



## NOTE

Internal Medicine

# Evaluation of monitoring methods in asymptomatic dogs with high serum cystatin C concentrations

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**ABSTRACT.** This study evaluated the monitoring methods in asymptomatic dogs with high serum cystatin C (Cys-C) concentrations. Ten dogs with high serum Cys-C were divided into two groups based on the owner's choice; one receiving clinical pathology-based monitoring at an animal hospital specialised in chronic kidney disease, and the other receiving symptom-based monitoring at home, partly because they showed no clinical symptoms. The dogs that received the clinical pathology-based monitoring led to an early treatment intervention, resulted in a longer survival period than dogs received the symptom-based monitoring ( $P < 0.05$ ). It became clear that early treatment intervention by clinical pathology-based monitoring extends the renal survival period even in asymptomatic dogs with increased serum Cys-C concentrations.

**KEY WORDS:** chronic kidney disease, cystatin C, dog, monitoring

It is widely agreed that the gold standard for the evaluation of renal function in dogs is the glomerular filtration rate (GFR), measured by an inulin or iothexol clearance test [4, 7]. Unfortunately, because it is complex and technically difficult, this approach of measuring the GFR is not often used, and instead, in veterinary medicine, renal function is commonly evaluated by measuring serum urea nitrogen (UN) and serum creatinine (Cre). Indeed, serum Cre concentration is used as part of the International Renal Interest Society (IRIS) classification for chronic renal disease in dogs. However, serum UN and Cre concentrations could be affected by other factors, such as feeding status, body weight, muscle mass and breed [3, 5, 10, 17, 20, 22]. Additionally, the serum Cre concentration does not increase until the GFR is reduced by 75% [3, 8]. For the afore-mentioned reasons, more sensitive markers for kidney function have been sought and developed.

Cystatin C (Cys-C) is a cysteine-proteinase inhibitor that is formed from 120 amino acid residues into a single polypeptide chain. It is continuously produced by nucleated cells [1, 25] and filtered by the glomeruli, with approximately 99% of filtered Cys-C being reabsorbed by tubular epithelial cells, meaning that it does not return to the circulation system, neither is it secreted by renal tubules [6, 15, 25]. These realities suggest that serum Cys-C concentration can be a biomarker of the GFR [2, 9].

In humans, serum Cys-C concentration is a better GFR marker for chronic kidney disease than serum Cre concentration [16, 21], since it can detect chronic kidney disease in earlier phase [11]. Even better, serum Cys-C concentration can be used to reliably predict the outcome of renal diseases [27]. In a veterinary study, serum Cys-C concentrations were found to be significantly higher in dogs with renal failure as compared with the serum Cys-C concentration in healthy dogs [14]. Additionally, the serum Cys-C concentrations were superior to serum Cre concentrations in estimating the GFR in dogs [26], especially in those dogs with a body weight of less than 15 kg [18]. These evidences suggest that serum Cys-C concentrations can detect the early phase of canine chronic kidney disease. In fact, serum Cys-C concentration was a better prognostic marker than serum Cre concentration [13]. Additionally, asymptomatic dogs with high serum Cys-C concentrations had a shorter renal disease-specific survival period than those with low serum Cys-C concentrations [13]. One of the lessons learned from this study is that despite a lack of clinical symptoms, it is important to closely monitor dogs if they have increased serum Cys-C concentrations. However, owners of asymptomatic dogs with high serum Cys-C concentrations sometimes decline a close follow-up visit because they do

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not see any clinical signs of difficulty, especially with regard to azotaemia. In the present study, dogs showing a high serum Cys-C concentration in a periodic health examination were retrospectively studied to evaluate the monitoring methods of their renal function.

This was a retrospective cohort study, where we defined increased serum Cys-C concentration ( $>0.55$  mg/dl) according to our previous investigation in this area [13]. Dogs that showed increased serum Cys-C concentration in a routine periodic health examination at a primary veterinary hospital between December 2013 and April 2017 were included in this study, and the clinical course of the eligible dogs was followed. The dogs that underwent a serum Cys-C measurement were limited to those with a body weight of  $<15$  kg, because Cys-C is known to be an inferior kidney marker in larger breed dogs [18]. We excluded dogs that showed clinical symptoms of renal disease, such as polyuria and polydipsia, vomiting, dehydration, weight loss and appetite decrease, as well as those with a heart murmur in the auscultation. Because owners of dogs with severe kidney disease generally elect intensive treatment for their animals, regardless of the presence of clinical symptoms, we excluded dogs with serum Cre concentration  $>2.0$  mg/dl. All dog owners were offered clinical pathology-based monitoring at hospital for chronic kidney disease at the time of the routine evaluation. All the dogs in this study were divided into two groups; one group, which accepted the offer to start regular follow-up visits every 2 to 4 weeks (Clinical pathology-based monitoring group; CPBM), and another group, which refused the regular follow-up visits (symptom-based monitoring group; SBM).

Using an automated biochemical analyser (Labospect 003; Hitachi High-Technologies, Tokyo, Japan), we measured numerous biomarkers in the subject animals, which included: serum total protein (TP); albumin (ALB); alkaline phosphatase (ALP); alanine aminotransferase (ALT); aspartate aminotransferase (AST); UN; Cre; total bilirubin; triglyceride;  $\gamma$ -glutamyl transferase (GGT); total cholesterol; calcium; inorganic phosphorus; glucose and lipase, as well as C-reactive protein levels. Serum Cys-C concentration and urinary protein and urinary creatinine levels were measured using several techniques: a latex immunoturbidimetric assay designed for human use (Iatro Cys-C, LSI Mediation, Tokyo, Japan); a colorimetric assay (Micro TP-AR, FUJIFILM WAKO Pure Chemical Co., Osaka, Japan) and an enzyme assay (L-type Wako Cre-M, FUJIFILM WAKO Pure Chemical Co.) with an automatic analyser (JCA-BM 2250, JEOL Co., Tokyo, Japan). Urinary specific gravity was evaluated using a clinical refractometer (MASTER-URC, ATAGO Co., Tokyo, Japan). The assay for serum Cys-C concentration has previously been validated in dogs [23].

All data are described as medians (with ranges: minima-to-maxima), and statistical analyses were performed using the analysis package R, version 3.2.1 (The R Foundation for Statistical Computing). The differences in parameters between groups were analysed using the Mann-Whitney *U*-test. The survival rate was evaluated using the Kaplan-Meier method and log-rank test.  $P<0.05$  was considered statistically significant.

We determined serum Cys-C concentrations in 394 dogs, and 130 of these dogs had clinical signs and were excluded from this study. Of the 264 asymptomatic dogs, 14 had high serum Cys-C concentrations ( $>0.55$  mg/dl) and four of these were excluded with serum Cre concentration  $>2.0$  mg/dl. Ultimately 10 dogs were included in the present study. A clinical pathology-based monitoring at hospital was accepted by owners of five dogs (CPBM group), while the owners of the other five refused such monitorings due to the absence of clinical symptoms in their animals (SBM group). The CPBM group included four females and one male, while the SBM group consisted of two females and three males. The CPBM group had two Miniature Dachshunds, a Pomeranian, a Shih Tzu and a Spitz. The SBM group included two Shetland Sheep Dogs, one Pomeranian, a Miniature Dachshund and a Yorkshire Terrier. The parameters for the dogs in the SBM and the CPBM groups are shown in Table 1. There were no significant differences in age, body weight, serum inorganic phosphate, serum Cys-C, serum UN or serum Cre concentrations between the groups at the time of detection of high serum Cys-C concentrations. Serum TP and ALB were higher in the SBM group than in CPBM group ( $P<0.05$ ).

For the evaluation of the CPBM group, we undertook a physical examination every 2 to 4 weeks, performed blood tests, urinalyses and measured blood pressure. The treatment of renal disease was initiated according to the guidelines of IRIS for canine chronic kidney diseases. Dogs in the SBM group started to receive treatment for chronic kidney disease only when the owners complained of clinical signs. High serum Cys-C concentrations were found  $45.0 \pm 23.8$  and  $168.8 \pm 105.6$  days prior to the initiation of the treatment in the CPBM and SBM groups, respectively. On the day of treatment initiation, the parameters for the dogs in the SBM and the CPBM groups are shown in Table 2. Dogs in the CPBM group were treated with fluid therapy, and ACE inhibitor (Benazepril or Enalapril maleate) and a low protein diet as needed according to the IRIS guidelines. Also, Dogs in SBM group were mainly treated with fluid therapy such as saline solution.

The follow-up period varied between 106 to 882 days (the median was 300). During the follow-up period, all dogs included in this study died from causes related to renal disease. The survival period in the CPBM group (median 441 days) was significantly longer than the survival period of the SBM group (median 262 days) ( $P<0.05$ ) (Fig. 1).

The results of this study show that the clinical pathology-based monitoring and resulting early treatment intervention in asymptomatic dogs with high concentrations of serum Cys-C extends the life of animals. It appears that the present study is the first report to show the value of early treatment intervention by CPBM of dogs with high serum Cys-C concentration.

We found that dogs in the CPBM group had a longer survival period than dogs in the SBM group. In humans, serum Cys-C concentration is a superior marker in the evaluation of kidney function and reliably predicts renal prognosis [21, 23]. High serum Cys-C concentration suggests an increased risk for subsequent mortality, even in patients with normal estimated GFR [27]. In dogs, an increased serum Cys-C concentration indicates a decrease in renal function [18, 26], and predicts a worse renal disease-specific prognosis even in dogs without clinical signs [13]. Moreover, the present study has showed that the CPBM in dogs with high serum Cys-C concentration could detect pathological change of CKD in an early stage, and start treatment before the appearance of the renal symptom.

**Table 1.** Comparison of variables according to the close monitoring group vs. the declined group

Parameters		SBM group (n=5)	CPBM group (n=5)	P
Body weight	(kg)	6.1 ± 2.1	7.9 ± 5.1	NS
Age	(years)	12.6 ± 3.8	13.2 ± 2.2	NS
Cystatin C	(mg/dl)	0.78 ± 0.20	0.76 ± 0.10	NS
Total protein	(g/dl)	7.8 ± 0.6	6.7 ± 0.5	<0.05
Albumin	(g/dl)	3.1 ± 0.2	2.7 ± 0.2	<0.05
Alkaline phosphatase	(U/l)	1,053.2 ± 1,563.2	615.4 ± 420.3	NS
Alanine aminotransferase	(U/l)	89.0 ± 17.9	63.6 ± 53.4	NS
Aspartate aminotransferase	(U/l)	35.8 ± 7.0	25.8 ± 9.4	NS
Gamma-glutamyl transpeptidase	(U/l)	7.1 ± 3.0	7.2 ± 3.8	NS
Urea nitrogen	(mg/dl)	41.7 ± 17.0	48.6 ± 17.6	NS
Creatinine	(mg/dl)	1.38 ± 0.50	1.40 ± 0.32	NS
Total bilirubin	(mg/dl)	0.14 ± 0.09	0.10 ± 0.00	NS
Triglyceride	(mg/dl)	99.0 ± 57.9	138.0 ± 63.5	NS
Total cholesterol	(mg/dl)	275.6 ± 138.7	325.0 ± 199.2	NS
Calcium	(mg/dl)	9.3 ± 2.7	10.6 ± 0.4	NS
Inorganic phosphate	(mg/dl)	4.2 ± 0.8	5.0 ± 1.7	NS
Glucose	(mg/dl)	93.8 ± 15.6	99.8 ± 10.3	NS
Lipase	(U/l)	112.4 ± 47.2	217.0 ± 150.7	NS
C-reactive protein	(mg/dl)	0.56 ± 0.40	0.50 ± 0.62	NS

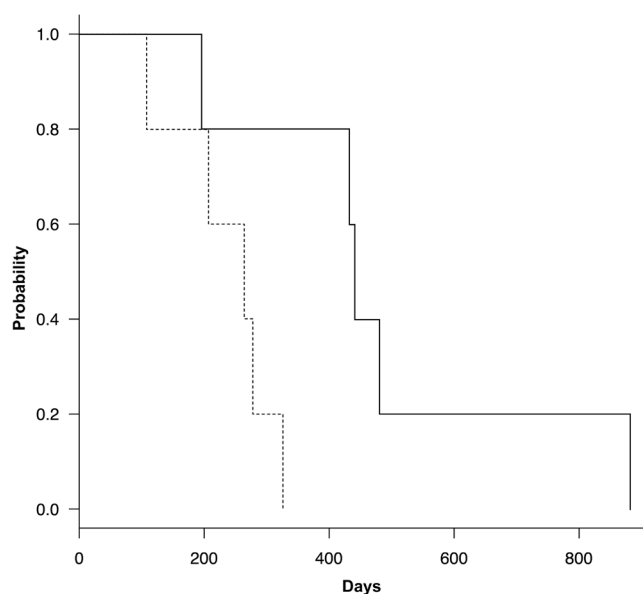
SBM, Symptom-based monitoring; CPBM, Clinical pathology-based monitoring; NS, Not significant.

**Table 2.** Comparison of variables between groups before treatment for chronic kidney disease

Parameters		SBM group	CPBM group	P
Cystatin C	(mg/dl)	1.19 (0.86–2.97)	0.86 (0.65–1.27)	NS
Urea nitrogen	(mg/dl)	129.4 (61.3–205.5)	55.1 (45–83.9)	<0.01
Creatinine	(mg/dl)	4.9 (4.0–7.0)	1.9 (1.2–2.5)	<0.01
Urine protein/creatinin ratio		2.2 (1.62–3.2)	1.1 (0.51–4.57)	NS
Urine specific gravity		1.010 (1.006–1.012)	1.012 (1.010–1.020)	NS
Blood pressure (systolic)	(mm Hg)	158 (137–206)	124 (117–129)	<0.01

SBM, Symptom-based monitoring; CPBM, Clinical pathology-based monitoring; NS, Not significant.

In veterinary studies, the serum Cre concentration is considered to be a standard renal function marker for dogs [19, 20]. However, serum Cre concentration does not increase until the GFR is reduced by 75% [3, 8]. Additionally, small-breed dogs with renal disease are less susceptible to the elevation of serum Cre concentrations because of small muscle mass [10, 17]. In humans, serum Cys-C concentration correlates with GFR better than does serum Cre concentration [16, 21], or estimated GFR using the calculated serum Cys-C concentration [12, 24]. Likewise, in veterinary studies, serum Cys-C concentration is a superior marker for the GFR than is serum Cre concentration [18, 26]. Although all dogs in the present study had high serum Cys-C concentrations, many of them nonetheless had serum Cre concentrations within the reference range (<1.6 mg/dl). These dogs were expected to have a significant decrease in renal function because high serum Cys-C concentration reflects a reduced GFR, even though serum Cre concentration levels could not detect the reduced GFR. Results from the present study suggest that the early treatment intervention by CPBM of asymptomatic dogs with normal serum Cre concentration levels could extend the prognosis of chronic kidney disease if serum Cys-C concentrations are high.



**Fig. 1.** Kaplan–Meier survival curves for the Clinical pathology-based monitoring (CPBM) group and symptom-based monitoring (SBM) group. Solid line; the CPBM group. Dashed line; the SBM group.

One of the limitations of this study was an absence of data regarding both urinalysis and symmetric dimethylarginine (SDMA). According to the IRIS CKD guidelines, an increased UPC or serum SDMA concentration might indicate the need for intensive treatment even in dogs with lower concentrations of serum Cre. If these data had been obtained, we could have analysed more information regarding the renal function of the dogs in this study. Another limitation of this study was the small sample size, being due to a rarity of asymptomatic dogs with increased concentration of serum Cys-C. Although the small sample size might diminish the power of statistical analysis for extrapolating the result to the general population, the present study provides constructive preliminary evidence. Further investigations should include a larger number of animals to help to strengthen the value of CPBM based on the concentration of serum Cys-C. Another limitation of this study was the differences in levels of serum TP and ALB between the two groups. Because higher values of these metrics might be associated with dehydration, the SBM group might originally have had ancillary factors that worsened the prognosis. However, serum TP and ALB concentrations were within the reference range in both groups, therefore the difference in these parameters was not likely to be related with the prognosis.

In conclusions, an increased concentration of serum Cys-C can reliably predict a poorer prognosis of renal disease in dogs. Fortunately, the present study demonstrated that early treatment intervention by CPBM extend the renal survival period even in asymptomatic dogs that have increased serum Cys-C concentrations. Additionally, we also found that while many dogs in the present study had serum Cre concentration that were within the reference range, they all showed increased concentration of serum Cys-C. Although this evidence is confined to small-breed dogs, the present results suggest that CPBM should be started in dogs with high serum Cys-C concentrations; even if they have no symptoms and have normal concentration of serum Cre.

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