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

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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 (R^2_{adj} = 0.42; $P < .0001$). Age, body weight (BW), and SBP were independently associated with cTnI (R^2_{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.[7–16] One example is kidney disease, which might influence biomarker concentrations by several mechanisms.[17–20]

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
Cl	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%inc	increase in LVIDd compared to expected LVIDd
LVIDd	left ventricular end-diastolic internal diameter
LVIDs%inc	increase in LVIDs compared to expected LVIDs
LVIDs	left ventricular end-systolic internal diameter
mGFRi	measured glomerular filtration rate with scintigraphy, integral method
mGFR	measured glomerular filtration rate
mGFRpv	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

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(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.[6,22–25] 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.[29–31] 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, cats, 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 prerenal 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 prerenal, renal, 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 (PVf) derived from scintigraphic examination (PVf), 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[a] 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 hematologic (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 [chol], C-reactive protein [CRP], total protein [TP], albumin, and fibrinogen). After analysis, serum and EDTA plasma were frozen (−20°) in aliquots and subsequently (within 24 hours) transferred to storage at −70°. 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[b] 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.[50] 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 $0.95 \times BW^{0,315}$ and expected LVIDd using the formula $1.53 \times BW^{0,294}$. The increase in LVIDs compared to the expected LVIDs (LVIDs %inc) was determined by use of the formula ((LVIDs − LVIDs expected)/LVDs) × 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.[53,54] 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[d] 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 NT-proBNP concentration lower than the detection limit of the assay was assigned a concentration of 125 pmol/L.

Cardiac Troponin I Analysis

Frozen EDTA plasma was analyzed in batch for cTnI using a high-sensitivity assay[c] previously validated for use in dogs.[55] The lower detection limit of the assay was 0.01 ng/mL. Each sample was run in duplicate, and the mean cTnI 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.[e] 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 (and 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) with a P-value of <.2 were included. Thereafter, the variable with the highest P-value was removed 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 ($R^2_{adj} = 0.42$; $P < .0001$). 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

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

	Healthy	Stage I CKD	Stage II CKD	Stage III CKD
Number	23	14	7	6
Sex (F/M)	17/6	6/8	3/4	3/3
mGFRpv (mL/min/L)	50 (45 to 69)	41 (24 to 55)	24 (15 to 35)	11 (6 to 13)
mGFRi (mL/min/kg)	3.4 (3.0 to 3.9)	2.9 (2.1 to 3.3)	2.1 (2.0 to 2.3)	1.5 (1.4 to 1.8)
Creatinine (mmol/L)	78 (72 to 95)	91 (69 to 101)	136 (127 to 147)	233 (221 to 244)
UPC ratio	0.07 (0.04 to 0.11)	0.13 (0.06 to 1.74)	0.44 (0.15 to 1.67)	0.90 (0.24 to 3.72)
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)[ab]	0.38 (0.31 to 0.40)[b]
PVf (mL/kg)	63.7 (60.0 to 73.5)[a]	74.8 (64.8 to 87.6)[ab]	89.8 (79.6 to 135.1)[bc]	139.9 (123.7 to 258.0)[c]
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)[ab]	833 (125 to 1,222)[ab]	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)[ab]	0.02 (0.01 to 0.06)[ab]	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; LVIDd inc%, increase in left ventricular end-diastolic internal diameter compared to expected LVIDs; NTproBNP, amino-terminal pro B-type natriuretic peptide; cTnI, cardiac troponin I; CKD, chronic kidney disease.

Summary and comparison of characteristics between healthy dogs and CKD dogs, stages I through III. Significant differences ($P < .008$) are noted where superscripted letters differ between groups. Values are reported as median and interquartile range.

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 ($R^2_{adj} = 0.50$; $P < .0001$). This result was identical despite replacing mGFRpv with mGFRi in the multiple regression analysis. Figure 2 illustrates the univariate regression analyses regarding the 3 variables that were significant in the final model. Complete results from the univariate and multiple regression analyses regarding cTnI are presented in Table 2.

Discussion

In our study of healthy dogs and dogs with stable CKD, PCV and PVf/kg were independently associated with NT-proBNP concentration. Age, SBP, and BW were the variables independently associated with cTnI concentration. Importantly, kidney function, assessed by mGFR, was not independently associated with NT-proBNP or 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.[56] In that study, a correlation between mGFR and plasma NT-proBNP concentration ($r = -0.47$, $P < .01$) 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 concentration in humans with CKD.[29–31] 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 do healthy humans.[57–60] Human patients in CHF with a decreased eGFR also have higher concentrations of NT-proBNP than those in CHF with normal eGFR.[30,61] Consequently, slightly higher cutoff concentrations of NT-proBNP and BNP, respectively, are used to exclude CHF in human CKD patients.[25,30,62] However, if age-adjusted cutoff concentrations of NT-proBNP are used

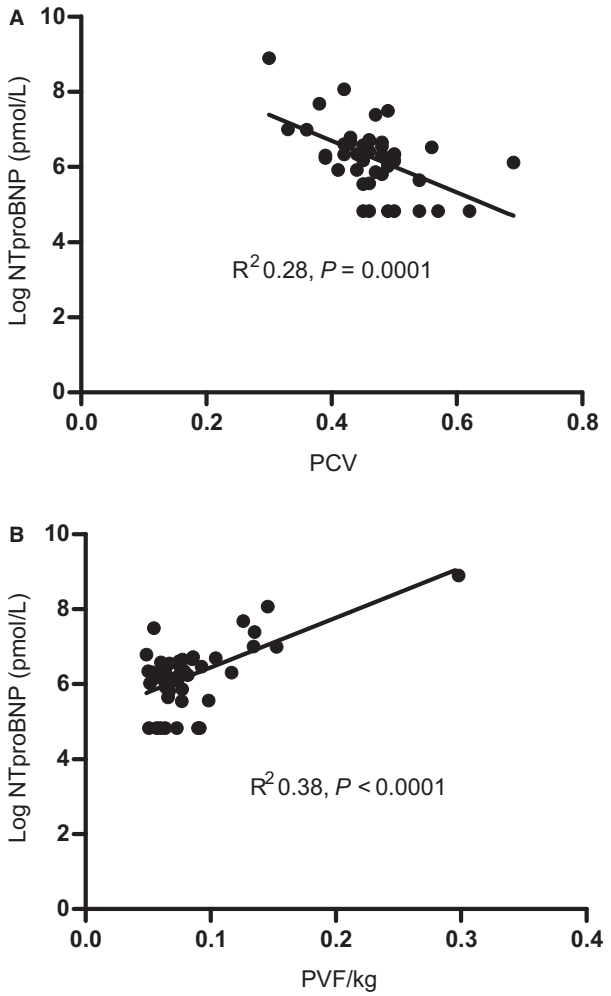

Fig 1. Association between (**A**) PCV and (**B**) PVF/kg (a factor related to plasma volume, indexed to BW) and log NT-proBNP concentration. These were the 2 significant variables in the final multiple regression model regarding NT-proBNP. BNP, B-type natriuretic peptide; BW, body weight; PVf, plasma volume factor.

for the diagnosis of CHF, further adjustment because of a decreased GFR is not necessary.[32] 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.[28] To what extent this occurs in dogs with CKD is yet unknown.

Findings of the present study were not consistent with a passive accumulation of NT-proBNP in this group of dogs with CKD. Although it was previously thought that increased NT-proBNP concentrations in humans with CKD result from decreased renal clearance of NT-proBNP, recent reports suggest that the main reason is increased secretion of this peptide by the heart.[28,31,65] Measurement of NT-proBNP and its metabolites in urine from people with CHF and differing kidney function shows that urine excretion of NT-proBNP increases rather than decreases with decreasing eGFR.[61,66–68] Also, when renal clearance of NT-proBNP was invasively measured in 1 study on subjects undergoing cardiac catheterization, the results indicated that the kidney extracts a similar percentage of plasma content of NT-proBNP regardless of kidney function.[69] This further implies that the absolute amount of circulating NT-proBNP removed by the kidneys actually increases with decreasing kidney function and that increased concentrations of NT-proBNP in these human patients are due mainly to increased production by the heart. This also might be true for dogs. Intravascular volume homeostasis is 1 of the major responsibilities of the kidneys, and with decreasing renal function, changes in intravascular volume may occur. Extracellular volume excess starts early in the course of CKD in humans,[70] and this could represent 1 reason for release of BNP into the circulation in dogs with CKD.

In our study, NTpro-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]

Table 2. Univariate and multiple regression analysis.

	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)	
Variable	R^2	*P*-Value	ß	*P*-Value	R^2	*P*-Value	ß	*P*-Value
Age	.003	.72			.35	<.0001	.14	<.0001
BW	.06	.096			.08	.041	.02	.04
Sex		.95				.061		
mGFRpv	.22	<.001			.06	.079		
mGFRi	.18	.0032			.05	.14		
PVf/kg	.38	<.0001	9.8	.001	.09	.039		
SBP	.003	.75			.18	.006	.01	.02
Creatinine	.35	<.0001			.08	.043		
P	.013	.45			.009	.52		
UPC	.10	.029			.07	.062		
Albumin	.10	.028			.03	.26		
EVF	.28	<.0001	−3.4	.04	.01	.40		
La/Ao	.00004	.97			.006	.60		
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.

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.[19] Subclinical cardiac pathology may be common in dogs with kidney disease, in accordance with the situation in humans.[75,76] The uremic human myocardium is known to be vulnerable to ischemia, 1 possible reason being increased cardiomyocyte area and diameter in combination with insufficient capillary growth (myocyte/capillary mismatch).[77] Another explanation for this vulnerability is an increased oxygen demand of the uremic myocardium.[78] 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.[79] 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.[70] Cardiac remodeling previously has been suggested to be associated with cardiac release of cTnI in dogs with MMVD.[40] 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.[31,40,80,81] 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.[19] An association between SBP and cTnI has been described in studies of humans.[82,83] 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.[40] 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,

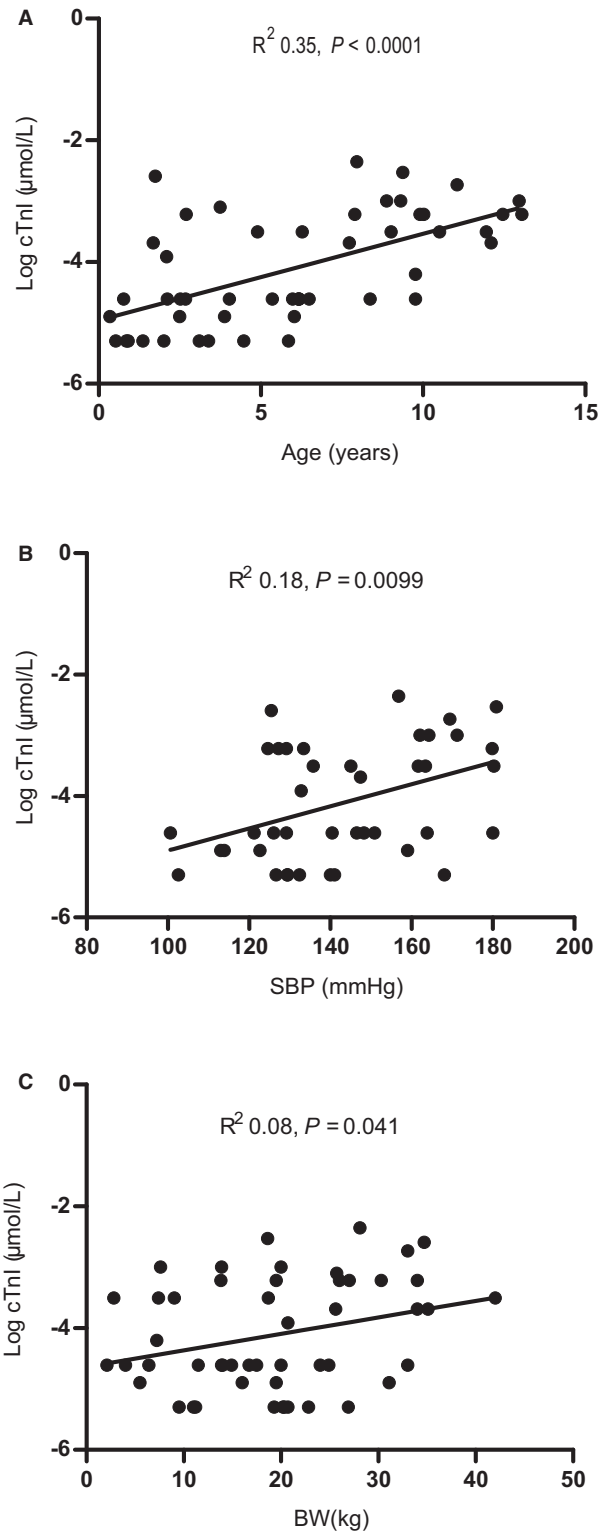

Fig 2. Association between (**A**) age, (**B**) systolic blood pressure, and (**C**) body weight and log cTnI concentration. These were the 3 significant variables in the final multiple regression model regarding cTnI.

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 PVf/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
[d] Cardiopet Pro-BNP, IDEXX laboratories Incorporated, Westbrook, ME
[c] Access AccuTnI+3 troponin I assay, Beckman Coulter, Brea, CA
[e] JMP Pro 11, SAS Institute, Cary, NC

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

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