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ORIGINAL ARTICLE

Cystatin C for managing diuretic-induced kidney dysfunction in MMVD dogs

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Cystatin C, a low-molecular-weight protein synthesized by cells, is being explored as a valuable biomarker for assessing renal function in veterinary medicine. Although the relationship between cystatin C and heart disease remains unclear, some studies suggest a possible association. This retrospective case-control study aimed to investigate the role of cystatin C as a biomarker for heart disease and its correlation with diuretic use in veterinary clinical practice. A total of 39 dogs were included in this study, comprising 9 control dogs without a predisposition to heart disease and 30 dogs in the study group diagnosed with heart disease. Among the 30 dogs with heart disease, 18 exhibited symptoms indicative of heart failure. Results showed significantly higher cystatin C levels in the heart disease group compared to the control group ($P<0.05$). However, no significant differences were observed among different stages of heart disease severity in the control group. Furthermore, cystatin-C showed statistically positive correlations with BUN ($r=0.478$, $P<0.01$), creatinine ($r=0.506$, $P<0.01$), and furosemide ($r=0.338$, $P<0.05$). Diuretics are essential for managing congestive heart failure, and the observed associations between cystatin C and furosemide suggest potential impacts of diuretic use on renal function in dogs with heart failure. Monitoring renal function markers, such as cystatin C, can aid in predicting and managing potential renal complications, ultimately improving the overall health and quality of life of dogs with heart disease.

Key Words: Cystatin C, Heart failure, Renal dysfunction, Furosemide, Dog

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INTRODUCTION

Myxomatous mitral valve disease (MMVD) is a prevalent cardiac condition in dogs, particularly in small to medium-sized breeds that affects the mitral valve and leads to congestive heart failure (CHF) (Atkins et al, 2009). Pulmonary edema, resulting from increased hydrostatic pressure in the pulmonary capillaries due to left-sided cardiac dysfunction, is a hallmark clinical sign of CHF (Drum et al, 2015).

In veterinary medicine, diuretic therapy is commonly employed to alleviate pulmonary edema and other congestive symptoms associated with MMVD. Diuretics are drugs that increase urine production and help eliminate excess fluid from the body by inhibiting the reabsorption of sodium and water in the kidneys (Oh and Han, 2015). Furosemide is one of the most widely used diuretics in the treatment of CHF in dogs. It acts on the thick ascending limb of the loop of Henle in the kidneys, leading to significant diuresis and natriuresis, resulting in a decrease in plasma volume and blood pressure (Felker, 2012).

Although diuretics are effective in managing pulmonary edema and other signs of congestion related to MMVD, their judicious use is critical as excessive diuresis can result in electrolyte imbalances, dehydration, and hypotension (Atkins et al, 2009). Furthermore, diuretic resistance may develop in some dogs, necessi-

tating higher doses or additional medications to achieve adequate diuresis (Atkins, 2012).

Despite the adverse effects reported, diuretics remain a crucial tool in the management of congestive heart failure in dogs. Nevertheless, their use can have negative effects on kidney function, including decreased glomerular filtration rate and increased serum creatinine levels (Elmahdy et al, 2021). These adverse effects are linked to the mechanisms of action of diuretics, which can result in reduced blood flow to the kidneys and altered renal tubular function (Ronco et al, 2008). Careful monitoring of renal function and alternative treatment options should be considered to minimize the risk of adverse renal outcomes, despite the importance of diuretics in the treatment of congestive heart failure in dogs.

Cystatin C (Cys-C) is a promising biomarker for the early detection of renal dysfunction in humans and animals (Dharnidharka et al, 2002; Miyagawa et al, 2009; Ghys et al, 2014). Studies have shown that Cys-C is a more sensitive and specific marker for early renal dysfunction than traditional markers of renal function, such as serum creatinine (Dharnidharka et al, 2002; Herget-Rosenthal et al, 2007). Cys-C is also less affected by factors such as age, sex, and muscle mass (Laterza et al, 2002).

In dogs, Cys-C has been shown to be a sensitive and specific marker for early renal dysfunction, with levels increasing before the onset of clinical signs of renal disease (Nabity et al, 2015). Similarly, in cats, Cys-C has been demonstrated to be a better marker for the early detection of renal dysfunction than serum creatinine (Hall et al, 2014). Cys-C has been shown to be a sensitive and specific marker for early renal dysfunction in dogs, with levels increasing before the onset of clinical signs of renal disease (Hall et al, 2014; Iwasa et al, 2018). Similarly, in cats, Cys-C has been demonstrated to be a better marker for early detection of renal dysfunction than serum creatinine (Hall et al, 2014).

Recent studies have suggested a potential link between Cys-C and heart disease in both humans and animals (Shlipak et al, 2005; Shlipak et al, 2009; Huerta et al, 2016; Zhao et al, 2016). While the exact mechanisms underlying this association are not fully understood, Cys-C has been suggested as a potential biomarker for identifying individuals at risk of heart disease and for monitoring disease progression (Koenig et al, 2005; Luo et al, 2015). However, it is essential to note that further research is necessary to investigate the relationship between Cys-C, heart disease, and diuretics used for treatment in veterinary clinical practice.

This study aimed to explore the relevance of Cys-C as a marker for heart disease with MMVD and its relationship with diuretics used for treatment in veterinary clinical practice.

MATERIALS AND METHODS

Study population

The subjects of this study were divided into two groups, the MMVD group and the control group. The data on the control group were analyzed through a retrospective investigation that analyzed the medical data of healthy dogs that had undergone a health checkup for heart and kidney conditions. Data collection was performed with the consent of the dogs' owners, and all examinations, tests, and treatments were conducted in accordance with established veterinary practices. The dogs' medical records were retrospectively examined to obtain information on relevant parameters.

The normal control group consisted of a total of 9 healthy dogs with weights and ages ranging from 1.9 to 15.6 kg and 1 to 8 years, respectively. Physical examinations of all normal controls showed no specific heart murmurs, and they exhibited no signs of physical disease based on complete blood count, serum chemistry test, and radiological examination. Echocardiography was not performed on dogs in this group.

The study group included a total of 30 dogs diagnosed with MMVD. Their ages and weights ranged from 7 to 17 years and 2.2 to 13.2 kg, respectively (Table 1). Di-

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Table 1. Demographic characteristics of the study population

	n (39)	Age (years)	BW (kg)	BCS (9-point)
Groups	39			
Control	9	4.9±0.9	5.4±1.4	5.1±0.4
Heart disease	30	11.1±0.5	4.6±0.5	5.2±0.2
ACVIM stage for heart disease	30			
B1	5	11.2±0.9	3.8±1.1	5.2±0.4
B2	7	13.1±1.0	4.9±1.0	5.6±0.4
C	15	10.4±0.6	4.8±0.7	5.1±0.2
D	3	9.7±0.9	3.8±1.3	5.3±0.9
IRIS stage	39			
1	6	4.5±1.3	3.7±0.5	4.7±0.4
2	16	10.3±0.9	4.4±0.9	5.2±0.2
3	11	11.6±0.6	5.8±1.0	5.4±0.3
4	6	9.7±0.6	4.7±1.0	5.5±0.5

All data expressed with the mean value±standard error. The control group and the asymptomatic stages of heart disease (ACVIM B1 and B2) did not receive any medication. However, the symptomatic heart failure groups (ACVIM C and D) were prescribed a treatment regimen consisting of pimobendan, furosemide, benazepril, and spironolactone.

agnosis of MMVD was confirmed based on clinical signs and echocardiography following American College of Veterinary Internal Medicine (ACVIM) guidelines, with subsequent classification of dogs into distinct heart failure severity stages according to ACVIM criteria (Keene et al, 2019). All dogs exhibited a heart murmur in the left chest, and underwent complete blood count and serum chemistry tests, urinalysis, echocardiography, and a general physical examination. Dogs with other systemic diseases, such as liver or pancreas disease, were excluded from the study. Additionally, dogs showing obvious symptoms of renal failure such as anorexia, vomiting, halitosis suspected of uremia, and those requiring hospitalization due to severe pulmonary edema resulting from heart failure were also excluded. Symptomatic dogs were treated with drugs such as pimobendan, furosemide, benazepril, and spironolactone depending on the severity.

As a criterion for the analysis of kidney function, the serum creatinine level recommended in IRIS classification was used (Brown, 2013; Society IRI, 2023). The dogs participating in the statistical analysis were not divided into control and study groups.

Echocardiography

Echocardiography was performed according to the recommended standard for all subjects in the study group with MMVD. Dogs were not administered sedation or anesthesia and were placed in right and left recumbency positions. Structural and hemodynamic evaluation of the heart was performed using commercially available transthoracic echocardiography (Vivid 7, GE Healthcare, Milwaukee, WI, USA).

Body weight (BW) normalized left ventricular internal diameter in diastole (LVIDDn) was measured at the end of diastole, and was measured by bisecting the chamber between the papillary muscles using 2D immediately before the R wave on the electrocardiogram (Morgan et al, 2020). The left atrium-to-aorta ratio (LA/Ao R) was measured at the end of ventricular systole just before the end of the T wave on the electrocardiogram (Hansson et al, 2002). Ao was measured along the junction of the non-coronary artery and the left coronary sinus, and LA was measured along the same line from inner edge to inner edge without extending the line to the pulmonary vein (Hansson et al, 2002). Trans-mitral blood flow velocity was measured using a pulse doppler (PD) with a sample volume placed at the tips of the leaflets of the

mitral valve. The early diastolic mitral inflow (E-peak) velocity was measured, and the early diastolic mitral inflow velocity to late diastolic mitral inflow velocity ratio (E/A ratio) could be measured using the lately diastolic mitral inflow (A-peak) velocity.

Analysis of serum Cys-C concentration

Cys-C in serum was measured with a commercial particle-enhanced turbidimetric immunoassay (PETIA) using Cys-C reagents (Tina-quant Cystatin C, Roche Diagnostics GmbH, Germany) designed for human samples. The concentration of Cys-C was measured by measuring the turbidity caused by the formation of aggregates, and the method in which Cys-C is bound to rabbit anti-human recombinant Cys-C polyclonal antibody coated latex particles was used. Assay for Cys-C was implemented on Mindray BS-330 analyzer (Mindray Bio-Medical Electronics Co., Ltd, Shenzhen, China) according to the manufacturer's user manual.

Statistical analysis

The statistical analysis of the collected data was performed using Jamovi for Windows, version 2.3.12. Continuous variables are presented as mean±standard error (SE). Differences in various indices that changed between the normal control group and the study group with heart disease, the ACVIM stage classified according to the severity of MMVD, and the IRIS stage according to the levels of creatinine were evaluated using one-way analysis of variance (ANOVA) with Welch's test, and the post-hoc analysis was performed using Games-Howell test. In addition, Pearson's coefficient of bivariate correlation analysis was used to test the strength of association between blood urea nitrogen (BUN), creatinine, and furosemide doses with Cys-C. In all comparisons, a probability value of $P<0.05$ was considered statistically significant, unless stated otherwise.

RESULTS

Demographic characteristics of the study population

The study compared various parameters between a healthy control group and a heart disease study group, as well as within the stages based on ACVIM and IRIS classification. The statistical analysis used a mean±SE format, and the significance of the differences between the groups was determined using a statistical analysis program. The statistical significance was determined by the P values, where $P<0.05$ was considered statistically significant, and $P<0.001$ was considered highly statistically significant. The mean and SE of age, weight, and body condition score for each group are summarized in Table 1.

Significance of each parameters between the control group and the heart disease group

The study compared the results of the control group with those of the study group. The control group had a systolic blood pressure (SBP) of 146±6 mmHg, BUN of 27.3±1.6 mg/dL, creatinine of 1.2±0.1 mg/dL, Cys-C of 2.3±0.4 mg/dL, urine protein-to-creatinine ratio (UPC) of 0.08±0.02, and urine specific gravity (USG) of 1.040±0.003 (Table 2).

In the comparison, the study group had significantly higher SBP (165±4 mmHg, $P<0.05$), BUN (45.5±4.0 mg/dL, $P<0.001$), creatinine (3.8±0.3 mg/dL, $P<0.001$), and Cys-C (4.1±0.6 mg/dL, $P<0.05$) compared to the control group. The UPC was also significantly higher (0.86±0.12, $P<0.001$), and USG was significantly lower (1.030±0.002, $P<0.05$) in the study group compared to the control group (Table 2).

Significance of each parameters according to ACVIM stage

The study group was classified into stages B1, B2, C, and D according to ACVIM classification. Compared

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Table 2. Mean±standard error values of test parameters according to control and heart disease group

		SBP (mmHg)	BUN (mg/dL)	Creatinine (mg/dL)	Cystatin C (mg/L)	UPC	USG
Control	Mean	146	27.3	1.2	2.3	0.08	1.040
	SE	6	1.6	0.1	0.4	0.02	0.003
Heart disease	Mean	165*	45.5***	3.8***	4.1*	0.86***	1.030*
	SE	4	4.0	0.3	0.6	0.12	0.002

*P<0.05, ***P<0.001.
SBP, systolic blood pressure; BUN, blood urea nitrogen; UPC, urine protein-to-creatinine ratio; USG, urine specific gravity.

to the control group, the following parameters showed statistical significance (Table 3): SBP (control to stage C, P<0.05), Creatinine (control to stage B1, P<0.05; control to stage C, P<0.01), UPC (control to stage C, P<0.01; control to stage D, P<0.001; stage C to D, P<0.05). USG (control to stage C, P<0.05), LA/Ao Ratio (stage B1 to C, B1 to D, B2 to C, P<0.001; stage B2 to D, P<0.01; stage C to D, P<0.05), E-peak velocity (stage B1 to D, B2 to C, P<0.01; stage B2 to D, P<0.001; stage C to D, P<0.05), E/A ratio (stage B1 to D, P<0.05; stage B2 to D, P<0.01), furosemide (control to stage C, B1 to C, 2 to C, P<0.001; stage C to D, P<0.05).

Statistical significance was confirmed between the control and study groups for Cys-C, but not according to the ACVIM classification (Fig. 1).

Significance of each parameters according to IRIS stage

The study involved dogs that were classified into different stages (1~4) based on the IRIS classification. The following parameters were measured in the study group and the statistical significance was determined using statistical analysis (Table 4): BUN, creatinine, UPC, USG, and furosemide. BUN showed statistical significance for the following stage comparisons: stage 1 to 2 (P<0.05), stage 1 to 3 (P<0.05), stage 1 to 4 (P<0.01), stage 2 to 4 (P<0.01), and stage 3 to 4 (P<0.05). Creatinine showed statistical significance for the following stage comparisons: stage 1 to 2 (P<0.001), stage 1 to 3 (P<0.001), stage 1 to 4 (P<0.001), stage 2 to 3 (P<0.001), stage 2 to 4 (P<0.01), and stage 3 to 4 (P<0.01). UPC showed statistical significance for the following stage comparisons: stage 1 to 2 (P<0.01) and stage 1 to 3 (P<0.05). USG showed statistical significance for the following stage comparison: stage 1 to 3 (P<0.01). Furosemide showed statistical significance for the following stage comparisons: stage 1 to 2 (P<0.001) and stage 1 to 3 (P<0.05). The echocardiography parameters measured in the study group did not show statistical significance.

The statistical correlation analysis for Cys-C included all dogs in the study without differentiating them into control and study groups or according to the stages of the disease in the study group. Cys-C showed statistically significant positive correlations with BUN (r=0.478, P<0.01), creatinine (r=0.506, P<0.01), and furosemide (r=0.338, P<0.05) (Fig. 2).

DISCUSSION

Compared to the control group, the study group had significantly higher values for SBP, BUN, creatinine, and Cys-C, while the control group had lower ranges for all these parameters. The study group also exhibited a significant increase in UPC, indicating proteinuria, which is commonly found in humans with heart disease and is considered a prognostic factor for survival (Currie and Delles, 2013). Additionally, the study group had a significantly lower USG, which is a widely used measure of urine concentration to assess renal function. A reduction in USG suggests a decline in renal function and has been associated with poor outcomes in dogs with heart failure (Brown et al, 2007). Of particular concern is the significantly elevated levels of BUN and creatinine in the

Table 3. Mean±standard error values of test parameters according to ACVIM stage

		SBP (mmHg)	BUN (mg/dL)	Creatinine (mg/dL)	Cystatin C (mg/L)	UPC	USG	LVIDDn	LA/Ao	E-peak velocity (m/s)	E/A	Furosemide (mg/kg)
ACVIM stage												
B1	Mean	167	33.0	2.9*	2.9	0.78	1.030	1.5	1.4	0.9	1.1	0.0
	SE	7	5.3	0.3	0.3	0.31	0.005	0.1	0.1	0.1	0.1	0.0
B2	Mean	166	58.2	4.3	3.6	0.70	1.030	1.6	1.6	0.8	0.9	0.0
	SE	9	10.1	0.9	0.8	0.21	0.003	0.1	0.1	0.1	0.1	0.0
C	Mean	169†	43.2	3.7[a)]	4.2	1.05[1)]	1.020†	1.8	2.0[2,4)]	1.3[d)]	1.3	1.8[1,2,4)]
	SE	4	5.1	0.5	0.9	0.19	0.002	0.1	0.0	0.1	0.1	0.1
D	Mean	143	47.7	4.1	7.2	0.45[§,b)]	1.030	2.0	2.5[§,c,3)]	1.7[§,c,5)]	1.6‡,e)]	2.0[§]
	SE	19	16.7	1.4	4.1	0.03	0.007	0.1	0.1	0.1	0.1	0.0

*Statistically significance between Control and B1 stage group, $P<0.05$. †Statistically significance between Control and C stage group, $P<0.05$. ‡Statistically significance between B1 and D stage groups, $P<0.05$. §Statistically significance between C and D stage groups, $P<0.05$. [a)]Statistically significance between Control and C stage group, $P<0.01$. [b)]Statistically significance between Control and D stage group, $P<0.01$. [c)]Statistically significance between B1 and D stage group, $P<0.01$. [d)]Statistically significance between B2 and C stage group, $P<0.01$. [e)]Statistically significance between B2 and D stage group, $P<0.01$. [1)]Statistically significance between Control and C stage group, $P<0.001$. [2)]Statistically significance between B1 and C stage group, $P<0.001$. [3)]Statistically significance between B1 and D stage group, $P<0.001$. [4)]Statistically significance between B2 and C stage group, $P<0.001$. [5)]Statistically significance between B2 and D stage group, $P<0.001$.

The parameter values of the normal control group are described in Table 1; SBP, systolic blood pressure; BUN, blood urea nitrogen; UPC, urine protein-to-creatinine ratio; USG, urine specific gravity; LVIDDn, body weight (BW) normalized left ventricular internal diameter in diastole; LA/Ao, left atrium-to-aorta ratio; E-peak velocity, early diastolic mitral inflow velocity; E/A, early diastolic mitral inflow velocity to late diastolic mitral inflow velocity ratio.

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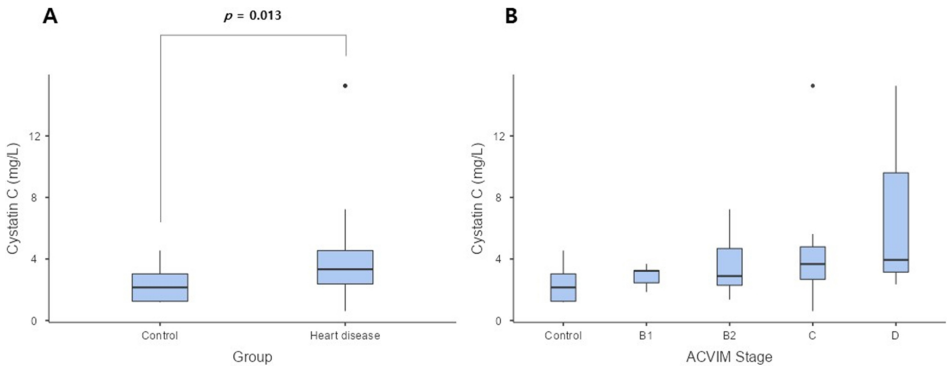

Fig. 1. Significance between the control group, the heart disease group, the control group, and the control group and each stage in the ACVIM classification. Significance (P=0.013) was shown between the control group and the heart disease group (A), but there was no significance between the control group and each stage in the ACVIM classification (B). ● Outliers within a data set.

study group, which indicate impaired kidney function. Diuretics are a standard treatment for heart failure, but they can cause kidney dysfunction in some patients. These findings align with previous studies indicating that renal function is commonly affected in dogs with heart failure, and elevated BUN and creatinine levels are indicative of renal damage (Liang et al, 2008; Pouchelon et al, 2015).

Each stage of study group by ACVIM classification showed statistically significant differences on several parameters, compared to the control group. Stage C dogs had significantly higher systolic blood pressure (SBP) than the control group, while stages B1 and C had significantly higher creatinine, indicating impaired kidney function (Brown et al, 2013). The UPC was significantly higher in stages C and D, indicating proteinuria and kidney dysfunction, while urine specific gravity (USG) was significantly lower in stage C, suggesting reduced kidney function (Brown et al, 2013). These findings were attributed to congestive heart failure in stage C and D dogs, and dehydration and impaired renal function due to diuretic treatment. As the heart disease progresses, the LA/Ao ratio significantly increases in stages B1, B2, and C compared to controls, indicating an enlarged left atrium relative to the aorta (Hansson et al, 2002). The E-peak rate, representing the speed of blood flowing between mitral valve leaflets, was significantly higher in stages B1 and D than controls, and the E/A ratio, indicating heart relaxation, was significantly higher in stages B1 and D, and significantly higher in stage B2, due to the increased blood flow and pressure in the dilated left atrium (Morgan et al, 2020). The administration of furosemide, a commonly used diuretic for heart disease treatment, was significantly higher in stages C and D compared to the control group. This is noteworthy since diuretics may result in kidney dysfunction in some patients, and the heart disease group already demonstrated impaired kidney function, as evidenced by elevated levels of creatinine and proteinuria (Ronco et al, 2008; Pouchelon et al, 2015).

Statistical analysis of each stage of IRIS classification revealed significant differences in the parameters of BUN, USG, and furosemide, but not in echocardiography. BUN levels were significantly different between stages 1 and 2, 3, and 4, between stages 2 and 4, and between stages 3 and 4. Creatinine levels showed significant differences between stages 1 and 2, 3, and 4, between stages 2 and 3, between stages 2 and 4, and between stages 3 and 4. USG levels were significantly different between stages 1 and 3. Furosemide levels showed significant differences between stages 1 and 2 and between stages 1 and 3. These findings suggest that measurements of BUN, creatinine, UPC, USG and furosemide may be useful in assessing renal function in dogs with heart disease, and provide important information about the progression of renal dysfunction. However, echocardiography does not reflect the severity of kidney disease.

Cys-C is a marker of kidney function (Dharnidharka et al, 2002), and is frequently employed in veterinary medicine to indicate renal dysfunction (Iwasa et al, 2018). Cys-C is a low-molecular-weight protein that is

Table 4. Mean±standard error values of test parameters according to IRIS stage

		SBP (mmHg)	BUN (mg/dL)	Creatinine (mg/dL)	Cystatin C (mg/L)	UPC	USG	Furosemide (mg/kg)
IRIS stage								
1	Mean	145	25.2	1.0	1.9	0.07	1.040	0.0
	SE	7	1.7	0.1	0.3	0.02	0.003	0.0
2	Mean	158	33.8*	2.2[1)]	2.6	0.73[a)]	1.030	0.9[a)]
	SE	4	2.5	0.1	0.3	0.15	0.002	0.2
3	Mean	170	41.5[†]	3.6[2,4)]	4.4	0.71[†]	1.020[b)]	1.0[†]
	SE	6	5.2	0.2	1.1	0.20	0.003	0.3
4	Mean	168	77.1[‡,c,d)]	7.1[d,e,3)]	7.1	1.12	1.020	1.3
	SE	14	7.6	0.6	1.7	0.34	0.004	0.4

*Statistically significance between 1 and 2 stage group, $P<0.05$. [†]Statistically significance between 1 and 3 stage group, $P<0.05$. [‡]Statistically significance between 3 and 4 stage groups, $P<0.05$. [a)]Statistically significance between 1 and 2 stage group, $P<0.01$. [b)]Statistically significance between 1 and 3 stage group, $P<0.01$. [c)]Statistically significance between 1 and 4 stage group, $P<0.01$. [d)]Statistically significance between 2 and 4 stage group, $P<0.01$. [e)]Statistically significance between 3 and 4 stage group, $P<0.01$. [1)]Statistically significance between 1 and 2 stage group, $P<0.001$. [2)]Statistically significance between 1 and 3 stage group, $P<0.001$. [3)]Statistically significance between 1 and 4 stage group, $P<0.001$. [4)]Statistically significance between 2 and 3 stage group, $P<0.001$.
SBP, systolic blood pressure; BUN, blood urea nitrogen; UPC, urine protein-to-creatinine ratio; USG, urine specific gravity.

produced by cells throughout the body (Abrahamson et al, 1990), and has emerged as a valuable biomarker for evaluating kidney function in veterinary medicine. Cys-C is considered superior to creatinine in terms of sensitivity and specificity, and is widely used in clinical practice due to its ease of measurement (Ghys et al, 2014). Cys-C is primarily used as a marker of kidney function, as it is filtered out of the blood by the glomeruli in the kidneys and then broken down and reabsorbed by the tubules (Dharnidharka et al, 2002). This means that levels of Cys-C in the blood are a good indicator of how well the kidneys function. Because Cys-C is less affected by factors such as age, sex, and muscle mass than other markers of kidney function, such as creatinine, it is often used in combination with other tests to estimate the glomerular filtration rate (GFR), which is a measure of how well the kidneys are filtering waste products out of the blood (Dharnidharka et al, 2002).

In this study, statistical analysis was performed to examine the correlation between Cys-C levels and other parameters in dogs with heart disease. The results revealed that although there was a statistically significant difference in Cys-C levels between the control and study groups, it was not significant according to the ACVIM classification guidelines. This suggests that Cys-C may not be a sensitive enough marker to identify early stages of kidney dysfunction in dogs with heart disease. However, statistically significant positive correlations were demonstrated between Cys-C levels and BUN, creatinine, and furosemide for all dogs included in this study. These findings suggest that Cys-C could be a valuable biomarker for evaluating kidney function in dogs with heart disease, particularly those who are receiving diuretic therapy. The positive correlation between Cys-C and BUN and creatinine levels implies that Cys-C levels could be a reliable indicator of renal dysfunction. Furthermore, the positive correlation between Cys-C and furosemide suggests that diuretic therapy can affect Cys-C levels, which can result in kidney dysfunction in some patients.

The study group exhibited statistically significant Cys-

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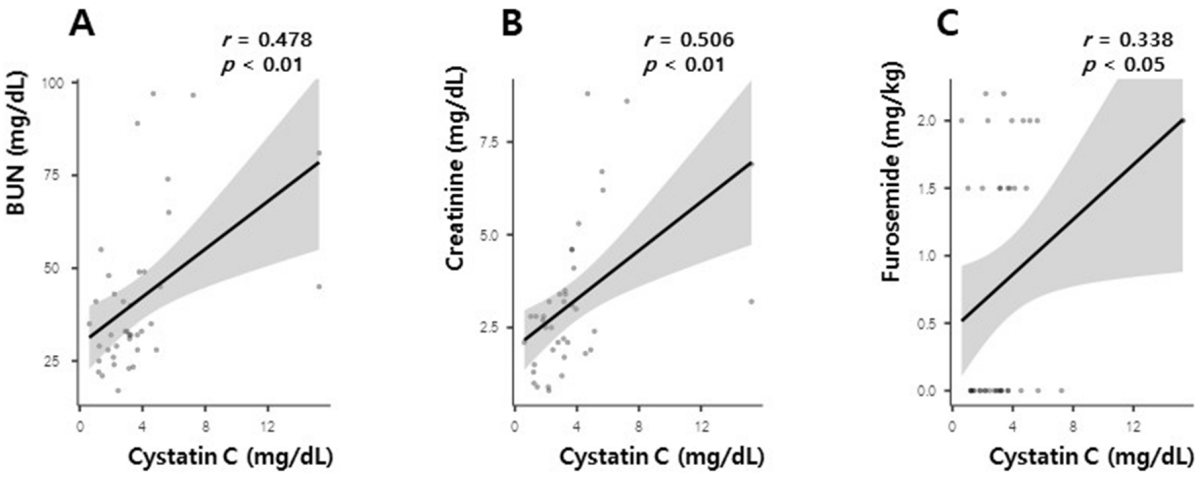

Fig. 2. Correlation of cystatin C with BUN (A), creatinine (B), and furosemide (C) in this study. Cysatin C showed significance and correlation with BUN (r=0.478, P<0.01), creatinine (r=0.506, P<0.01), and furosemide (r=0.338, P<0.05).

C levels in comparison to the control group. Although there is no conclusive and reliable evidence, this finding is consistent with previous studies that suggest a potential association between Cys-C and heart disease (Shlipak et al, 2005; Shlipak et al, 2009; Huerta et al, 2016; Zhao et al, 2016).

Creatinine was found to be statistically significant for each stage based on the IRIS classification, as it is a standard parameter of the classification system (Brown, 2013; Society IRI, 2023). Dogs with clinical symptoms of kidney dysfunction typically exhibit anorexia, vomiting, and halitosis, along with elevated blood creatinine levels. Creatinine is an important parameter used to evaluate renal function using IRIS classification and is commonly used in veterinary clinics. However, in this study, the statistical significance of creatinine in relation to echocardiography parameters was found to be insufficient for comparing the stages of IRIS classification. Additionally, none of the dogs with heart disease in this study presented clear clinical symptoms of renal dysfunction. This finding is consistent with previous studies that have suggested that heart disease and subsequent diuretic therapy may not directly induce renal disease (Martinelli et al, 2020). Therefore, the absence of significant correlations between creatinine and the stages of heart disease observed in this study provides further evidence to support the hypothesis that high levels of creatinine in dogs with heart disease may be due to factors other than primary renal dysfunction, such as dehydration resulting from diuretic therapy.

Cys-C did not show statistical significance in the IRIS classification, which may lead to the misconception that Cys-C is an inadequate marker for evaluating kidney dysfunction in this study. Instead, it is more appropriate to infer from the statistical correlation between Cys-C and furosemide. Since diuretics are essential for managing congestive heart failure but may cause kidney dysfunction (Ellison, 2001), the statistical values of creatinine in dogs with heart disease receiving diuretics may not adequately reflect the severity of primary renal disease. Furthermore, the positive correlation between Cys-C and furosemide is a noteworthy finding in this study. It is possible that the relationship between Cys-C and heart disease is attributed to the use of diuretics as a therapeutic drug. Thus, Cys-C may be an excellent marker for evaluating renal dysfunction induced by inappropriately prescribed diuretics in dogs with heart failure.

The study has some limitations that need to be addressed. Firstly, the IRIS classification parameter used in the study was creatinine alone, even though SDMA is a more reliable kidney marker (Hall et al, 2016), and is also used in conjunction with creatinine to classify severity in IRIS (Brown, 2013). Unfortunately, due to

the small number of dogs tested for SDMA in the blood analysis of the experimental population, it was not appropriate as an analysis item. Secondly, there was no histopathological examination of renal damage in dogs treated with diuretics. In veterinary clinical settings, it is challenging for dog owners to perform biopsies beyond routine exams or treatments. Thirdly, the potential influence of prescribed medications on the kidneys of dogs with heart failure participating in this study cannot be entirely disregarded. Patients with heart failure received prescribed medications, including pimobendan, furosemide, benazepril, and spironolactone, aimed at alleviating symptoms. Notably, dogs in the refractory stage (ACVIM D) were administered high doses of furosemide and pimobendan. Variations in the usage of these drugs could potentially impact renal function. Additionally, in a typical veterinary clinical setting, owners frequently exhibit hesitancy towards performing kidney biopsies on living patients, often due to concerns regarding potential exacerbation of kidney damage following the procedure. While post-mortem biopsies can be conducted through autopsy in cases where patients have deceased, all patients included in the study group were still alive. Thirdly, the size of the population at each stage of the analysis was relatively small. Although further research is necessary, the statistical significance and correlation found in this study suggest that diuretics administered to heart disease patients can affect kidney function, which can be confirmed through early Cys-C screening.

In conclusion, the relationship observed between furosemide, BUN, creatinine, and Cys-C is particularly relevant for understanding kidney function in dogs with heart disease who are receiving diuretic therapy. The correlation between furosemide and these kidney function markers suggests that the amount of diuretic used may affect renal function in these patients. Therefore, the use of Cys-C and other biomarkers can be an important tool for managing kidney dysfunction in dogs with heart disease who are receiving diuretic therapy.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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