

Cystatin C: A New Renal Marker and Its Potential Use in Small Animal Medicine

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The occurrence of chronic kidney disease is underestimated in both human and veterinary medicine. Glomerular filtration rate (GFR) is considered the gold standard for evaluating kidney function. However, GFR assessment is time-consuming and labor-intensive and therefore not routinely used in practice. The commonly used indirect GFR markers, serum creatinine (sCr) and urea, are not sufficiently sensitive or specific to detect early renal dysfunction. Serum cystatin C (sCysC), a proteinase inhibitor, has most of the properties required for an endogenous GFR marker. In human medicine, numerous studies have evaluated its potential use as a GFR marker in several populations. In veterinary medicine, this marker is gaining interest. The measurement is easy, which makes it an interesting parameter for clinical use. This review summarizes current knowledge about cystatin C (CysC) in humans, dogs, and cats, including its history, assays, relationship with GFR, and biological and clinical variations in both human and veterinary medicine.

Key words: Cat; Dog; Glomerular filtration rate; Human; Renal diseases.

Chronic kidney disease (CKD) is common not only in humans, with an overall prevalence of 13%,¹ but also in veterinary medicine. The estimated prevalence of CKD is between 0.5 and 7% in dogs, between 1.6 and 20% in the general cat population, and approximately 30% in geriatric cats.^{2,3} Chronic kidney disease is progressive and irreversible. Early detection and treatment is of great importance and may increase median survival time by preventing or delaying additional renal damage.^{4,5} Direct measurement of GFR is considered the best overall index for evaluating kidney function.⁶ However, this procedure is labor-intensive and time-consuming, making it an inappropriate method for routine use in daily practice.⁷

Indirect markers of GFR, sCr and blood urea nitrogen (BUN) concentration, can easily be measured and are widely available. Their serum concentrations increase when approximately 75% of the functional renal mass is lost.⁶ These markers, especially BUN, are influenced by nonrenal factors, such as age, diet, hydration status, and muscle mass.⁶ Cystatin C is a low molecular weight (LMW) 13 kilodalton (kDa) protein and proteinase inhibitor involved in intracellular protein catabolism that is produced at a constant rate because it is encoded by a housekeeping gene.⁸ Studies in rats have shown that there is no plasma protein binding, which

Abbreviations:

⁵¹ Cr-EDTA	chromium ethylenediamine tetraacetic acid
^{99m} Tc-DTPA	diethylenetriamine pentaacetic acid
AKI	acute kidney injury
BUN	blood urea nitrogen
CKD	chronic kidney disease
Cr	creatinine
CV	coefficient of variation
CysC	cystatin C
Da	dalton
DM	diabetes mellitus
GFR	glomerular filtration rate
LMW	low molecular weight
PENIA	particle-enhanced nephelometric immuno-assay
PETIA	particle-enhanced turbidimetric immuno-assay
sCr	serum creatinine
sCysC	serum cystatin C
uCr	urinary creatinine
uCysC	urinary cystatin C

allows glomerular filtration without restriction.^{9,10} Cystatin C is reabsorbed in the proximal tubules by megalin-mediated endocytosis and is completely catabolized.¹¹ It is generally accepted that no tubular secretion of CysC occurs.^{10,12} Cystatin C has many properties that are ideal for endogenous GFR marker applications, such as constant production and plasma concentration in the absence of GFR variation, low intraindividual variability, no plasma protein binding, no tubular secretion, no tubular reabsorption without catabolism, and no extrarenal clearance.¹² Cystatin C is considered superior to sCr in detecting renal dysfunction in humans.¹³ Furthermore, urinary CysC (uCysC) concentrations are extremely low in healthy individuals compared with individuals with renal tubular damage.^{14,15} Therefore, uCysC can be used as a marker for proximal tubular damage.

This review provides more information regarding the use of CysC in human medicine, available assays, biological and clinical variation, and its potential use in veterinary medicine.

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History

In the early 1960s, a new protein was discovered in normal human cerebrospinal fluid¹⁶ and in the urine of patients with proteinuria.¹⁷ The highest concentration of this protein was measured in cerebrospinal fluid, followed by plasma, saliva, and urine,¹⁸ which suggested production in the central nervous system and catabolism by the kidney.^{19–21} The single polypeptide chain contained 120 amino acids, and the molecular mass was 13.260 kDa.¹⁹ Abrahamson et al observed expression in every examined tissue, including kidney, liver, pancreas, intestine, stomach, lung, placenta, seminal vesicles, and parotid salivary gland.⁸ Because of similar activity as cystatin A and B, this new protein was named cystatin C.²² These cystatins inhibit the activity of cysteine proteinases and therefore protect host tissue against destructive proteolysis.²³

Cystatin C in serum was investigated as a potential marker for GFR because a better correlation was observed between the reciprocal of CysC and GFR compared with the serum concentrations of other LMW proteins such as beta-2 microglobulin, retinol-binding protein, and factor D.^{24,25}

Assays

Human Medicine

In 1994, a fully automated particle-enhanced turbidimetric immuno-assay (PETIA)^a for CysC was developed and validated in serum²⁶ and urine.²⁷ A few years later, a particle-enhanced nephelometric immuno-assay (PENIA)^b was validated in serum²⁸ and urine.²⁹ Concentrations of CysC measured in serum using PENIA showed good correlation with those obtained with the PETIA.^{28,30,31} However, this correlation was not observed above concentrations of 2 mg/L, with the PETIA yielding lower concentrations.³²

Both turbidimetry and nephelometry are based on the dispersion of light caused by immune complexes formed by CysC and latex particles coated with polyclonal antibodies. In the turbidimetric assay, the

particles are polystyrene particles that are 38 nm in diameter,²⁶ and in the nephelometric assay, the particles are chloromethylstyrene particles that are 80 nm in diameter.³³ Both assays use polyclonal rabbit anti-human CysC antibodies.

The major difference between these 2 methods is that PENIA^b can only be used with a specialized automated immunonephelometer, whereas PETIA^a can be used with several analyzers, including the Cobas Fara analyzer,^{c,34} Hitachi analyzer,^{d,35} Cobas 6000 analyzer,^{e,36} and Abbott Architect ci8200.^{f,37} Newer devices are available but are limited for veterinary use because of high cost. No interferences of triglycerides (≤ 8.5 mmol/L), bilirubin (≤ 150 μ mol/L), hemoglobin (≤ 1.2 g/L), or rheumatoid factors ($< 3,230$ kIU/L) were observed for PETIA.^{a,26} PENIA^b showed even less interference.³³

Similar to creatinine (Cr), standardization has been accomplished, and certified reference material (ERM-DA471/IFCC) is available for both PENIA^b and PETIA^a analyzers and enzyme-amplified single radial immunodiffusion.^{38–41}

Veterinary Medicine

Currently, veterinary assays for measurement of CysC are not available. Therefore, results in animals obtained using the assays designed for humans do not reflect exact CysC concentrations. An amino acid sequence homology of approximately 70% between human and feline CysC has been reported.^{42,43} In dogs, homology between 46 and 79% has been reported,⁴⁴ but others have reported a maximum and minimum amino acid sequence homology of 63 and 22%, respectively.⁴³ Cystatin C was first demonstrated in canine amyloid plaques.⁴⁵ This finding was of major importance because the authors demonstrated cross-reactivity between the rabbit antihuman CysC antibody from human PETIA^a and canine CysC present in cerebrospinal fluid. Based on those findings and studies in humans, Jensen et al⁴⁶ performed the first validation study using PETIA^a to measure sCysC in dogs (Table 1).

Table 1. Validation parameters of human CysC assays in veterinary medicine.

Species	Authors	Assay-Analyzer	Samples	Interassay CV (%)
Dog	Jensen et al ⁴⁶	PETIA (Cobas Fara II, Hoffman-La Roche, Switzerland)	Low sCysC (< 1.1 mg/L)	9.6
			Medium sCysC (1–2 mg/L)	5.9
			High sCysC (> 2 mg/L)	1.7
Dog	Almy et al ⁴⁸	PETIA (Hitachi 912, Roche)	Low sCysC	4.7
			Medium sCysC	4.7
			High sCysC	2.9
Dog	Wehner et al ⁵¹	PETIA (Hitachi 911, Roche, Germany)	High sCr	2.9
			Normal sCr	3.6
Cat	Ghys et al ⁵⁴	PENIA	Serum	12.5
			Urine	4.1

CysC, cystatin C; CV, coefficient of variation; PETIA; particle-enhanced turbidimetric immuno-assay; sCr, serum creatinine; PENIA, particle-enhanced nephelometric immuno-assay.

Several other authors also have measured sCysC with PETIA^a in healthy dogs and in dogs with renal failure.^{47–51} Cross-reactivity between sCysC and the polyclonal rabbit antihuman CysC antibody by western blotting was only shown in 1 report,⁴⁸ and analytical validation parameters were sufficient for PETIA.^{a,46,48,51} PETIA^a also was validated for measurement of canine urinary CysC.⁵² Miyagawa et al⁵³ also measured canine sCysC with a noncommercially available ELISA using the same antibody from PETIA,^a but this technique is not suitable for everyday practice. PENIA^b recently was validated in feline serum and urine,⁵⁴ with acceptable validation parameters (Table 1).⁵⁵ Jonkisz et al⁵⁶ observed significantly different results for serum CysC as measured by PENIA^b among dogs of all International Renal Interest Society (IRIS) stages, which was not observed with PETIA.^a Based on those findings, the authors suggested that PENIA^b is more precise. In our opinion, parallel validation of both PENIA^b and PETIA^a and correlation with GFR measurements are necessary to determine which assay is most appropriate for veterinary use.

Nakata et al⁴² developed recombinant feline CysC in *Escherichia coli* and 3 monoclonal antibodies against the protein. These antibodies also were able to recognize native feline CysC. These authors aimed to design a sensitive and specific sandwich ELISA to detect feline CysC, but this assay is not yet available.

CysC and GFR

The best method to evaluate kidney function is measurement of the renal clearance of a substance that is freely filtered but not reabsorbed, secreted, or catabolized.⁵⁷ Exogenous markers can be used, and GFR is calculated by measuring their concentration in plasma or urine. Inulin is considered the gold standard,⁵⁸ but isotopically labelled compounds are frequently used, including iothalamate,⁵⁹ iodothalamate,⁶⁰ chromium ethylenediamine tetraacetic acid (⁵¹Cr-EDTA),⁶¹ and technetium diethylenetriamine pentaacetic acid (^{99m}Tc-DTPA).⁶² The contrast agent iohexol also has become a commonly used marker.⁶³ Endogenous and exogenous Cr clearance tests also can be used by measuring Cr concentrations in blood and urine.^{64,65} These clearance tests are time-consuming and labor-intensive. Therefore, the measurement in plasma of indirect markers of GFR, BUN, and sCr is routinely used to estimate GFR. However, they are influenced by muscle mass, age, feeding status, sex,⁶⁶ and intraindividual variation.⁶⁷ In addition, tubular secretion of Cr occurs in humans, which leads to an overestimation of GFR based on sCr in patients with a moderate-to-severe decrease in GFR.⁶⁸ Urea is reabsorbed from the tubules, and this occurs to a greater extent at slow tubular flow rates. Therefore, BUN is not a reliable indicator of GFR.⁵⁷ Furthermore, production and excretion of urea is not constant.⁶⁹ Serum Cr often is used as a more reliable measure of GFR than BUN in patients with CKD.⁷⁰

Human Medicine

Several studies in humans have shown that the reciprocal of sCysC correlates more closely with GFR, as measured by exogenous clearance tests, than the reciprocal of sCr (Table 2). In addition, no significant correlation was observed between the reciprocal of sCr and GFR in patients with normal GFR, whereas the correlation with the reciprocal CysC concentration extended to the entire GFR range and remained significant.²⁶ However, the correlation between GFR and the reciprocal of sCysC is weak in healthy individuals.⁷¹

The sensitivity and specificity of the 2 variables were compared by receiver operating curve (ROC) analysis, and sCysC had higher sensitivity and negative predictive value in detecting a decreased Cr clearance as compared with sCr.³³ Serum CysC concentration began to increase when the GFR decreased, whereas sCr did not change.^{33,72}

In human medicine, equation formulas were developed in patients with CKD and are commonly used to estimate GFR based on sCr^{67,73–78} or sCysC.^{79–82} Equation formulas based on sCysC provided a more accurate and precise GFR estimate than those obtained with sCr concentration⁷⁹ and did not underestimate measured GFR.⁸¹ However, an equation including both plasma Cr and sCysC provided better results than all of the other equations, especially in patients with early-stage renal impairment.^{83,84}

In humans, sCysC has larger intraindividual variation and smaller interindividual variation compared with sCr, which leads to a higher critical difference for the comparison of sequential serum concentrations for

Table 2. Correlation data for comparisons between the reciprocal of sCysC or sCr and exogenous marker clearance in humans.

Author	Clearance Technique	Correlation Coefficient (r)	
		sCysC	Cr
Grubb et al ²⁴	⁵¹ Cr-EDTA	0.77	0.75
Simonsen et al ²⁵	⁵¹ Cr-EDTA	0.75	0.73
Kyhse-Andersen ²⁶	Iohexol	0.87	0.73
Newman et al ³³	⁵¹ Cr-EDTA	0.81	0.50
Bökenkamp et al ¹⁷⁷	Inulin	0.88	0.72
Randers et al ⁹⁸	⁹⁹ Tc-DTPA	0.87	0.81
Risch et al ⁹⁹	[¹²⁵ I]iodothalamate	0.83	0.67
Stickle et al ¹⁰⁰	Inulin	0.77	0.84
		(4–12 years)	(4–12 years)
		0.87	0.89
		(12–19 years)	(12–19 years)
Nitta et al ⁹⁷	Inulin	0.84	0.72

sCysC, serum Cystatin C; sCr, serum creatinine; Cr, creatinine; ⁵¹Cr-EDTA, 51-chromium-labeled ethylenediamine tetra-acetic acid; ⁹⁹Tc-DTPA, 99-metastable-technetium-labeled diethylenetriamine penta-acetic acid.

CysC.⁸⁵ These findings lead to the assumption that sCysC is better as a screening test for decreased GFR and that sCr is better for monitoring changes in established renal disease.⁸⁶ Serum CysC showed no advantages over sCr in patients with advanced CKD.^{86,87} In addition, in the general healthy population, GFR equations based on CysC were not superior compared with those based on Cr.⁸⁸ Authors have attributed the large bias of GFR equations to the fact that the equations were developed in populations with CKD and low GFR because nonrenal factors may differ between patients with CKD and healthy individuals.⁸⁸ Nonrenal elimination and lack of CysC measurement standardization may contribute to the observed differences.⁸⁶ Therefore, in human medicine, sCysC is used as an additional marker for GFR evaluation without replacing sCr.

Veterinary Medicine

Cystatin C has been evaluated as an endogenous indirect marker for GFR in dogs.^{48,51,53} Dogs with CKD had significantly higher CysC concentrations compared with healthy dogs^{48,49,53} and dogs with various nonrenal diseases (immune-mediated, endocrine, dermatologic, cardiologic, neoplastic)^{46,47,50,51,89}

(Table 3). There was overlap in sCysC concentrations between dogs with nonrenal disease and healthy dogs^{46,53,89} and between healthy dogs and dogs with CKD.^{47,50,53} These results indicate that, currently, sCysC is not a good marker for kidney damage. However, no GFR measurement was performed. Thus, early kidney impairment in healthy dogs or dogs with nonrenal diseases cannot be excluded.

Furthermore, very limited information is available for dogs with clinical signs of CKD but without azotemia. In 1 study, plasma CysC was increased in only 1 of 7 dogs that met those criteria.⁴⁷ No clearance test was performed in that dog, and thus it remains unclear whether or not GFR was decreased.⁴⁷ In a remnant kidney model in young adult Beagle dogs, correlation with GFR was better for the reciprocal of CysC ($r = 0.79$) than sCr ($r = 0.54$) in the first week after the procedure, when GFR was lowest (0.50 ± 0.15 mL/min/kg). At 10 weeks after the procedure, when GFR was higher (1.00 ± 0.27 mL/min/kg) but still below the reference interval (3.50–4.50 mL/min/kg),⁴⁸ equal correlation was observed for sCysC and sCr.⁴⁸ The authors hypothesized that the equal correlation of sCysC and sCr with increasing GFR was caused by a difference in inter- and intraindividual variation. The inter- and intraindividual variation for sCysC and sCr

Table 3. Overview of studies evaluating the use of sCysC in small animal medicine. Serum CysC (mg/L) was expressed as the mean \pm SD, median sCysC or (range).

Species	Authors	Status	Age (years)	n	sCysC
Dog	Jensen et al ⁴⁶	Healthy (sCr <130 μ mol/L)	1–9	17	1.06 (0.4–1.38)
		Nonrenal disease (sCr <130 μ mol/L)	0.5–13	12	1.62 (0.4–2.24)
		CKD (sCr >130 μ mol/L)	0.5–9	8	5.01 (3.39–7.35)
Dog	Almy et al ⁴⁸	Healthy (sCr <141.4 μ mol/L)	Adult	25	1.08 \pm 0.16
		CKD (sCr <141.4 μ mol/L)	Adult	25	4.37 \pm 1.79
Dog	Braun et al ⁴⁷	Healthy	0.16–16.5	179	0.60 \pm 0.31
		CKD (sCr >133 μ mol/L)		27	(0–8.6)
		Signs of CKD, no azotemia		7	(0.2–1.2)
		Azotemia, no signs of CKD		13	(0–1.2)
Dog	Wehner et al ⁵¹	Healthy (sCr 55.31–108.5 μ mol/L)	0.25–13	99	(0.68–1.6)
Dog	Gonul et al ⁴⁹	Reduced ECPC (<3 mL/min/kg)	0.5–15	15	>1.6
		Healthy (sCr 69.8 \pm 22.1 μ mol/L)	1–9	10	1.2 \pm 0.42
Dog	Miyagawa et al ⁵³	CKD (sCr 588.7 \pm 373 μ mol/L)	2–13.5	20	2.96 \pm 1.09
		Healthy dogs (EIPC >30 mL/min/m ²)		76	0.85 \pm 0.15
		CKD (EIPC <30 mL/min/m ²)		88	1.23 \pm 0.21
		Neoplasia		5	0.93 \pm 0.13
Cat	Martin et al ⁹²	Congestive heart disease		5	0.80 \pm 0.12
		Healthy (PCr <229 μ mol/L)		99	1.60 (0.19–4.37)
		Signs of CKD and azotemia		75	2.64 (0.35–9.52)
		Signs of CKD, no azotemia		35	1.595 (0.4–4.36)
Cat	Poświatowska-Kaszczyszyn ⁹⁴	Azotemia, no signs of CKD		24	1.74 (0.69–3.48)
		Healthy (EIPC 2.4 \pm 0.8 mL/min/kg)		24	0.7 \pm 0.2
		CKD (EIPC 1.2 \pm 0.7 mL/min/kg)		46	1.3 \pm 0.6
		IRIS I		16	1.1 \pm 0.3
Cat	Ghys et al ⁵⁴	IRIS II		16	10 \pm 0.5
		IRIS III		6	14 \pm 0.3
		IRIS IV		8	1.25 \pm 0.6
		Healthy (sCr <141.4 μ mol/L)	1.8–19	10	0.79 (0.43–1.05)
Cat		CKD		10	1.24 (0.63–2.99)

sCysC, serum Cystatin C; SD, standard deviation; CKD, chronic kidney disease; sCr, serum Creatinine; ECPC, exogenous creatinine plasma clearance; EIPC, exogenous iohexol plasma clearance; PCr; plasma creatinine; IRIS, International Renal Interest Society.

was investigated in the dog by calculating the index of individuality (IoI) determined by the analytical, inter-individual and intraindividual coefficient of variation.⁹⁰ For parameters with a low IoI, the repeat test results will be similar to the first result and will not provide new information.⁹¹ If parameters have a high IoI, the ratio of true positives/false positives will increase.⁹¹ In humans, this explains the higher sensitivity of sCysC (high IoI) in detecting renal impairment, but in dogs, sCysC and sCr showed comparable IoI.⁹⁰ However, the authors attributed the difference in IoI of sCysC and sCr between humans and dogs to different storage times, different food, different physical activity index, and different breeds, which require further investigation.⁹⁰

A higher sensitivity of sCysC (76%) than sCr (65%) and comparable specificity (87% for sCysC and 91% for sCr) for detecting decreased GFR (<3.0 mL/min/kg), as measured by an exogenous Cr clearance test, was observed in dogs by Wehner et al.⁵¹ In this study, dogs with normal GFR (≥ 3 mL/kg/min; $n = 23$), slightly decreased GFR (2.00–2.99 mL/min/kg; $n = 22$), and markedly decreased GFR (<1.99 mL/min/kg; $n = 15$) were included. Cystatin C and sCr had comparable positive predictive values, but sCysC had higher negative predictive value (69%) compared with Cr (62%) for detecting early CKD.⁵¹ There was a slightly better negative correlation between sCysC ($r = -0.630$) and exogenous Cr clearance compared with sCr ($r = -0.572$).⁵¹ There was also a better correlation between sCysC ($r = -0.704$) and plasma iohexol clearance compared with sCr ($r = -0.598$). In that study, 88 dogs with CKD and 43 healthy control dogs were included.⁵³

In cats, CysC was evaluated in 3 reports, and contradictory results were observed. Martin et al.⁹² concluded that plasma CysC was not a valuable marker for the detection of renal impairment because only 14 of the 75 cats that had clinical signs of CKD and azotemia had CysC concentrations above the upper reference limit of 4.11 mg/L, which was determined by the authors. However, group allocation in this study did not take into account IRIS guidelines, and the reference interval was not calculated according to the American Society of Clinical Veterinary Pathology guidelines.⁹³ In contrast, a significant difference in sCysC and uCysC (uCysC/uCr) ratios between healthy cats and cats with CKD was found by our group.⁵⁴ One possible explanation for this result could be the use of different assays and the measurement of plasma CysC and sCysC. Until now, GFR has only been measured in 1 report on feline CysC; Poświatowska-Kaszczyszyn⁹⁴ found a significantly better correlation between GFR and sCysC ($r = -0.51$) than between GFR and sCr ($r = -0.46$), which is comparable to findings in humans^{24–26,33,95–100} and dogs.^{48,51,53} An interesting and common finding in all 3 studies is the overlap in sCysC concentrations between healthy cats and cats with CKD. In Ghys et al.⁵⁴ and Martin et al.,⁹² no GFR measurement was performed; thus, early kidney impairment in the healthy cats cannot be excluded. In the study of Poświatowska-Kaszczyszyn,⁹⁴

GFR was calculated, and GFR also was found to overlap between healthy cats and cats with CKD, potentially explaining the overlap of sCysC for both groups. However, this study lacked information on urine specific gravity (USG) and used the 1-compartment model for GFR calculation. It is generally accepted that 1-compartment models may overestimate true GFR,¹⁰¹ which recently was confirmed by Finch et al.¹⁰² Thus, correlation between sCysC and GFR should be further investigated.

Urinary CysC

Human Medicine

Cystatin C is freely filtered through the glomerulus, reabsorbed, and catabolized in the tubules, as has been shown in rats.^{10,103} With normal renal function, CysC can be found in small quantities in the urine.¹⁸ With proximal tubular damage, uCysC increases.^{14,15} Urinary CysC was higher in human patients with renal tubular damage compared with patients with proteinuria without tubular damage and a healthy control group.^{15,104,105} Urinary CysC might be more sensitive than other LMW proteins, such as α_1 -microglobulin and β_2 -microglobulin, because uCysC showed the highest correlation coefficient with sCr.¹⁰⁶ However, it is mandatory to measure total proteinuria because massive proteinuria has been shown to inhibit tubular reabsorption of CysC in experimentally induced nephropathies¹⁰⁷ and in children with idiopathic nephropathy,¹⁰⁸ causing higher uCysC concentrations and therefore underestimating tubular function.

Small Animal Medicine

To the authors' knowledge, only 1 report has validated PETIA^a for measuring canine uCysC in healthy dogs, dogs with renal impairment and dogs with nonrenal disease.⁵² The assay was linear and precise, and the uCysC/uCr ratio was significantly higher in dogs with renal disease compared with healthy dogs and dogs with nonrenal disease. In cats, PENIA^b was validated for measuring feline uCysC, and a significant difference in uCysC/uCr ratio between healthy cats and cats with CKD was observed.⁵⁴ Although the results for uCysC seem promising in both dogs and cats, additional studies are required. First, uCysC has not yet been investigated as a marker of early renal damage. Second, canine and feline purified CysC were not available, and therefore, the accuracy of the method could not be evaluated. Third, follow-up to evaluate uCysC/uCr as a prognostic marker was not performed. In addition, the effect of proteinuria on uCysC concentration was not investigated.

Biological Variations of CysC

Human Medicine

Age and Sex. Because the estimation of renal function by sCr requires adjustment for height and body

composition, sCysC was studied as an alternative marker for GFR in children and the elderly. Serum CysC showed diagnostic superiority over sCr as a marker for decreased GFR in the pediatric population,⁹⁶ and the CysC-based GFR equation was better than the Schwartz formula,^{109–111} except in individuals >60 years old.¹¹² The superiority of CysC and more common use of an enzymatic assay instead of the Jaffe method to measure Cr resulted in a new Schwartz formula.⁷⁸ Interestingly, several studies have shown that sCysC was high during the first days of life, rapidly declined during the first 4 months, and then stayed constant beyond the first year of life.^{113,114} In contrast, sCr falls to a nadir at 4 months and gradually increases to adult concentrations by 15–17 years of age.¹¹⁵ The decrease in both parameters during the first year can be explained by developing renal function, which causes an increase in GFR. The increase in sCr beyond the first year of life is mainly attributable to increasing muscle mass and body weight,¹¹⁵ in contrast with sCysC, which is not correlated with muscle mass.^{71,116} In an adult population, increasing age, male sex, greater weight, greater height, cigarette smoking, and higher C-reactive protein concentrations were independently associated with higher sCysC concentration before¹¹⁷ and after adjusting for age, sex, and weight of individuals for whom GFR was estimated by a urinary Cr clearance test.¹¹⁸ The latter indicates that these factors may influence sCysC independent of their effects on renal function. However, others have observed no difference between healthy male and female individuals.^{119,120}

Serum CysC concentrations were significantly higher in individuals >80 years of age compared with individuals between 65 and 80 years of age, which corresponds to the inverse change in the predicted Cr clearance.¹²¹ However, no benefit was found for sCysC compared with sCr in detecting early renal impairment.¹²²

Interindividual Variation. A larger intraindividual variation has been reported for CysC compared with sCr in healthy individuals and in individuals with impaired kidney function,^{99,123,124} and a smaller interindividual variation has been found.⁸⁵ Therefore, some authors propose using sCr as the marker of choice for detecting temporal changes in renal function.⁸⁵ A possible explanation for the greater intraindividual variation for CysC is the better ability of CysC to reflect small changes in GFR.⁹⁹

Food. Serum CysC was unaffected after intake of a cooked meal, whereas sCr concentration was significantly higher after eating.¹²⁵

Storage. Cystatin C generally is considered a stable protein.¹² Cystatin C was stable in serum for 6 months at -80°C and for 7 days at temperatures ranging from 20 to -20°C .³¹ Others have reported stability up to 1 month at $2-8^{\circ}\text{C}$,¹²⁶ but only 1 day at ambient temperature ($19-23^{\circ}\text{C}$) and 2 days at 4°C .²⁷ No significant differences in sCysC concentrations were observed when comparing concentrations of selected proteins in samples stored at -25°C for 2 years and 25 years with samples stored for 1 month.¹²⁷

Urinary CysC was stable at urine pH ≥ 5 at both -20 and 4°C for 7 days and at 20°C for 48 hours.²⁹

Veterinary Medicine

Serum Cr concentration in dogs is influenced by breed, age, diet, and exercise, which may result in errors in diagnosing CKD.⁶ Because sCysC appeared to be a sensitive GFR marker, some authors have investigated the effect of physiological factors on sCysC. Plasma CysC was shown to be lower in adult dogs compared with younger and older dogs and lower in dogs with body weight <15 kg compared with heavier dogs.⁴⁷ In this study, 179 dogs were included: 89 young dogs (<1 year), 39 adult dogs (1–8 years), and 51 old dogs (8–16.2 years). An overlap in plasma CysC concentration was observed (0.12–1.10 mg/L in the adult dogs, 0–1.73 mg/L in the young dogs, and 0–1.60 mg/L in the old dogs). Moreover, it remained unclear whether all of the dogs were healthy because complete CBC, serum biochemistry, and urinalysis were not performed. Other studies did not find a correlation between sCysC and age or weight.^{51,90} No circadian rhythm or sex difference was observed.^{47,51} In Wehner et al, 99 healthy dogs were included, with an equal sex distribution (52 female, 47 male dogs) and a wide range in age and body weight (3 m–13 year; 5–42 kg).⁵¹ In contrast, the study of Pagitz was limited by including only 24 healthy dogs (16 female and 8 male) with an age range of 10–97 months.⁹⁰ Because contradictory results were reported regarding the effect of age and body weight on sCysC in dogs, additional studies in a larger number of healthy dogs, preferably in which GFR is measured, are required.

In contrast to plasma Cr concentration, which increases in dogs during the first 12 hours after a meal, plasma CysC concentration showed a dramatic decrease during the first hour after a meal. This decrease lasted for 9 hours and then returned to baseline after 12 hours.⁴⁷ Based on these results, dogs should be fasted for at least 12 hours before taking blood samples to measure CysC concentration. Creatinine originates primarily from the amino acids glycine, arginine, and methionine but also from the gastrointestinal tract, which can explain the increase after a meal.¹²⁸ Because plasma CysC concentration is mainly determined by GFR, and it has been shown that a meal causes a significant increase in GFR,¹²⁹ the increased clearance of CysC could explain the decreased concentration, but this has not yet been confirmed.

To the authors' knowledge, no studies about the biological variation in sCysC in cats have been performed.

Clinical Variation in CysC

Human Medicine

CysC in Patients with Diabetes Mellitus. Diabetic nephropathy is a common complication in human

diabetes patients and is characterized by persistent albuminuria and an associated decrease in GFR.¹³⁰ Several studies have reported that sCysC is a better GFR marker than sCr for the early detection of incipient diabetic nephropathy.^{131,132} Moreover, the correlation between GFR measured with ⁵¹Cr-EDTA and sCysC ($r = 0.84$) was significantly stronger compared with using estimated GFR ($r = 0.70$).¹³² However, others have reported that sCysC is equal to sCr as a GFR marker in micro- and macro-proteinuric diabetes patients.¹³³ This difference can be explained by the different methods used to measure sCr, differing GFR reference methods, and varying diabetes populations studied.

CysC and AKI. Acute kidney injury (AKI) is associated with high mortality. Therefore, early detection is critical to prevent further progression.¹³⁴ Serum CysC concentration could detect development of AKI 1 or 2 days earlier than sCr concentration in intensive care patients with ≥ 2 predisposing factors of AKI.¹³⁴ A limitation of this study was that GFR was not measured. Interestingly, the uCysC concentration also may predict renal replacement requirement in patients initially diagnosed with nonoliguric acute tubular necrosis.¹³⁵ In similar studies, CysC was as effective as¹³⁶ or less sensitive than¹³⁷ sCr in the detection of AKI. However, similar to sCr, CysC could not discriminate between CKD and AKI.¹³⁸ In conclusion, several authors^{139,140} have suggested that the use of CysC to detect AKI must be evaluated in larger studies and with different types of AKI and that the prognostic value also must be determined.

CysC and Thyroid Function. In patients with hyperthyroidism, renal blood flow is stimulated, which causes increased GFR.¹⁴¹ Serum Cr concentration decreases, which masks patients with concurrent CKD.¹⁴² Contrasting effects have been observed in patients with hypothyroidism.^{143,144} As sCysC was introduced as a new marker of kidney function, the impact of thyroid dysfunction on sCysC also was investigated. With treatment, sCysC concentration increased in patients with hypothyroidism and decreased in patients with hyperthyroidism.¹⁴⁵⁻¹⁴⁷ However, others did not observe higher or lower sCysC concentrations in patients with untreated hyper- or hypothyroidism, respectively.¹⁴⁸ When considering sCysC concentrations in patients with hyperthyroidism, GFR is underestimated, and, in patients with hypothyroidism, GFR is overestimated.¹⁴⁹ Den Hollander suggested that there is increased or decreased production of CysC in hyper- and hypothyroidism, respectively, because of the influence of the thyroid state on general metabolism.¹⁵⁰ Serum concentrations of CysC and transforming growth factor $\beta 1$ (TGF- $\beta 1$) were significantly higher in patients with hyperthyroidism, and a positive correlation among sCysC, thyroid hormones, and TGF- $\beta 1$ was observed.¹⁵¹ After treatment, sCysC and TGF- $\beta 1$ decreased. In vitro findings have suggested an increase in TGF- $\beta 1$ concentrations in hyperthyroidism and a stimulatory effect of thyroid hormones and TGF- $\beta 1$ on CysC production.¹⁵¹

CysC and Cardiovascular Risk. Chronic kidney disease is a known risk factor for ischemic heart disease. In contrast with sCr, CysC was associated with an increased risk of heart failure.¹⁵² Serum CysC tends to be a stronger predictor of mortality than sCr in elderly individuals with heart failure,¹⁵³ as well as in the wider elderly population.¹⁵⁴ Because CysC is a proteinase inhibitor that plays an important role in tissue remodeling, a higher CysC concentration also could represent a compensatory mechanism in vascular injury.¹⁵⁵

CysC and Cancer. Because renal disease has a high prevalence in the elderly, concurrent neoplasia may be present. Decreased regulation by cystatins is responsible for increased cysteine protease activity in tumor cells.¹⁵⁶⁻¹⁵⁸ Cystatin C has 2 antitumor effects. First, it is a major inhibitor of the cathepsins, enzymes that cause degradation of basal membranes by tumor cells. Therefore, CysC suppresses the metastatic process.¹² Second, CysC inhibits TGF- β and the TGF- β signaling pathway.^{159,160} The specific role of CysC in oncogenesis has not yet been elucidated. However, individuals with untreated carcinomas¹⁶¹ and leukemia¹⁶² had significantly higher sCysC concentrations compared with patients after treatment. However, 2 other studies^{95,163} did not find a difference in sCysC concentrations between patients with malignancy and a healthy control group.

CysC and Inflammation. In vitro, CysC regulates certain aspects of immune function¹⁶⁴ because IL-10 controls CysC synthesis in response to inflammation.¹⁶⁵ Several reports have shown a good correlation between sCysC and other inflammatory markers,^{118,166,167} but these studies were performed in populations with either cardiovascular¹⁶⁷ or renal impairment,¹⁶⁶ which can cause bias. Dexamethasone caused a dose-dependent increase in CysC secretion in vitro¹⁶⁸; in vivo, sCysC is influenced by prednisolone administration.^{169,170}

Veterinary Medicine

One study in dogs showed no influence of inflammation on sCysC.⁵¹ However, only a limited number of dogs was examined; therefore, additional research is needed to examine the impact of inflammation on sCysC. Because glucocorticoids are commonly administered to small animals, future studies are needed to evaluate whether corticosteroids falsely increase sCysC. Serum CysC concentration and GFR should be measured in healthy dogs and cats before, during, and after glucocorticoid administration.

In a study comprising 10 volume-depleted dogs and 1 dog with AKI, a weaker correlation between sCysC and GFR than sCr and GFR was observed.⁴⁸ These results indicate that CysC is not a good GFR marker for decreased GFR because of prerenal causes. However, caution is warranted. Only a few dogs were sampled, which could have influenced the regression analysis. Furosemide administration used to achieve volume depletion also could have affected CysC kinetics.⁴⁸ In the same study, the sCysC concentrations of

the dog with AKI fell within the reference interval established for healthy dogs,⁴⁸ which is in contrast to observations in human patients.⁷² This suggests that in dogs with AKI, sCysC is not a sensitive indicator of decreased GFR. However, in critically ill dogs, sCysC concentrations were significantly higher in dogs in shock compared with healthy dogs, but this result was not observed in multiple-trauma dogs,¹⁷¹ which is in contrast to reports in humans.¹⁷² To date, no large-scale study in dogs with AKI has been performed to evaluate sCysC.

One of the diseases leading to AKI in dogs is babesiosis, and diagnosis of this serious complication is difficult. Photochemistry assays can cause false-positive results in babesiosis attributable to free hemoglobin or bilirubin.¹⁷³ In 1 study, no difference between sCysC and sCr was observed. Studies investigating correlations between GFR and sCr and sCysC should be performed to identify the most appropriate marker for screening for renal damage in dogs with babesiosis.¹⁷³ In our opinion, additional studies in dogs with AKI or prerenal azotemia are needed.

Cystatin C also was of particular interest in dogs with visceral leishmaniasis, a disease that results in CKD caused by immune-complex disposition and glomerular injury.¹⁷⁴ In humans, sCysC concentrations were positively correlated with circulating immune complexes and the production of granulocyte-macrophage colony stimulating factor (GM-CSF), 2 factors leading to glomerular dysfunction in leishmaniasis.¹⁷⁵ In dogs with visceral leishmaniasis, mean sCysC concentration was significantly higher than in the control groups, and sCr concentration was lower than in the control group, although not significantly. However, the mean sCysC concentration was in the reference interval proposed by 2 other authors using a turbidimetric assay.^{47,89} GFR should be determined, and renal biopsies should be performed to determine if the increased sCysC concentration in dogs with leishmaniasis is caused by immune-complex deposition or an extrarenal factor.

Cystatin C has not yet been investigated in cats with nonrenal disease, except for hyperthyroidism. Serum CysC was evaluated in cats with hyperthyroidism using PETIA.^a No correlation was observed between GFR measured by exogenous inulin clearance and 1/sCysC concentration, although a significant correlation between GFR and 1/sCr was observed.¹⁷⁶ In addition, no significant decrease in sCysC concentration was observed after treatment with ¹³¹I.¹⁷⁶ Although preliminary, the study of Jepson et al¹⁷⁶ suggests a potentially similar influence of thyroid function in cats as in humans, with hyper- and hypothyroidism causing increased or decreased sCysC concentrations, respectively. Additional studies to clarify the impact of thyroid function on CysC are warranted.

In human medicine, contradictory reports have been published regarding the effect of different tumors on sCysC concentration. Therefore, studies in small animals evaluating the effect of neoplasia on sCysC are essential. Cystatin C is an antitumor marker because it

is a protease inhibitor, and therefore, it inhibits damage from tumor cells and the metastatic process. Serum Cr concentration is not a good GFR marker in patients with neoplasia attributable to the decreased muscle mass, and sCysC potentially may be a valuable alternative.

Conclusion

Cystatin C has the potential to become a valuable biomarker in small animal medicine, but adequate analytical, biological, and clinical validation is needed first.

A few studies using canine serum have been performed, but studies in cats are scarce. There is a need to perform a thorough analytical validation of the nephelometric and turbidimetric assays for determining CysC in serum and urine of both cats and dogs. These studies will identify which assay is most suitable for CysC measurement.

To evaluate whether sCysC is a better GFR marker than sCr, it is necessary to evaluate the biological factors that may influence sCysC and to establish a reference range.

In addition, the correlations of GFR with sCysC and sCr must be compared. To use sCysC as GFR marker in practice, conditions that contribute to CysC production, such as neoplasia and inflammation, also must be investigated.

Finally, further investigations of uCysC should be performed to assess its value for the detection of tubular dysfunction.

Footnotes

^a PETIA Cystatin C assay, Dako, Glostrup, Denmark

^b PENIA Cystatin C assay, Siemens, Marburg, Germany

^c Cobas Fara analyser, Roche Diagnostics, Basel, Switzerland

^d Hitachi analyser, Roche Diagnostics, Indianapolis, IN

^e Cobas 6000 analyser, Roche Diagnostics, Basel, Switzerland

^f Abbott Architect ci8200 analyser, Abbott Laboratories, Abbott Park, IL

^g PETIA Cystatin C assay, Gentian AS, Moss, Norway

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