Serum cystatin C concentration can be used to evaluate glomerular filtration rate in small dogs

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ABSTRACT. Serum cystatin C levels (CysC) are used in human medicine to document progressive kidney failure. Although CysC are not thought to be useful for the diagnosis of kidney dysfunction in dogs, there has been no specific consideration of body weight as a confounding issue. The aim of this study was to assess that the utility of CysC for the diagnosis of decreased glomerular filtration rate (GFR) in smaller *vs*. larger dogs. In clinically healthy dogs, serum creatinine (Cre) and CysC correlate directly with body weight; we found that dogs weighing <20 kg had significantly lower CysC than those weighing ≥20 kg (0.27 ± 0.07 *vs*. 0.34 ± 0.05 mg/l, respectively, *P*<0.001). In dogs weighing <20 kg, CysC had superior diagnostic accuracy for the detection of mildly decreased plasma iohexol clearance (PCio) (<1.8 ml/min/kg) compared with Cre (sensitivity 100% *vs*. 80.9% and specificity 100% *vs*. 85.7%); this was not true for dogs weighing ≥20 kg. Additionally, using a cut-off PCio of <1.8 ml/min/kg, the area under receiver-operating characteristics curve (AUC) of CysC was significantly higher than that of Cre in dogs weighing <20 kg (*P*<0.05); this was not true for dogs weighing ≥20 kg (*P*=0.695). In conclusion, CysC is a useful marker for the detection of a mild decreasing GFR compared with Cre in dogs weighing <20 kg.

KEY WORDS: chronic kidney disease, creatinine, cystatin C, dog, glomerular filtration rate

Chronic kidney disease (CKD) is defined as persistently decreasing glomerular filtration rate (GFR) and/or chronic renal damage, especially in the presence of renal proteinuria. GFR can be measured directly by urine or plasma clearance methods using inulin, creatinine or iohexol [7, 9, 14, 25, 26]. In current veterinary practice, iohexol clearance from plasma is recognized as a standard, clinical available method for assessing GFR in both dogs and cats [15]. However, this test requires sophisticated technology and cannot be used for screening in a primary care practice. As such, GFR is typically assessed indirectly using measurements of serum creatinine (Cre) and symmetric dimethylarginine (SDMA) concentrations [15]. Currently, Cre is the most common GFR biomarker, as it can be measured easily and the test is comparatively inexpensive. However, Cre are lower in elderly, small, or thin dogs, as levels of this marker are directly related to muscle mass [12, 20, 21]. There are large differences in physical size among dog breeds. As such, it is reasonable to consider the possibility that smaller and larger dogs may have different Cre. There are currently no breed- or size-specific reference ranges for Cre, and it is difficult to rely just on this measurement for diagnosis and monitoring of GFR.

Cystatin C (CysC) is low molecular weight (13 kDa) protein that is produced at a stable rate by all nucleated cells. CysC is freely filtered by the glomeruli and reabsorbed by the proximal tubule cells [1, 30]. Serum CysC concentration-based equations for estimation of GFR are used widely in human medicine [18]; compared to serum Cre levels, serum CysC concentrations do not vary substantially with respect to ethnicity, age, and sex. In veterinary medicine, several studies have also shown that serum CysC concentrations in dogs are a better marker for GFR than Cre concentrations [22, 33]. Recently, Pelander *et al*. [29] demonstrated that serum CysC concentrations were less reliable than Cre or SDMA for the detection of decreased GFR in dogs. However, it is possible that using serum CysC levels to detect decreased GFR in dogs will need to consider differences in levels secondary to body weight. Notably, Braun *et al*. [5] found that serum CysC concentrations in dogs with a body weight of >15 kg were significantly higher than those in dogs with a body weight of <15 kg. Furthermore, in our previous study, serum CysC concentrations correlated directly with body weight in a cohort of healthy dogs [22]. Furthermore, Iwasa *et al*. [16] reported that elevated serum CysC concentrations predicted a shorter survival in dogs with CKD with a body weight of <15 kg.

As such, we hypothesize that the utility of CysC for the diagnosis of decreased GFR will be revealed if smaller and larger dogs

are evaluated separately. The aim of this study was to examine both smaller and larger dogs in order to determine the utility of serum CysC measurements in the detection of decreased GFR.

MATERIALS AND METHODS

Study design and target population

This study was a cross-sectional study. This study involved 102 dogs owned by clients and included those diagnosed with CKD (n=56) as well as dogs (n=7) with suspected CKD, and clinically healthy dogs (n=39). The dogs were evaluated at the Nephrology Service of the Veterinary Medical Teaching Hospital at Nippon Veterinary and Life Science University, Tokyo, Japan. The diagnosis of CKD was based on kidney damage (urinary protein to creatinine ratio >0.5, and/or abnormal kidney size, irregular shape, or loss of corticomedullary distinction evaluated by ultrasonography) or abnormality of function (serum Cre >1.4 mg/dl or decreased GFR as estimated by plasma iohexol clearance [PCio] <1.8 ml/min/kg with isosthenuria) that persisted for >3 months. Sixty three dogs with CKD and suspected CKD underwent PCio test. In seven dogs, CKD were suspected by increased blood urea nitrogen (BUN) (n=3) and polyuria and polydipsia (n=4), but these dogs were not diagnosed with CKD based on normal PCio value, kidney structure and urinalysis findings. Dogs diagnosed with other systemic diseases, including cardiac diseases, respiratory diseases, neurological diseases, hyperadrenocorticism, hypothyroidism, and hypertension caused by extrarenal disease; those undergoing treatment with prednisolone; those with unstable CKD (as evaluated during the 3 months prior to initiation of the study); those with clinical signs such as dehydration, loss of appetite, and vomiting; and those who were obese or thin (body condition score of 1/5, 2/5, or 5/5 and/or mild to severe muscle loss using the muscle condition score system to assign a score of normal muscle mass or mild, moderate, or severe muscle loss created by World Small Animal Veterinary Association Global Nutrition Committee [10, 34]) were excluded from this study.

Data from clinically healthy dogs (n=39) who were seen at the Veterinary Medical Teaching Hospital at Nippon Veterinary and Life Science University for regular medical examinations were used to evaluate the effect of body weight on serum CysC concentrations. Dogs were considered to be clinically healthy if they had no abnormalities reported in past medical history and had normal physical examination, complete blood count, serum biochemistry, urinalysis, radiography, and ultrasonography. None of the dogs had undergone PCio test. The 39 dogs in the study were classified into three groups according to body weight: <20 kg (n=27), and ≥20 kg (n=12). The use of data from medical records has been approved by all clients.

Blood sample collection and analysis

Blood was collected via the jugular vein or cephalic vein and serum was separated within 30 min of collection. Serum levels of urea, Cre, phosphate, and calcium were determined enzymatically using an automatic analyzer (7180 Automatic Analyzer; Hitachi High-tech, Tokyo, Japan). Serum levels of CysC were measured by a latex agglutination method (Iatro Cys-C, LSI Mediation, Tokyo, Japan) [16] per manufacturer's instructions within 30 min of blood collection. This assay, which utilized a rabbit polyclonal anti-human CysC antibody, was also performed by an automatic analyzer (7180 Automatic Analyzer; Hitachi High-tech, Tokyo, Japan).

Determination of PCio

In 63 dogs with CKD and suspected CKD, PCio was performed to determine GFR as previously described [24]. All dogs were well hydrated and fasted for half a day prior to the PCio study. Iohexol (Omnipaque 300; 90 mg iodine/kg) was administered via the cephalic vein (time 0); heparinized blood was sampled at 120, 180, and 240 min. Plasma iodine concentrations were determined by the cerium arsenite colorimetric method. PCio was calculated using the 1-compartment model. The area under the curve (AUC) was estimated from the slope (a) and intercept (A) of the elimination phase as determined by linear regression analysis of the final three plasma samples. Clearance values (Cl) were calculated as Cl (ml/min)=dose of iohexol/AUC (AUC=A/a). Plasma iohexol clearance was then calculated as PCio (ml/min)=$0.990778 \times Cl - 0.001218 \times Cl^2$ [6]. PCio (ml/min) was standardized for body weight. A PCio of <1.8 ml/min/kg was considered to represent decreased GFR in the present study based on our previous study using clinical healthy dogs (mean ± 2 SD, 1.85−8.50 ml/min/kg) [24].

Measurement of blood pressure

Blood pressure was non-invasively measured by the oscillometric method using a hemomanometer (BP100D, Fukuda M-E Kogyo, Tokyo, Japan). Blood pressure measurements were based on the 2018 ACVIM guidelines [2]. Dogs were individually housed in dimly lit rooms and calmed. Blood pressure was repeatedly measured until stable values were obtained.

Statistical analysis

All statistical analysis was performed using the SPSS statistical software package (IBM SPSS Statistics ver.25, IBM Japan, Tokyo, Japan). Sixty-three dogs underwent PCio test were categorized into two groups (body weight <20 kg, n=35; body weight ≥20 kg, n=28) based on the results from healthy dogs. Normality of data was examined by the Shapiro–Wilk test. All data were normality distributed and presented as means ± SD. Pearson's correlation coefficient was used to assess the relationship between serum CysC concentration and body weight in 39 clinical healthy dogs. One-way analysis of variance and post hoc Tukey test were used to evaluate differences in serum CysC concentrations among dogs with body weights of <20 kg and ≥20 kg. Pearson's correlation coefficient was used to assess the association of PCio with the following parameters: reciprocal of Cre concentration (1/Cre), reciprocal of CysC concentration (1/CysC), body weight, age, urea, phosphate, calcium, and systolic blood pressure. Multiple regression analysis

with forward-backward stepwise selection method (entered criteria were P=0.1, and removed criteria were P=0.15) was performed to assess associations of 1/Cre and 1/CysC with each of the following variables: PCio, body weight, age, urea, phosphate, calcium, and systolic blood pressure. A PCio of <0.9 ml/min/kg, one half of cut-off value (1.8 ml/min/kg), were considered severe decreased GFR in this study. Receiver-operating characteristics (ROC) curve analysis was performed using two different cut-off values for PCio (mild and severe decrease, PCio <1.8 and <0.9 ml/min/kg, respectively) to evaluate the diagnostic accuracy of serum CysC and Cre concentrations at two different levels of reduced PCio. The AUC from the ROC analysis for each marker was compared using the method reported by Hanley and McNeil [13]. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), and negative likelihood ratio (LR−) were calculated for each marker at their cut-off values determined by the ROC analysis.

RESULTS

Serum Cre and CysC concentrations in all clinical healthy dogs were 0.79 ± 0.26 mg/dl and 0.29 ± 0.08 mg/l, respectively. Within the defined body weight categories of <20 kg and ≥20 kg, serum Cre concentrations were found 0.70 ± 0.20 and 1.0 ± 0.24 mg/dl, and serum CysC concentrations were 0.27 ± 0.07 and 0.34 ± 0.05 mg/l, respectively. Serum Cre and CysC concentrations both correlated directly with body weight (r=0.617 and 0.616, respectively, P<0.001). Dogs that weighed <20 kg had significantly lower serum Cre and CysC concentrations than dogs that weighed ≥20 kg (P<0.001; Fig. 1).

Among the dogs that weigh <20 kg (n=35; Table 1) (mean body weight, 10.2 kg; range 3.9−15.8 kg), 1/CysC was significantly associated with age (r=−0.511; P<0.01), BUN (r=0.491; P<0.01), 1/Cre (r=0.639; P<0.001), and PCio (r=0.868; P<0.001). Additionally, 1/Cre was also significantly correlated with BUN (r=0.452; P<0.01) and PCio (r=0.752; P<0.001; Fig. 2). By stepwise multivariable linear regression analysis, the final model for 1/CysC included only PCio (adjusted r^2=0.794, P<0.001) and the model for 1/Cre included only PCio (adjusted r^2=0.477, P<0.001). Dogs with decreased PCio (<1.8 ml/min/kg) had significantly higher levels of serum Cre (1.44 ± 0.53 *vs.* 0.85 ± 0.21 mg/dl) and serum CysC concentrations (0.73 ± 0.32 *vs.* 0.28 ± 0.06 mg/l) than dogs with normal GFR (P<0.001). In ROC analysis, the AUCs of CysC and Cre for the detection of a PCio of <1.8 ml/min/kg were 1.00 (95% confidence interval [CI], 1.00–1.00) and 0.88 (95% CI, 0.76–0.99), respectively, and the AUCs for detection of a PCio of <0.9 ml/min/kg were 0.94 (95% CI, 0.87–1.00) and 0.84 (95% CI, 0.66−1.00), respectively (Fig. 4). The AUC of CysC for detecting a PCio of <1.8 ml/min/kg was significantly higher than that of Cre (P<0.05). In contrast, the AUC for detecting a PCio of <0.9 ml/min/kg was not significantly different between CysC and Cre (P=0.258). The sensitivity and specificity of CysC for the detection of a PCio <1.8 ml/min/kg (100% and 100%, respectively; cut-off value, 0.39 mg/l) were higher than those of Cre (76.2% and 92.9%, respectively; cut-off value, 1.08 mg/dl) (Table 2).

In dogs weighing ≥20 kg (n=28; Table 1) (mean body weight, 30.9 kg; range, 20.0−44.3 kg), 1/CysC was associated with BUN (r=0.478; P<0.01), 1/Cre (r=0.489; P<0.001), and PCio (r=0.810; P<0.001). Additionally, 1/Cre was correlated with BUN (r=0.627; P<0.01) and PCio (r=0.651; P<0.001; Fig. 3). By stepwise multivariable linear regression analysis, the final model for 1/CysC in this group of dogs included only PCio (adjusted r^2=0.710, P<0.001) and the model for1/Cre included age and PCio (adjusted r^2=0.553, P<0.001). Dogs with a mildly decreased PCio (<1.8 ml/min/kg) had significantly higher levels of serum Cre (1.89 ± 0.80 *vs.* 0.92 ± 0.23 mg/dl) and serum CysC (0.79 ± 0.37 *vs.* 0.37 ± 0.13 mg/l) concentrations than those with a normal PCio (P<0.001). In ROC analysis, the AUCs of CysC and Cre for the detection of a PCio <1.8 ml/min/kg were 0.92 (95% CI, 0.82−1.00) and 0.89 (95% CI, 0.76−1.00), respectively, and the AUCs of CysC and Cre for the detection of a PCio of <0.9 ml/min/kg were 0.96 (95% CI, 0.90−1.00) and 0.90 (95% CI, 0.79−1.00), respectively (Fig. 4). The AUCs for the detection of a PCio of <1.8 or <0.9 ml/min/kg were not significantly different between CysC and Cre (P=0.695 and 0.276, respectively). The sensitivity and specificity of
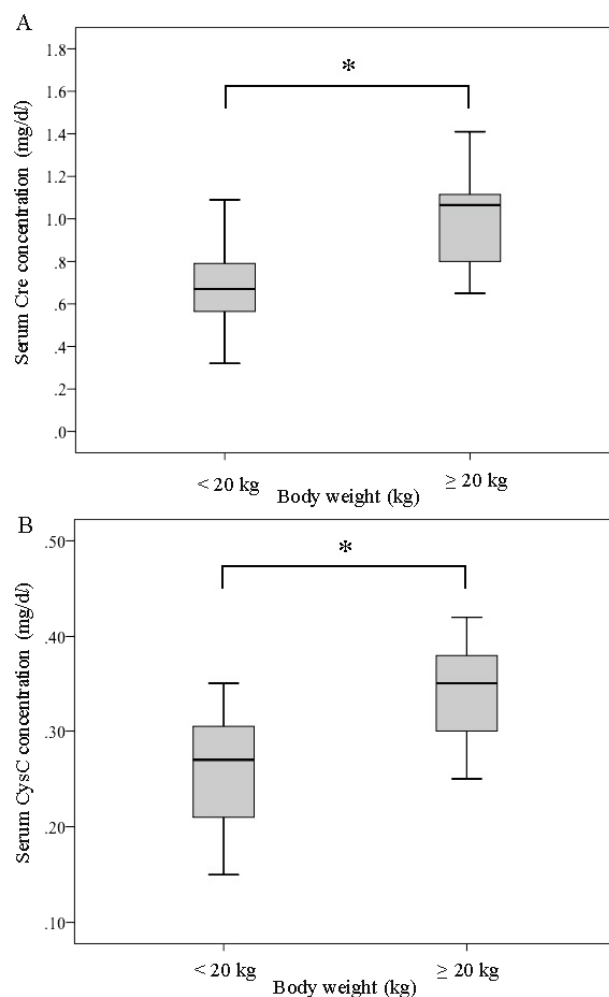
Fig. 1. Serum creatinine (Cre) (A) and cystatin C (CysC) (B) concentrations in clinically healthy dogs within each body weight group. The boxes include 50% of the serum Cre or serum CysC levels within each weight group; lines within the boxes represent the median value, and the top and bottom of each box correspond to the 75th and the 25th percentiles, respectively. The whiskers (error bars) indicate the 95% confidence interval; *P<0.001.

Table 1. Clinical and demographic characteristics

	All dogs	Dogs with body weight <20 kg	Dogs with body weight >20 kg
n	63	35	28
Age (year)	7.5 ± 4.2	7.9 ± 4.6	7.0 ± 3.7
Body weight (kg)	19.4 ± 11.5	10.2 ± 4.7	30.9 ± 5.3
Sex (n, %)			
Male	11, 17.5	8, 22.9	3, 10.7
Neuterd male	14, 22.2	7, 20.0	7, 25.0
Female	19, 30.2	10, 28.6	9, 32.1
Neuterd female	19, 30.2	10, 28.6	9, 32.1
Breeds (n, %)	Labrador Retriever (10, 15.9)	Beagle (6, 17.1)	Labrador Retriever (9, 32.1)
	Beagle (7, 11.1)	Border Collie (4, 11.4)	Golden Retriever (6, 21.4)
	Golden Retriever (7, 11.1)	Miniature Dachshund (4, 11.4)	German Shepherd (5, 17.8)
	Border Collie (4, 6.3)	Mongrel (3, 8.6)	Chesapeake Bay Retrieber (2, 7.1)
	Miniature Dachshund (4, 6.3)	Toy Poodle (2, 5.7)	Grey Hound (2, 7.1)
Urea (mg/dl)	23.5 ± 17.8	24.3 ± 19.3	22.5 ± 16.0
Creatinine (mg/dl)	1.35 ± 0.68	1.20 ± 0.52	1.54 ± 0.80
Phosphate (mg/dl)	3.5 ± 1.0	3.6 ± 1.3	3.5 ± 0.8
Calcium (mg/dl)	10.8 ± 0.8	10.6 ± 0.8	11.0 ± 0.9
Cystatin C (mg/l)	0.59 ± 0.35	0.55 ± 0.33	0.64 ± 0.37
PCio (ml/min/kg) [a]	1.67 ± 0.94	1.80 ± 0.98	1.51 ± 0.87
Systolic BP (mmHg) [b]	135 ± 22	138 ± 23	130 ± 19

Values are expressed as mean ± SD.
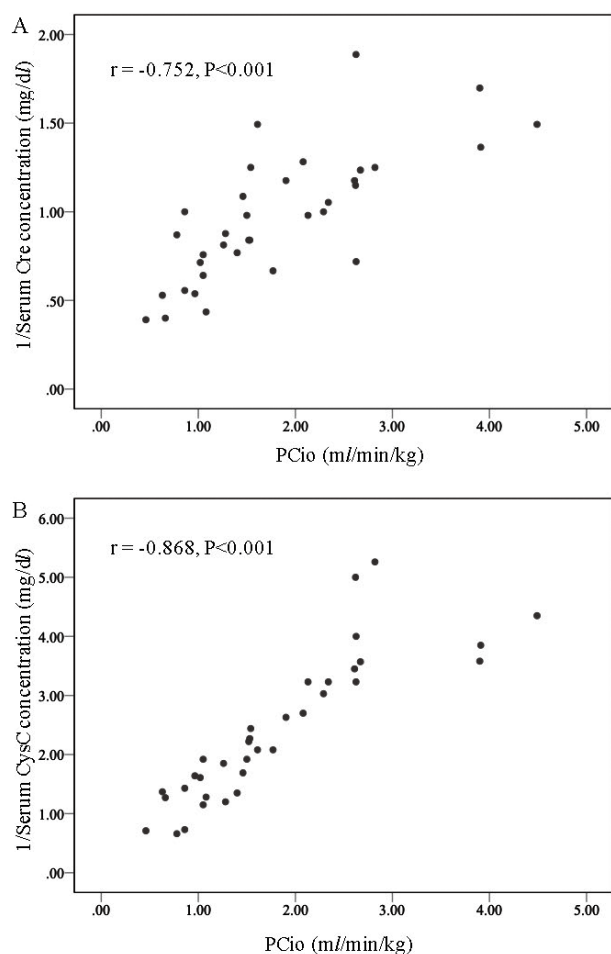
Fig. 2. The correlation between reciprocal of serum creatinine (1/Cre) (A) and reciprocal of serum cystatin C (1/CysC) (B) concentrations and plasma iohexol clearance (PCio) in dogs weighing <20 kg.
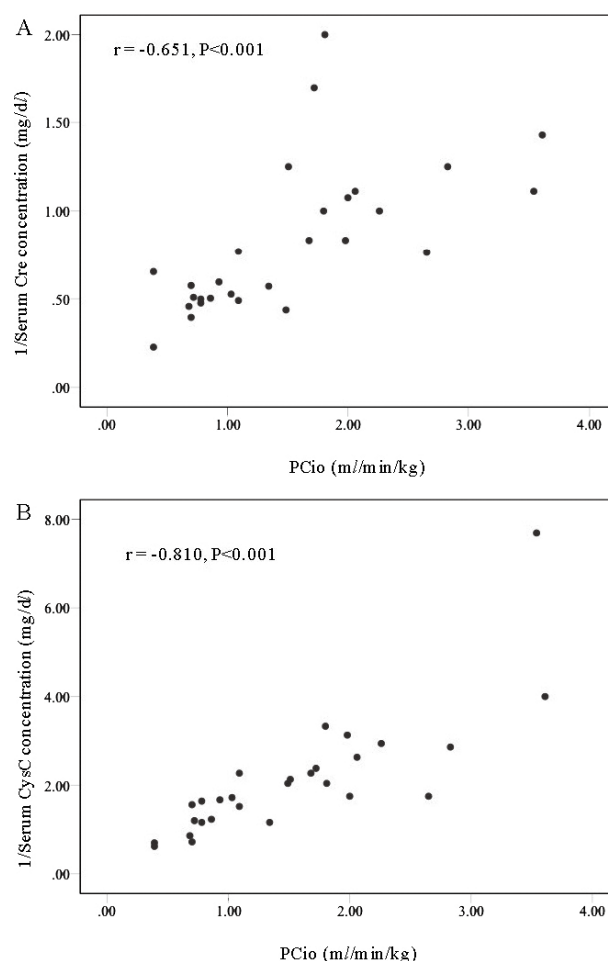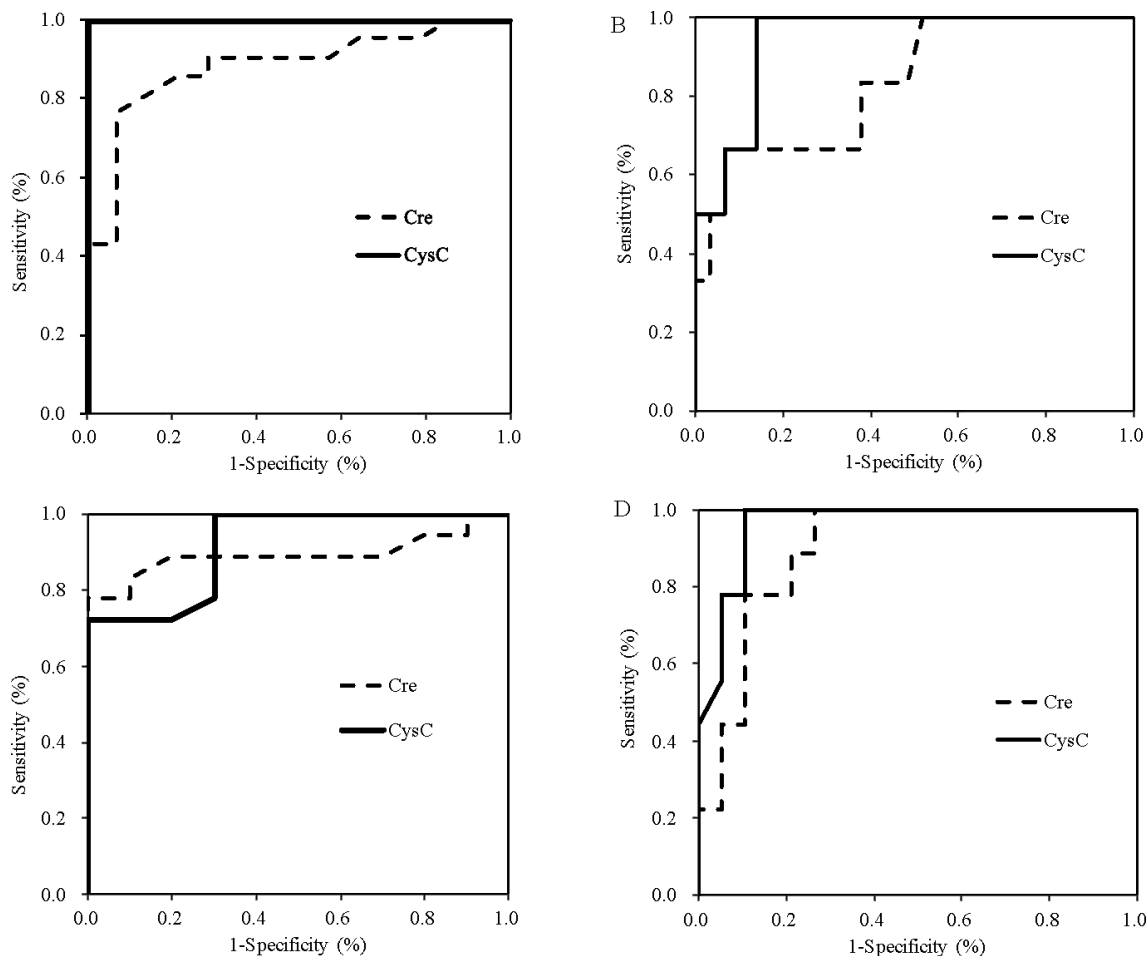

Fig. 3. The correlation between reciprocal of serum creatinine (1/Cre) (A) and reciprocal of serum cystatin C (1/CysC) (B) concentrations and plasma iohexol clearance (PCio) in dogs weighing ≥20 kg.

Fig. 4. Receiver-operating characteristic (ROC) plots of serum creatinine (Cre) and cystatin C (CysC) concentrations in dogs that weigh <20 kg (A, B) and dogs that weigh ≥20 kg (C, D) classified into those with normal and reduced plasma iohexol clearance at two different cut-off values (<1.8 ml/min/kg [A, C], and <0.9 ml/min/kg [B, D]).

Table 2. The area under curve from receiver operating characteristic, and sensitivity, specificity, predictive value, and likelihood ratio of 2 markers for detection of dcreased plasma iohexol clearance at 2 different cut-off value

PCio cut-off	Body weight >20 kg		≥ 20 kg	
	Creatinine	Cystatin C	Creatinine	Cystatin C
<1.8 ml/kg/min				
Cut-off value	1.02	0.39	1.31	0.57
AUC	0.88 (0.76–0.99)	1.00 (1.00–1.00)	0.89 (0.76–1.00)	0.92 (0.82–1.00)
Sensitivity (%)	80.9 (58.1–94.5)	100 (83.9–100.0)	77.8 (52.4–93.6)	72.2 (46.5–90.3)
Specificity (%)	85.7 (57.2–98.2)	100 (76.8–100.0)	100 (69.2–100)	80.0 (44.4–97.5)
PPV (%)	89.1 (69.9–96.8)	100 (−)	100 (−)	86.6 (64.4–95.8)
NPV (%)	75.0 (54.8–88.1)	100 (−)	71.6 (51.5–85.7)	61.7 (41.9–78.3)
LR+	5.7 (1.5–20.8)	∞	∞	3.6 (1.0–12.9)
LR-	0.2 (0.1–0.6)	0 (−)	0.2 (0.1–0.5)	0.4 (0.2–0.8)
<0.9 ml/kg/min				
Cut-off value	1.5	0.73	1.9	0.8
AUC	0.84 (0.66–1.00)	0.94 (0.87–1.00)	0.90 (0.79–1.00)	0.96 (0.90–1.00)
Sensitivity (%)	66.7 (22.3–95.7)	83.3 (35.9–99.6)	77.8 (40.0–97.2)	77.8 (40.0–97.2)
Specificity (%)	89.7 (72.7–97.8)	92.6 (75.7–99.1)	89.5 (66.9–98.7)	94.7 (74.0–99.9)
PPV	57.1 (28.4–81.7)	69.9 (36.7–90.2)	77.7 (47.4–93.1)	87.5 (72.6–96.6)
NPV	92.9 (80.7–97.6)	96.4 (81.8–99.4)	89.5 (71.3–96.7)	90.0 (72.6–96.9)
LR+	6.4 (1.9–21.6)	11.3 (2.8–44.8)	7.4 (1.9–28.7)	14.8 (2.1–102.8)
LR-	0.4 (0.1–1.2)	0.2 (0.0–1.1)	0.3 (0.1–0.9)	0.2 (0.1–0.8)

Values are given with 95% confidence interval (CI) in parenthesis: PCio; plasma iohexol clearance, AUC; area under the curve, PPV; positive predictive value, NPV; negative predictive value, LR-, negative likelihood ratio; LR+, positive likelihood ratio.

serum CysC were not superior to those of serum Cre for the detection of decreased PCio at two different cut-off values (Table 2). The AUC for the detection of a PCio of <0.9 ml/min/kg was not significantly different between CysC and Cre (*P*=0.258).

DISCUSSION

Glomerular filtration rate is a critical factor for diagnosis of and evaluation of progression in CKD. Serum Cre concentrations, the most commonly used indirect marker of GFR is affected by muscle mass and may be different depending on the age, body weight, physical size, and breed of a given dog [12, 20, 23]. As such, the diagnostic accuracy of the serum Cre concentration is not sufficient for a screening test for CKD. However, serum Cre concentrations are more stable within a single individual than are measurements of SDMA and CysC [19, 28]. Serum CysC concentrations can be increased by various extrarenal factors, including administration of prednisolone [27], hyperadrenocorticism [21]. In our study, serum CysC concentrations correlated directly with body weight; dogs that weighed <20 kg had significantly lower levels of serum Cre and serum CysC than did dogs that weighed ≥20 kg. By contrast, body weight and muscle mass has no impact on serum CysC concentration in human subjects [4, 32]. This may relate to the fact that there is a very large range of physical size among different dog breeds, much more so that is typically detected among healthy human subjects. Previous studies suggested that serum CysC concentration was not superior to serum Cre in its utility for the detection of decreased GFR in dogs [3, 29]. One explanation for this finding might be the effect of body weight on serum CysC concentrations. In humans, serum CysC concentration is not associated with muscle mass but correlates with fat mass [8]. One study in obese dogs reported that serum CysC concentrations were significantly decreased after weight loss [31]. Thus, the utility of serum CysC concentration for the detection of decreased GFR is different between thin and obese dogs. The association between serum CysC concentration and body weight is unknown because dogs defined as obese based on the body condition and muscle condition scores were excluded in this study. The lower serum CysC concentrations observed in smaller dogs might be owing to a low number of nucleated cells producing CysC, which could not be addressed in the present study. However, our findings clearly demonstrated that serum CysC concentration is a useful marker for the detection of mildly decreased GFR in dogs weighing <20 kg, compared with serum Cre concentration.

In dogs that weighed ≥20 kg, serum CysC concentration did not exhibit superior utility to serum Cre concentration for the detection of decreased PCio at two cut-off values. The range of weights among the dogs in the ≥20kg group was quite wide (from 20.0 to 44.3 kg) compared to the weight range of dogs in the <20 kg group (range 3.9 to 15.8 kg); the degree to which body weights vary within the two groups may have an impact on determining the diagnostic accuracy of serum CysC levels. Dogs with lower weights have reduced serum Cre and CysC concentrations compared to heavier dogs [31]; as such, dogs that were unusually thin and those that had lost weight immediately prior to the study were excluded from consideration. However, we may have included some dogs who had recently undergone some loss of muscle mass as we did not evaluate lean body weight in our study cohorts.

We evaluated serum CysC concentrations using a latex agglutination method that generated a lower value (0.29 ± 0.08 mg/l in healthy dogs) than that determined by ELISA (0.85 ± 0.15 mg/l) [22] or by a polystyrene particle-enhanced turbidometric assay (0.68−1.6 mg/l) [33]; however, our values were similar to those measured with a particle-enhanced nephelometric immunoassay (0.25 ± 0.15 mg/l) [17]. The latex agglutination method, which can rapidly determine CysC concentrations, is widely use in human medicine. The cause of differences in CysC concentration based on different measurement methods is not unclear. A standard measurement method for determining serum levels of CysC will be needed to establish its use in veterinary practice.

In this study, we excluded dogs that had been treated with prednisolone [27] or diagnosed with hyperadrenocorticism [21] or thyroid disease [11, 34]; likewise, we excluded dogs that were obese, overly thin, or had a history of weight loss prior to the study initiation [31]. The elimination of these confounders increased overall probability of detection of CysC in association with decreased PCio. In other words, the clinical use of serum CysC concentration should be considered after the exclusion of these extrarenal factors. Nonetheless, evaluation of decreased GFR using biomarkers should be considered as a critical goal; in reality, all GFR markers may ultimately be affected by numerous variables (e.g., body weight, muscle mass, dog breed); indeed, the actual direct measure of baseline GFR can vary based on age, hydration status, and dietary intake. We believe that this study represents an important first step toward the recognition of serum CysC levels as an indirect biomarker for CKD and for monitoring GFR at least among smaller dog breeds.

In conclusion, serum CysC concentrations are the useful indirect markers for determining changes in GFR, notably among dogs that weigh <20 kg. Definition of a reference range and/or cut-off values for dogs of different weights and breeds will be needed in order to use this information to assess kidney function in dogs.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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