthy dogs in our study. However, NT-proBNP concentration in this canine population was not associated with mGFR when other variables were controlled. The only variables that were independently associated with NT-proBNP concentration in our study were PCV and PVf/kg. One previous retrospective study investigated the association between mGFR and NT-proBNP in dogs with stable CKD. In that study, a correlation between mGFR and plasma NT-proBNP concentration was found. This corresponds to a $R^2$ of 0.22 which is identical to the $R^2$ for mGFRpv reported in the present study. Furthermore, mGFR was an independent predictor of NT-proBNP in that same retrospective study. Reported associations between eGFR and NT-proBNP in humans also are often weak ($R^2 < 0.35$), but eGFR is an independent predictor of NT-proBNP concentrations in humans with CKD. Renal handling of NT-proBNP in humans with CKD is a topic of continuing discussion. People with CKD but without cardiovascular disease have higher circulating concentrations of NT-proBNP than non-CKD humans. Human patients in CHF with a decreased eGFR also have higher concentrations of NT-proBNP than those in CHF with normal eGFR. Consequently, slightly higher cutoff concentrations of NT-proBNP and BNP, respectively, are used to exclude CHF in human CKD patients. However, if age-adjusted cutoff concentrations of NT-proBNP are used

Table 1. Dog characteristics, clinical variables, cTnI, and NT-proBNP concentrations.

| Number | Healthy | Stage I CKD | Stage II CKD | Stage III CKD |
|--------|---------|-------------|--------------|--------------|
| Sex (F/M) | 23 | 14 | 7 | 6 |
| mGFRpv (mL/min/L) | 17/6 | 6/8 | 3/4 | 3/3 |
| mGFRi (mL/min/kg) | 50 (45 to 69) a | 41 (24 to 55) a | 24 (15 to 35) a | 11 (6 to 13) a |
| Creatinine (mmol/L) | 3.4 (3.0 to 3.9) a | 2.9 (2.1 to 3.3) a | 2.1 (2.0 to 2.3) a | 1.5 (1.4 to 1.8) a |
| Creatinine ratio | 78 (72 to 95) a | 91 (69 to 101) a | 136 (127 to 147) a | 233 (221 to 244) a |
| UPC ratio | 0.07 (0.04 to 0.11) a | 0.13 (0.06 to 1.74) a | 0.44 (0.15 to 1.67) a | 0.90 (0.24 to 3.72) a |
| Age (years) | 5.8 (2.7 to 9.0)a | 5.5 (1.7 to 10.0)a | 8.0 (2.1 to 8.9)a | 7.9 (3.5 to 9.9)a |
| BW (kg) | 19.5 (11.0 to 24.9)a | 19.1 (11.4 to 32.0)a | 20.0 (5.5 to 28.1)a | 19.8 (12.2 to 27.0)a |
| SBP (mmHg) | 129 (122 to 149)a | 143 (130 to 169)a | 157 (133 to 169)a | 154 (133 to 175)a |
| P (mmol/L) | 1.2 (1.0 to 1.3)a | 1.3 (1.2 to 1.4)a | 1.0 (1.0 to 1.2)a | 1.3 (1.2 to 1.6)a |
| Albumin (g/L) | 31 (29 to 33)a | 31 (28 to 34)a | 30 (29 to 32)a | 28 (26 to 35)a |
| PCV | 0.48 (0.45 to 0.50)a | 0.46 (0.45 to 0.49)a | 0.44 (0.43 to 0.49)b, | 0.38 (0.31 to 0.40)b, |
| PVf (mL/kg) | 63.7 (60.0 to 73.5)a | 74.8 (64.8 to 97.6)b, | 89.8 (79.6 to 135.1)b, | 139.9 (123.7 to 258.0)b, |
| LA/Ao | 1.18 (1.11 to 1.21)a | 1.18 (1.10 to 1.29)a | 1.14 (1.00 to 1.20)a | 1.25 (1.12 to 1.30)a |
| LVIDs inc% | 12.6 (–1.0 to 25.8)a | 4.2 (–9.8 to 9.8)a | 5.2 (–15.7 to 19.3)a | 4.1 (–6.5 to 15.0)a |
| LVIDd inc% | 2.5 (–6.5 to 14.6)a | –3.4 (–8.3 to 6.2)a | 0.9 (–5.0 to 2.7)a | 9.7 (2.4 to 14.8)a |
| NT-proBNP (pmol/L) | 425 (125 to 560)a | 570 (431 to 780)b, | 833 (125 to 1,222)b, | 2,167 (824 to 5,223)b, |
| cTnI (µg/L) | 0.01 (0.01 to 0.02)a | 0.03 (0.01 to 0.04)b, | 0.02 (0.01 to 0.06)b, | 0.04 (0.03 to 0.05)b |

UPC, urine protein-to-creatinine; mGFRpv, measured glomerular filtration rate by plasma volume method; mGFRi, measured glomerular filtration rate by integral method; BW, body weight; P, inorganic phosphorous concentration; SBP, systolic blood pressure; PVf, plasma volume factor; LA/Ao, left atrial-to-aortic diameter ratio; LVIDs inc%, increase in left ventricular end-systolic internal diameter compared to expected LVIDs; NT-proBNP, amino-terminal pro B-type natriuretic peptide; cTnI, cardiac troponin I; CKD, chronic kidney disease.
for the diagnosis of CHF, further adjustment because of a decreased eGFR is not necessary. Interestingly, the prognostic value of NT-proBNP in people with heart disease is valid regardless of patient eGFR.63,64 In a prospective study involving 3,483 human patients with CKD, individuals with NT-proBNP concentrations in the highest quintile were more likely to develop CHF, and the authors speculated that increased concentrations of NT-proBNP might indicate subclinical changes in volume and myocardial stress that subsequently contribute to clinical CHF. To what extent this occurs in dogs with CKD is yet unknown.

In our study, NT-pro-BNP concentration was independently associated with PVf/kg, a variable corresponding to plasma volume. This is an interesting and logical finding because NT-proBNP is released in response to myocardial wall stretch, usually secondary to increased circulating blood volume. The other variable independently associated with NT-proBNP concentration in our study was PCV. An independent association between PCV and NT-proBNP concentration has been described in healthy older humans,71 and hemoglobin concentration has been identified as an independent predictor of NT-proBNP concentration in several studies.72,73 In addition, NT-proBNP concentration is higher in anemic humans, sometimes high enough to exceed the clinical cutoff value for diagnosis of CHF.72 In the present study, only 4 of the CKD dogs were anemic, but dogs in IRIS stage III CKD had significantly lower PCV than both healthy dogs and dogs in stage I CKD, presumably partly because of decreased erythropoietin production consistent with CKD (Table 1). Anemia is associated with increased circulating volume in humans,74 and it is possible that even a mildly decreased PCV might contribute to the increase in circulating volume seen in some dogs in our study.

Dogs in IRIS stage III had higher plasma concentrations of cTnI than did healthy dogs in our study. There were, however, large overlaps in cTnI concentrations among groups (data not shown). In a previous study, dogs with azotemia (which included individuals with acute kidney injury, CKD, or both) had higher cTnI concentrations than did nonazotemic dogs, but there was no association between degree of azotemia and cTnI concentration, which is in agreement with the results of our study.20 Dogs with severe azotemia in another study also included both dogs with acute kidney injury and those with CKD, and although the association between cTnI concentration and degree of azotemia was not investigated, dogs with azotemia as a group had higher cTnI concentrations than did healthy dogs.19
There was no association between mGFR and cTnI in the univariate regression analysis in our study. In addition, mGFR was not an independent predictor of cTnI concentration in the multiple regression analysis. This finding suggests that passive accumulation of cTnI with decreasing GFR is not a major contributor to increased plasma concentrations of cTnI in dogs with stable CKD. Increased cTnI concentrations in these dogs therefore might result mainly from cardiac secretion. In 1 of the previously mentioned studies of dogs, cardiac histopathology was available for 3 dogs with azotemia, and all had cardiac lesions. Subclinical cardiac pathology may be common in dogs with kidney disease, in accordance with the situation in humans. The uremic human myocardium is known to be vulnerable to ischemia, I possible reason being increased cardiomyocyte area and diameter in combination with insufficient capillary growth (myocyte/capillary mismatch). Another explanation for this vulnerability is an increased oxygen demand of the uremic myocardium. Individuals with advanced CKD are predisposed to changes in plasma volume, as discussed earlier. Hyper- or hypovolemia as well as anemia might contribute to myocardial ischemia in dogs with CKD. These (and probably other) factors may compromise cardiomyocyte integrity, which could result in secretion of cTnI into the circulation. Also, cardiac remodeling, which begins early in humans with CKD, might contribute to increased cTnI concentrations. Cardiac remodeling previously has been suggested to be associated with cardiac release of cTnI in dogs with MMVD. Concentrations of cTnI in dogs in our study generally were quite low. If an earlier generation assay would have been used, most of these dogs probably would have had undetectable cTnI concentrations.

Age was an independent predictor of cTnI concentration in the dogs of our study. This finding is in agreement with earlier studies of cTnI in dogs and humans. Systolic blood pressure was another independent predictor of cTnI concentration in our study. In 1 previous study of dogs, no correlation was found between SBP and cTnI concentration. An association between SBP and cTnI has been described in studies of humans. Increased SBP over time could compromise myocardial cell integrity by increasing left ventricular afterload. Divergent results in previous studies regarding this association might reflect the inherent difficulty in acquiring a relevant blood pressure measurement in a clinical situation. Also, the relatively low number of conclusively hypertensive dogs in studies on cTnI and azotemia in dogs performed to date may influence results regarding a potential association between increased SBP and cTnI concentration.

In our study, an independent association between cTnI and BW in dogs was found. This observation has, to our knowledge, not been described previously. In 1 study of dogs, BW was not associated with cTnI concentration, but the dogs in that study all were of small breeds, and an association between these variables may have been obscured. One possible explanation for increased cTnI concentrations in larger dogs may be that their larger hearts release higher amounts of cTnI. Intravascular volume also is higher in larger individuals.

### Table 2. Univariate and multiple regression analysis.

| Variable          | NT-proBNP (n = 47) | cTnI (n = 50) |
|-------------------|-------------------|--------------|
|                   | Univariate Analysis | Multiple Regression Model ($R^2_{adj} = 0.42$) | Univariate Analysis | Multiple Regression Model ($R^2_{adj} = 0.50$) |
|                   | $R^2$ | $P$-Value | $\beta$ | $P$-Value | $R^2$ | $P$-Value | $\beta$ | $P$-Value |
| Age               | .003  | .72       |        |        | .35   | <.0001    | .14   | <.0001    |
| BW                | .06   | .096      |        |        | .08   | .041      | .02   | .04       |
| Sex               | .95   | .06       |        |        | .06   | .079      |       |           |
| mGFRpv            | .22   | <.001     |        |        | .05   | .14       |       |           |
| mGFRi             | .18   | .0032     |        |        | .09   | .039      |       |           |
| PVf/kg            | .38   | <.0001    | 9.8    | .001    | .18   | .006      | .01   | .02       |
| SBP               | .003  | .75       |        |        | .08   | .043      |       |           |
| Creatinine        | .35   | <.0001    |        |        | .09   | .52       |       |           |
| P                 | .013  | .45       |        |        | .07   | .062      |       |           |
| UPC               | .10   | .029      |        |        | .03   | .26       |       |           |
| Albumin           | .10   | .028      |        |        | .01   | .40       |       |           |
| EVF               | .28   | <.0001    | −3.4   | .04     | .06   | .60       |       |           |
| LA/Ao             | .00004| .97       |        |        | .002  | .74       |       |           |
| LVIDs inc%        | .002  | .77       |        |        | .002  | .74       |       |           |
| LVIDd inc%        | .009  | .55       |        |        | .006  | .61       |       |           |
| Storage time      | .08   | .053      |        |        | .07   | .058      |       |           |

cTnI, cardiac troponin I; SBP, systolic blood pressure; mGFR, measured glomerular filtration rate with plasma volume method; mGFRi, measured glomerular filtration rate integral method; BW, body weight; PVf/kg, plasma volume factor indexed to BW; P, inorganic phosphate; UPC, urine protein-to-creatinine ratio; EVF, erythrocyte volume fraction; La/Ao, left atrial-to-aortic diameter ratio; LVIDs inc%, increase in LVIDs compared to expected LVIDs; LVIDd inc%, increase in LVIDd compared to expected LVIDd; BNP, B-type natriuretic peptide; NTproBNP, amino-terminal pro B-type natriuretic peptide.

Results of linear regression analyses.
but the relationship between heart size and intravascular volume might be different in dogs of different sizes. Effects of BW on concentrations of various circulating biochemical variables in dog populations have been described previously, none of which is assumed to be of clinical relevance.84

Our study had some limitations. There were no CKD dogs in IRIS stage IV (a stable serum creatinine concentration >440 µmol/L)85 included. Urine concentrations of NT-proBNP or cTnI were not analyzed but could have been helpful for conclusions regarding renal handling of these peptides.

Conclusion

In our study, neither NT-proBNP nor cTnI was independently associated with mGFR. NT-proBNP was independently associated with PCV and PVI/kg. Age, BW, and SBP were the variables independently associated with cTnI. Thus, findings were not suggestive of passive accumulation of either marker in dogs with stable CKD. Clinically, based on our results, increased circulating concentrations of NT-proBNP and cTnI likely indicate myocardial stretch and myocardial cell damage, respectively, in dogs with stable CKD, similar to the situation in healthy dogs.

Footnotes

a High Definition Oscillometry (HDO), S+B medVET, Babenhausen, Germany
b iE33, Philips Ultrasound, Bothell, WA
c cTnI assay, Beckman Coulter, Brea, CA
d Access AccuTnI+3 troponin I assay, Beckman Coulter, Brea, CA
e JMP Pro 11, SAS Institute, Cary, NC

Acknowledgments

Grant support: The authors thank the Swedish research funds “AGRIA/SSK forskningsfond,” “Michael Forsgrens stiftelse” and “Thure F och Karin Forsbergs stiftelse” for funding this study.

Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

References

1. Bouquegneau A, Krzesinski JM, Delanaye P, et al. Biomarkers and physiopathology in the cardiorenal syndrome. Clin Chim Acta 2015;443:100–107.
2. Waldum B, Os I. The cardiorenal syndrome: What the cardiologist needs to know. Cardiology 2013;126:175–186.
3. Pouchelon JL, Atkins CE, Bussadori C, et al. Cardiovascular-renal axis disorders in the domestic dog and cat: A veterinary consensus statement. J Small Anim Pract 2015;56:537–552.
4. Boswood A. Biomarkers in cardiovascular disease: Beyond natriuretic peptides. J Vet Cardiol 2009;11(Suppl 1):S23–S32.
5. Oyama MA. Using cardiac biomarkers in veterinary practice. Clin Lab Med 2015;35:555–566.
6. Oyama MA, Boswood A, Connolly DJ, et al. Clinical usefulness of an assay for measurement of circulating N-terminal pro-B-type natriuretic peptide concentration in dogs and cats with heart disease. J Am Vet Med Assoc 2013;243:71–82.

7. Collins SA, Patteson MW, Connolly DJ, et al. Effects of sample handling on serum N-terminal pro-B-type natriuretic peptide concentration in normal dogs and dogs with heart disease. J Vet Cardiol 2010;12:41–48.

8. Hezzell MJ, Boswood A, Lotter N, et al. The effects of storage conditions on measurements of canine N-terminal pro-B-type natriuretic peptide. J Vet Cardiol 2015;17:34–41.

9. 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.

10. Adin DB, Oyama MA, Sleeper MM, et al. Comparison of canine cardiac troponin I concentrations as determined by 3 analyzers. J Vet Intern Med 2006;20:1136–1142.

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

12. Ruans C, Scollan K, Suchodolski JS, et al. Biologic variability in NT-proBNP and cardiac troponin-I in healthy dogs and dogs with mitral valve degeneration. Vet Clin Pathol 2015;44:420–430.

13. Daniels LB, Maisel AS. Cardiovascular biomarkers and sex: The case for women. Nat Rev Cardiol 2015;12:588–596.

14. Couto KM, Iazbik MC, Marin LM, et al. Plasma N-terminal pro-B-type natriuretic peptide concentration in healthy retired racing Greyhounds. Vet Clin Pathol 2015;44:405–409.

15. Kellihan HB, Oyama MA, Reynolds CA, et al. Weekly variability of plasma and serum NT-proBNP measurements in normal dogs. J Vet Cardiol 2009;11(Suppl 1):S93–S97.

16. LaVecchio D, Marin LM, Baumwurt R, et al. Serum cardiac troponin I concentration in retired racing Greyhounds. J Vet Intern Med 2009;23:87–90.

17. Boswood A, Dukes-McEwan J, Loureiro J, et al. The diagnostic accuracy of different natriuretic peptides in the investigation of canine cardiac disease. J Small Anim Pract 2008;49:26–32.

18. Daniels LB, Maisel AS. Natriuretic peptides. J Am Coll Cardiol 2007;52:2357–2368.

19. Sharkey LC, Berzina I, Ferasin L, et al. Evaluation of serum cardiac troponin I concentration in dogs with renal failure. J Am Vet Med Assoc 2009;234:767–770.

20. Porcello F, Rishniw M, Herndon WE, et al. Cardiac troponin I is elevated in dogs and cats with azotemia renal failure and in dogs with non-cardiac systemic disease. Aust Vet J 2008;86:390–394.

21. Maeda K, Tsutamoto T, Wada A, et al. Plasma brain natriuretic peptide as a biochemical marker of high left ventricular end-diastolic pressure in patients with symptomatic left ventricular dysfunction. Am Heart J 1998;135:825–832.

22. 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.

23. Christenson ES, Collinson PO, DeFilippi CR, et al. Heart failure biomarkers at point-of-care: Current utilization and future potential. Expert Rev Mol Diagn 2014;14:185–197.

24. Lalor SM, Connolly DJ, Elliott J, et al. Plasma concentrations of natriuretic peptides in normal cats and normotensive and hypertensive cats with chronic kidney disease. J Vet Cardiol 2009;11(Suppl 1):S71–S79.

25. Januzzi JL Jr, Camargo CA, Anwaruddin S, et al. The N-terminal pro-BNP investigation of dyspnea in the emergency department (PRIDE) study. Am J Cardiol 2005;95:948–954.

26. Raffan E, Loureiro J, Dukes-McEwan J, et al. The cardiac biomarker NT-proBNP is increased in dogs with azotemia. J Vet Intern Med 2009;23:1184–1189.

27. Schmidt MK, Reynolds CA, Estrada AH, et al. Effect of azotemia on serum N-terminal proBNP concentration in dogs with normal cardiac function: A pilot study. J Vet Cardiol 2009;11(Suppl 1):S81–S86.

28. Bansal N, Hyre Anderson A, Yang W, et al. High-sensitivity troponin T and N-terminal pro-B-type natriuretic peptide (NT-proBNP) and risk of incident heart failure in patients with CKD: The Chronic Renal Insufficiency Cohort (CRIC) Study. J Am Soc Nephrol 2015;26:946–956.

29. Vickery S, Webb MC, Price CP, et al. Prognostic value of cardiac biomarkers for death in a non-dialysis chronic kidney disease population. Nephrol Dial Transplant 2008;23:3546–3553.

30. Anwaruddin S, Lloyd-Jones DM, Baggish A, et al. Renal function, congestive heart failure, and amino-terminal pro-brain natriuretic peptide measurement: Results from the ProBNP Investigation of Dyspnea in the Emergency Department (PRIDE) Study. J Am Coll Cardiol 2006;47:91–97.

31. Bjurman C, Petzold M, Venge P, et al. High-sensitive cardiac troponin, NT-proBNP, hFABP and copeptin levels in relation to glomerular filtration rates and a medical record of cardiovascular disease. Clin Biochem 2015;48:302–307.

32. DeFilippi C, van Kimmenade RR, Pinto YM. Amino-terminal pro-B-type natriuretic peptide testing in renal disease. Am J Cardiol 2008;101:82–88.

33. Adams JE 3rd, Bodor GS, Davila-Roman VG, et al. Cardiac troponin I. A marker with high specificity for cardiac injury. Circulation 1993;88:101–106.

34. Wu AH, Jaffe AS, Apple FS, et al. National Academy of Clinical Biochemistry laboratory medicine practice guidelines: Use of cardiac troponin and B-type natriuretic peptide or N-terminal pro-B-type natriuretic peptide for etiologies other than acute coronary syndromes and heart failure. Clin Chem 2007;53:2086–2096.

35. Burger IA, Kovacevic A, Mauldin GN, et al. Cardiac troponins as indicators of acute myocardial damage in dogs. J Vet Intern Med 2006;20:277–283.

36. Pelander L, Hagman R, Haggstrom J. Concentrations of cardiac Troponin I before and after ovariectomy in 46 female dogs with pyometra. Acta Vet Scand 2008;50:35.

37. Pelander L, Ljungvall I, Haggstrom J. Myocardial cell damage in 24 dogs bitten by the common European viper (Vipera berus). Vet Rec 2010;166:687–690.

38. Gow DJ, Gow AG, Bell R, et al. Serum cardiac troponin I in dogs with primary immune-mediated haemolytic anaemia. J Small Anim Pract 2011;52:259–264.

39. Hannacher L, Dorfelt R, Muller M, et al. Serum cardiac troponin I concentrations in dogs with systemic inflammatory response syndrome. J Vet Intern Med 2015;29:164–170.

40. Ljungvall I, Hoglund K, Tidholm A, et al. Cardiac troponin I is associated with severity of myxomatous mitral valve disease, age, and C-reactive protein in dogs. J Vet Intern Med 2010;24:153–159.

41. Langhorn R, Oyama MA, King LG, et al. Prognostic importance of myocardial injury in critically ill dogs with systemic inflammation. J Vet Intern Med 2013;27:895–903.

42. Twerenbold R, Wildi K, Jaeger C, et al. Optimal cutoff levels of more sensitive cardiac troponin assays for the early diagnosis of myocardial infarction in patients with renal dysfunction. Circulation 2015;131:2041–2050.

43. Kanderian AS, Francis GS. Cardiac troponins and chronic kidney disease. Kidney Int 2006;69:1112–1114.

44. deFilippi C, Seliger SL, Kelley W, et al. Interpreting cardiac troponin results from high-sensitivity assays in chronic kidney disease without acute coronary syndrome. Clin Chem 2012;58:1342–1351.

45. Parikh RH, Seliger SL, deFilippi CR. Use and interpretation of high sensitivity cardiac troponins in patients with chronic kidney disease with and without acute myocardial infarction. Clin Biochem 2015;48:247–253.
46. Wolf J, Gerlach N, Weber K, et al. The diagnostic relevance of NT-proBNP and proANP 31–67 measurements in staging of myxomatous mitral valve disease in dogs. Vet Clin Pathol 2013;42:196–206.
47. Moesgaard SG, Falk T, Teerlink T, et al. Brain-natriuretic peptide and cyclic guanosine monophosphate as biomarkers of myxomatous mitral valve disease in dogs. Vet J 2011;189:349–352.
48. Tarnow I, Olsen LH, Kvart C, et al. Predictive value of natriuretic peptides in dogs with mitral valve disease. Vet J 2009;180:195–201.
49. Polzin DJ. Chronic kidney disease in small animals. Vet Clin North Am Small Anim Pract 2011;41:15–30.
50. Hansson K, Hagstrom J, Kvart C, et al. Left atrial to aortic root indices using two-dimensional and M-mode echocardiography in cavalier King Charles spaniels with and without left atrial enlargement. Vet Radiol Ultrasound 2002;43:568–575.
51. 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.
52. Westgren F, Ley CJ, Kampa N, et al. Effects of hydration on scintigraphic glomerular filtration rate measured using integral and plasma volume methods in dogs with suspected renal disease. Vet Radiol Ultrasound 2014;55:632–637.
53. Kampa N, Lord P, Maripuu E, et al. Effects of measurement of plasma activity input on normalization of glomerular filtration rate to plasma volume in dogs. Vet Radiol Ultrasound 2007;48:585–593.
54. Kampa N, Bostrom I, Lord P, et al. Day-to-day variability in glomerular filtration rate in normal dogs by scintigraphic technique. Vet Med A Physiol Pathol Clin Med 2003;50:37–41.
55. Oyama MA, Solter PF. Validation of an immunoassay for measurement of canine cardiac troponin-I. J Vet Cardiol 2004;6:17–24.
56. Miyagawa Y, Tominaga Y, Toda N, et al. Relationship between glomerular filtration rate and plasma N-terminal pro-B type natriuretic peptide concentrations in dogs with chronic kidney disease. Vet J 2013;197:445–450.
57. Srisawasdi P, Vanavanan S, Charoenpanichkit C, et al. The effect of renal dysfunction on BNP, NT-proBNP, and their ratio. Am J Cardiol Pathol 2010;133:14–23.
58. Das SR, Abdullah SM, Leonard D, et al. Association between renal function and circulating levels of natriuretic peptides (from the Dallas Heart Study). Am J Cardiol 2008;102:1394–1398.
59. Vickers S, Price CP, John RI, et al. B-type natriuretic peptide (BNP) and amino-terminal proBNP in patients with CKD: Relationship to renal function and left ventricular hypertrophy. Am J Kidney Dis 2005;46:610–620.
60. DeFilippi CR, Fink JC, Nass CM, et al. N-terminal pro-B-type natriuretic peptide for predicting coronary disease and left ventricular hypertrophy in asymptomatic CKD not requiring dialysis. Am J Kidney Dis 2005;46:35–44.
61. Manzano-Fernandez S, Januzzi JL, Boronat-Garcia M, et al. Impact of kidney dysfunction on plasma and urinary N-terminal pro-B-type natriuretic peptide in patients with acute heart failure. Congest Heart Fail 2010;16:214–220.
62. McCullough PA, Duc P, Omland T, et al. B-type natriuretic peptide and renal function in the diagnosis of heart failure: An analysis from the Breathing Not Properly Multinational Study. Am J Kidney Dis 2003;41:571–579.
63. Bosselmann H, Egstrup M, Rossing K, et al. Prognostic significance of cardiovascular biomarkers and renal dysfunction in outpatients with systolic heart failure: A long term follow-up study. Int J Cardiol 2013;170:202–207.
64. Bruch C, Fischer C, Sindermann J, et al. Comparison of the prognostic usefulness of N-terminal pro-brain natriuretic peptide in patients with heart failure with versus without chronic kidney disease. Am J Cardiol 2008;102:469–474.
65. Wieshammer S, Dreyhaupt J, Basler B. Elevated levels of N-terminal pro-brain natriuretic peptide in patients with chronic dyspnea and moderate renal dysfunction: Decreased clearance or increased cardiac stress? Cardiorenal Med 2011;1:156–163.
66. Ng LL, Geeranavar S, Jennings SC, et al. Diagnosis of heart failure using urinary natriuretic peptides. Clin Sci 2004;106:129–133.
67. Palmer SC, Endre ZH, Richards AM, et al. Characterization of NT-proBNP in human urine. Clin Chem 2009;55:1126–1134.
68. Cortes R, Portoles M, Rosello-Lleti E, et al. Impact of glomerular filtration rate on urinary BNP and NT-proBNP levels in heart failure. Peptides 2012;33:354–358.
69. Palmer SC, Yandle TG, Nicholls MG, et al. Regional clearance of amino-terminal pro-brain natriuretic peptide from human plasma. Eur J Heart Fail 2009;11:832–839.
70. Essig M, Escoubet B, de Zuttere D, et al. Cardiovascular remodelling and extracellular fluid excess in early stages of chronic kidney disease. Nephrol Dial Transplant 2008;23:239–248.
71. Muscari A, Berzegotti A, Bianchi G, et al. Non-cardiac determinants of NT-proBNP levels in the elderly: Relevance of haematocrit and hepatic steatosis. Eur J Heart Fail 2006;8:468–476.
72. Willis MS, Lee ES, GrenACHE D. Effect of anemia on plasma concentrations of NT-proBNP, Clin Chim Acta 2005;358:175–181.
73. Hogenhuis J, Voors AA, Jaarsma T, et al. Anaemia and renal dysfunction are independently associated with BNP and NT-proBNP levels in patients with heart failure. Eur J Heart Fail 2007;9:787–794.
74. Anand IS, Chandrashekhar Y, Ferrari R, et al. Pathogenesis of oedema in chronic severe anaemia: Studies of body water and sodium, renal function, haemodynamic variables, and plasma hormones. Br Heart J 1993;70:357–362.
75. Schirrfin EL, Lipman ML, Mann JC. Chronic kidney disease: Effects on the cardiovascular system. Circulation 2007;116:85–97.
76. Sharma R. Screening for cardiovascular disease in patients with advanced chronic kidney disease. J Ren Care 2010;36(Suppl 1):68–75.
77. Amann K, Breitbach M, Ritz E, et al. Myocytes/capillary mismatch in the heart of uremic patients. J Am Soc Nephrol 1998;9:1018–1022.
78. Raine AE, Seymour AM, Roberts AF, et al. Impairment of cardiac function and energetics in experimental renal failure. J Clin Invest 1993;92:2934–2940.
79. Kogika MM, Lustoza MD, Hagiwara MK, et al. Evaluation of oxidative stress in the anemia of dogs with chronic kidney disease. Vet Clin Pathol 2015;44:70–78.
80. Silvestrini P, Piziani M, Alberola J, et al. Serum cardiac troponin T concentrations in dogs with leishmaniasis: Correlation with age and clinicopathologic abnormalities. Vet Clin Pathol 2012;41:568–574.
81. Cilli F, Alibhai HI, Armitage-Chan E, et al. Incidence of elevation of cardiac troponin I prior to and following routine general anaesthesia in dogs. Vet Anaesth Analg 2010;37:409–416.
82. Bubuin L, Jaffe AS. Troponin: The biomarker of choice for the detection of cardiac injury. CMAJ 2005;173:1191–1202.
83. Aeschbacher S, Schoen T, Bossard M, et al. Relationship between high-sensitivity cardiac troponin I and blood pressure among young and healthy adults. Am J Hypertens 2015;28:789–796.
84. Misbach C, Cherbul B, Concorlet D, et al. Basal plasma concentrations of routine variables and packed cell volume in clinically healthy adult small-sized dogs: Effect of breed, body weight, age, and gender, and establishment of reference intervals. Vet Clin Pathol 2014;43:371–380.
85. Polzin DJ. Evidence-based step-wise approach to managing chronic kidney disease in dogs and cats. J Vet Emerg Crit Care (San Antonio) 2013;23:205–215.