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Evaluation of Serum Symmetric Dimethylarginine Concentrations in Dogs with Chronic Mitral Valve Insufficiency

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Abstract : Symmetric dimethylarginine (SDMA) is a new renal biomarker for kidney function. It is almost exclusively eliminated by renal filtration. The purpose of this retrospective study was to evaluate the changes in serum ceatinine (CREA), blood urea nitrogen (BUN) and SDMA concentrations in dogs with chronic mitral valve insufficiency (CMVI), according to the severity of CMVI. The evaluation of the severity of CMVI was performed according to the American College of Veterinary Internal Medicine (ACVIM) classification of heart failure. The dogs were classified into two groups: group 1 (ACVIM B; n = 11) and group 2 (ACVIM C; n = 15). In dogs with advanced CMVI, the serum SDMA concentrations were significantly increased above the normal reference range and were independent of body weight (BW), systolic blood pressure (SBP), or sex. No dog in either group had higher serum CREA concentrations than the upper limit. The serum SDMA concentration may be a better renal marker than serum CREA concentrations for the early diagnoses of renal dysfunction in dogs with CMVI.

Key words **:** CMVI, dog, renal dysfunction, creatinine, SDMA.

Introduction

Chronic heart failure (CHF) in humans and dogs often results in renal dysfunction (1,15,19). One study reported that 21% of human patients suffering from CHF developed renal dysfunction (12). Although the precise etiology remains uncertain, low cardiac output leading to decreased renal perfusion and hemodynamic alterations, as a result of neurohormonal activation, may be disadvantageous for renal function and reduce glomerular filtration rates (GFR) (1,15,17). Chronic or acute heart failure in dogs leads to low tissue perfusion and can cause ischemic damages to vital organs such as the kidneys (3). The prevalence rate of renal dysfunction in dogs with CHF has not been researched in veterinary practice. However, one study reported that renal dysfunction had ensued in dogs suffering from Chronic Mitral Valvular Insufficiency (CMVI) (15). CMVI that is caused by progressive myxomatous mitral valve disease is the most frequent cause of CHF in small breed dogs (2).

Renal dysfunction is commonly present and strongly correlates with mortality in people with CHF (1,21). In patients with CHF, azotemia and renal dysfunction result in hypertension, anemia, and volume overload, which are detrimental to the management of CHF (18). Therefore, early detection of CHF-associated renal dysfunction is important for long-term management of CHF in dogs and would allow timely administration of initial therapy. Early management of renal dysfunction may delay the progress of renal failure and extend the life of dogs with CMVI.

Direct determination of GFR is a valuable method for evaluating renal function and is currently accepted as the gold standard (4,8,10,20). However, direct measurement of GFR is infrequently used in veterinary practice because it is inconvenient to perform and stressful to patients with CMVI (4,8,10). Measurements of creatinine (CREA) and blood urea nitrogen (BUN) are commonly used for early diagnosis of renal disease because they are easily measured and are indirect indicators of GFR (8,10). However, the elevation of serum CREA and BUN concentrations are not detected until 75% of the kidney function has been lost (6,8,10). In addition, these renal markers are impacted by extra-renal factors such as age, hydration status and lean body mass (8,10,11,13).

Symmetric dimethylarginine (SDMA) is a new renal biomarker for kidney function. Since SDMA is almost exclusively eliminated by renal filtration, it is a good marker for estimating GFR and provides a method to access to renal function in dogs and cats (4,14). SDMA is not affected by extra-renal factors that affect CREA and other diseases including hepatic and cardiac diseases. A recently published study revealed that serum SDMA concentration increases earlier than serum CREA concentration in animals with chronic kidney disease (8).

The aim of this study was to evaluate the changes in serum CREA, BUN and SDMA concentrations in dogs with CMVI, according to the severity of CMVI.

Materials and Methods

Animals

This retrospective study was performed to estimate renal function in 26 client-owned dogs with CMVI. Twenty six

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Table 1. American Colleage of Veterinary Internal Medicine (ACVIM) classification of heart failure

ACVIM classification		
Stage A		Patients at high risk for developing heart disease but that currently have no structural disease of the heart
Stage B	B1	Asymptomatic patients with structural heart disease No evidence of cardiac remodeling
	B2	Asymptomatic patients with structural heart disease Left sided heart enlargement
Stage C		Symptomatic patients with structural heart disease
Stage D		Patients with end-stage disease with clinical signs Refractory to standard therapy

dogs of different breeds, sexes and ages were used in this study. Mean body weight was 4.87 kg (range: 1.67-10.5 kg) and the mean age was 11.65 years (range: 6-17 years). All dogs underwent physical, radiographic and echocardiographic examinations, as well as blood analysis and systolic blood pressure (SBP) measurements. CMVI was identified from the results of radiographic and echocardiographic examinations.

The evaluation of the severity of CMVI was performed according to the American College of Veterinary Internal Medicine (ACVIM) classification of heart failure (Table 1) (2). The dogs were classified into two groups: group 1 (ACVIM B; n = 11) and group 2 (ACVIM C; n = 15).

Diagnostic criteria of CMVI

CMVI in dogs was diagnosed on the basis of clinical signs, physical examinations, chest radiography and echocardiographic findings, in accordance with published guidelines for the diagnosis of CMVI in dogs. In all dogs, a systolic murmur, with the maximum point of intensity near the mitral valve area, was detected by auscultation. Pulmonary edema caused by CMVI was identified by pulmonary infiltrates and left atrial enlargement on thoracic radiographs, clinical signs related with CMVI, and the need for diuretics for symptomatic relief. Echocardiography was performed for each dog by veterinary medical imaging specialists using a Hitachi ARIETTA 70 system (Hitachi Aloka medical, Ltd., Tokyo, Japan). On color Doppler examinations, it was found that degenerative changes in the mitral valve leaflets caused mitral valve regurgitation. The inclusion criterion was left ventricular shortening fraction > 20%. Dogs suffering from other accompanying heart diseases, such as aortic stenosis or bacterial endocarditis, were notinvolved in this study.

Measurement of systolic blood pressure

A Doppler flow detectorwas used to measure systolic blood pressure (SBP). The Doppler flow probe was in contact with the palmar aspect of the forelimb (median artery) or the plantar aspect of the rear limb (tibial and metatarsal arteries), which were covered by anaqueous ultrasonic transmission gel (Eco gel 99, Seung Won Medical Corp., Korea). The cuff was placed around the limb close to the Doppler flow probe. The pressure of the cuff was increased until the sound of flow could not be heard, after which pressure was gradually released. The SBP measurement was recorded as the reading that coincided with the first audible signal; SBP measurements were repeated more than 5 times for mean calculations.

Blood samples

To control for the effect of feeding, all dogs were fasted for 12 hours prior to the blood test. Blood samples were withdrawn from either the jugular or cephalic veins with a 23-gauge needle attached to a syringe or a 24-gauge intravenous catheter. After collection, the blood samples were drawn into serum separating tubes (BD vacutainer®, Becton Dickinsonand Co, UK) for biochemical analyses. Serum samples were instantly separated by a centrifuge (Sigma, Sartorius AG, Germany) at 5000 rpm for 10 minutes. All serum samples were stored at 4°C and sent to a reference laboratory for the determination of serum SDMA concentrations.

Follow-up re-assessment of serum SDMA and CREA concentrations and implementation of renal ultrasonography

After the initial test to measure serum SDMA concentration, serum SDMA and CREA concentration were re-assessed in 6 animals with the consent of the owners (3 out of 11 in group 1, 3 out of 15 in group 2). Renal ultrasonography was performed simultaneously. Serum SDMA and CREA levels were measured using the same procedure as in the first test, and renal ultrasonography was performed by a radiology specialist using the Hitachi ARIETTA 70 system.

Analytical procedures

Serum samples for the measurement of SDMA concentration were sent to IDEXX laboratory (Idexx Korea) after storage at 4°C. The SDMA assay was conducted using a homogeneous enzyme immunoassay, while serum CREA and BUN levels were determined with an automated biochemistry analyzer (IDEXX Catalyst One™, Idexx Laboratories, USA) according to the manufacturer's instructions.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS version 23.0, IBM Corporation, USA) program was utilized for statistical analyses. Results were denoted as mean ± standard deviation (SD). The concentrations of serum BUN, CREA and SDMA between the two groups were compared using the Mann-Whitney U test. Correlations between SDMA and other renal markers and biological factors were evaluated by Spearman's correlation. For all analyses, a p-value of less than 0.05 ($P < 0.05$) was regarded as statistically significant.

Results

Baseline characteristics of the dogs

Twenty-six dogs with CMVI were used in this retrospective study (Table 2). Of the 26 dogs, 11 had asymptomatic mitral valve disease (group 1), while 15 had current or a history of

Table 2. Baseline characteristics and SBP of 26 dogs in this study

	Male (n)	Female (n)	Age (years)	BW (kg)	SBP (mmHg)
Group 1 Min - Max	4	7	7-12	4.18-13	102-160
Group 2 Min - Max	8	7	6-17	1.67-8	100-170

BW, body weight; SBP, systolic blood pressure; Min, minimum; Max, maximum

Table 3. Concentrations of serum BUN, CREA and SDMA, and the number of azotemic dogs in this study

Groups	BUN (mg/dl)	CREA (mg/dl)	SDMA (ug/dl)
Group 1 (n = 11) (Azotemic dogs*)	20.72 ± 12.95 (3/11)	0.78 ± 0.21 (0/11)	13.00 ± 3.82
Group 2 (n = 15) (Azotemic dogs*)	34.86 ± 18.31 (8/15)	1.06 ± 0.33 (0/11)	19.86 ± 6.68

Results expressed as mean values ± SD (group 1; n = 11, group 2; n = 15)
BUN, blood urea nitrogen; CREA, creatinine; SDMA, symmetric dimethylarginine
A dog was regarded as azotemic status if concentrations of BUN and CREA exceeded 26 mg/dL and 1.4 mg/dL, respectively

symptomatic heart failure related to mitral valve disease (group 2). In groups 1 and 2, Malteses (n = 11) and Shih Tzus (n = 6) were the most represented breeds. The average values of age and body weight (BW) were 11.65 ± 2.49 years (range: 6-17 years) and 4.87 ± 2.14 kg (range: 1.67-10.5 kg), respectively. SBP measurements ranged from 100 to 170 mmHg, with a mean ± SD of 130.38 ± 19.65 mmHg. The drugs for CMVI treatment were already being administered in 88% of the animals when their serum BUN, CREA, and SDMA concentrations were measured for this study.

Concentrations of serum BUN, CREA, and SDMA in dogs

The mean concentration of SDMA, CREA and BUN are shown in Table 3. The mean serum SDMA concentrations were 13.00 ± 3.82 μg/dl in group 1 and 19.86 ± 6.68 μg/dl in group 2. The upper limit of SDMA was set at 14 μg/dl in the basis of veterinary references. The mean serum BUN concentrations were 20.72 ± 12.95 mg/dl and 34.86 ± 18.31 mg/dl in group 1 and group 2, respectively, whereas the mean serum CREA concentrations were 0.78 ± 0.21 mg/dl in group 1 and 1.06 ± 0.33 mg/dl in group 2. The serum SDMA concentrations of groups 1 and 2 were statistically different ($p = 0.001$). Similarly, the serum BUN ($p = 0.015$) and CREA ($p = 0.024$) concentrations between the two groups were significantly different. Based on veterinary references, the upper limit of serum BUN, CREA, and SDMA concentrations were set at 25 mg/dl, 1.4 mg/dl, and 14 μg/dl, respectively. Three dogs in group 1, and eight in group 2, had concentrations over the upper limit of BUN. No dog in either group had higher CREA concentrations than the upper limit. However, the number of patients with concentrations over the upper limit of SDMA levels was two and thirteen for groups 1 and 2, respectively.

Table 4. Spearman's correlation coefficients between BUN, CREA and SDMA concentrations

	Correlation between SDMA and BUN	Correlation between SDMA and CREA
Group 1	r = 0.127	r = 0.249
Group 2	r = 0.765	r = 0.578
Group 1 & 2	r = 0.606	r = 0.638

BUN, blood urea nitrogen; CREA, creatinine; SDMA, symmetric dimethylarginine

The correlation of serum SDMA concentrations to serum BUN and CREA concentrations

The correlation coefficients of serum SDMA concentrations to serum BUN and CREA concentrations are presented in Table 4. In group 1, there were no statistically significant correlations between serum SDMA and BUN concentrations ($r = 0.127$, $p = 0.710$) nor between serum SDMA and CREA concentrations ($r = 0.249$, $p = 0.461$). In group 2, serum SDMA concentrations were positively correlated with serum BUN concentrations ($r = 0.765$, $p < 0.01$), and serum CREA concentrations ($r = 0.578$, $p < 0.05$). In both groups, serum SDMA concentrations were closely correlated to serum BUN ($r = 0.606$, $p < 0.01$) and CREA ($r = 0.638$, $p < 0.01$) concentrations.

Effects of age, sex, BW and SBP on serum SDMA concentrations

A significant correlation was identified between serum SDMA concentration and age ($r = 0.639$, $p < 0.01$); however, serum SDMA concentrations did not correlate with BW ($r = 0.170$, $p = 0.406$) or SBP ($r = 0.151$, $p = 0.462$). In addition, the serum SDMA concentrations of female and male dogs were not statistically different ($p = 0.595$).

Follow-up re-assessment of serum SDMA and CREA concentrations

Serum SDMA and CREA concentrations were re-examined in 6 out of 26 animals (three out of 11 in Group 1, three out of 15 in Group 2) as follow-up study. The SDMA concentration increased in 2 dogs and slightly decreased in another 2 dogs. The other 2 dogs showed similar SDMA concentrations on the initialand second follow-up tests. Two out of 3 dogs in Group 1 showed serum SDMA concentrations within the normal range on both initial and second tests and the other dogin Group 1 exceeded the normal range on the second test. All 3 dogs in Group 2 exceeded the normal range on both initial and second tests.

The CREA concentration was found to be within the normal range in 5 out of 6 re-tested dogs. Although no significant changes in concentration were observed between the two tests for 5 dogs, the animal with a high SDMA concentration exceeded the normal range for CREA concentration on the second test.

Renal ultrasonography results for six re-assessed animals

With the exception of the animal with significantly high SDMA concentrations on both tests, the ultrasound results for the other 5 dogs were within the normal range. Ultrasonography revealed structural changes in the kidney associated with aging of the animal with significantly high SDMA concentrations. Among the 5 dogs with normal renal ultrasound images, two had SDMA concentrations that exceeded the normal range on both tests, and one exceeded the normal range only upon re-examination. The SDMA concentrations for the other 2 dogs were within the normal range.

Discussion

In this study, renal function was estimated by determining serum SDMA concentrations. The correlations of serum SDMA concentrations with BUN and CREA concentrations, as well as the impact of physiological factors on serum SDMA concentration, were identified in dogs with CMVI.

Reduction of GFR significantly correlates with the severity of renal dysfunction. Quantification of serum CREA and BUN concentrations are commonly used biochemical tests for the indirect measurement of GFR and detection of renal dysfunction in dogs (11). Serum BUN concentrations can be easily influenced by several extra-renal factors such as dehydration, high protein diet, gastrointestinal bleeding, and liver failure (5,13,16). Quantification of serum CREA concentrations is currently the standard surrogate for the measurement of GFR (8,10). Recent studies report that a significant correlation was found between SDMA and GFR in dogs and cats (4,14). In comparison with serum CREA, serum SDMA increases much earlier than serum CREA in the early stages of chronic kidney diseases and its levels are not influenced by lean body mass (8,9).

As heart diseases progresses, the concentration of renal markers in dogs with CMVI progressively becomes elevated, suggesting that the decrease of GFR may worsen with the reduction of heart function. In a recent study, the serum SDMA concentrations increased, on average, with 40% loss of renal function (8), while the serum CREA concentrations did not increase until 75% of kidney function had been lost (11). In the present study, serum SDMA concentrations increased above the normal reference range (< 14 μg/dl) in 2 patients from group 1 and 10 patients from group 2, whereas serum CREA concentrations were within the reference range in all dogs. These results suggest that the measurement of serum SDMA concentrations allow us to detect renal dysfunction earlier than that of serum CREA concentrations.

In this study, the elevation of serum SDMA concentrations in dogs with advanced stage CHF indicated that the severe stage of heart disease and medications could be the risk factors of renal dysfunction. It is generally known that reduced GFR and azotemia are complications of advanced CHF patients on medication (15). Even though serum CREA and BUN concentrations are routinely measured to estimate GFR in dogs, these measurements are affected by extra-renal factors (11). In contrast, serum SDMA levels are not affected by extra-renal factors (10). Moreover, previous studies found a strong correlation between serum SDMA levels and GFR (8,9).

Considering the results from renal ultrasound and the reassessment of SDMA, serum SDMA concentration should be measured in patients with heart disease even if renal ultrasound reveals normal renal structure. Although on repeat evaluation, the serum SDMA levels were generally similar to the levels from the first test, some animals had increased levels on the second test. The serum SDMA concentration should be tested periodically, rather than only once, to assess changes in the concentration. This may be a more accurate method for measuring the glomerular filtration rate and renal function.

There are some limitations to this study. The study population in each group was not sufficient for statistical analyses to accurately reflect the correlation of serum SDMA concentrations to the severity of CHF in CMVI dogs. Additionally, there was no control group in this study, and the serum SDMA concentrations in normal dogs were not measured and compared with those of CMVI dogs. Direct measurements of GFR were not conducted due to technical and cost issues. Therefore, the correlation of GFR to renal markers such as SDMA and CREA was not calculated. The effect of medication on serum SDMA concentrations was also not reflected in this study. Dogs with CMVI were treated with long-term regimens of enalapril and furosemide and showed a tendency to have elevated serum BUN levels (7). As GFR can be reduced by furosemide, it can affect the concentration of renal markers in dogs treated with high doses. Therefore, further studies are required to elucidate the influence of cardiac medication on serum SDMA concentration.

Conclusion

The present study demonstrated the evaluation of serum SDMA concentrations as an endogenous marker of GFR in dogs with chronic mitral valve insufficiency. According to the present study, renal dysfunction tends to occur more frequently with an increase in the severity of CMVI. In dogs with advanced CMVI, the serum SDMA concentrations were significantly increased above the normal reference range. In addition, serum SDMA concentration was not influenced by BW, SBP, or sex; thus, it may serve as a better renal marker than serum CREA concentrations for the early diagnoses of renal dysfunction in dogs with CMVI.

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