TITLE: Repeated measurements of renal function in evaluating its decline in cats

AUTHORS: Natalie C Finch, Harriet M Syme, Jonathan Elliott

JOURNAL TITLE: Journal of Feline Medicine and Surgery.

PUBLICATION DATE: 16 February 2018 (online)

PUBLISHER: SAGE Publications

DOI: 10.1177/1098612X18757591

Repeated measurements of renal function in evaluating its decline in cats

N.C.Finch[1], H.M.Syme[2] and J.Elliott[3]

1. Bristol Renal, University of Bristol, Bristol, UK, BS1 3NY

2. Department of Clinical Science and Services, Royal Veterinary College, Hawkshead Lane, Hatfield, Herts, UK, AL9 7TA

3. Department of Comparative Biomedical Sciences, Royal College Street, London, UK, NW1 0TU

Corresponding author: Natalie. C. Finch BVSc PhD DipECVIM, Bristol Renal, Dorothy Hodgkin Building, University of Bristol, Whitson Street, Bristol, UK, BS1 3NY, natalie.finch@bristol.ac.uk, Tel no: 01173313106

Objectives: To describe the variability in renal function markers in non-azotaemic and azotaemic cats and also the rate of change in the markers.

Methods: Plasma creatinine concentration and its reciprocal, glomerular filtration rate (GFR) and urine specific gravity (USG) were studied as markers of renal function in client owned cats. GFR was determined using a corrected slope-intercept iohexol clearance method. Renal function testing was performed at baseline and a second time point. The within-population variability (coefficient of variation; CV%) was determined at the baseline time-point. Within-individual variability (CV%) and rate of change over time was determined from the repeated measurements.

Results: Twenty-nine cats were included in the study of which five had azotaemic chronic kidney disease. The within-individual variability (CV%) in creatinine concentration was lower in azotaemic cats compared to non-azotaemic cats (6.81% vs. 8.82%) whereas, the within-individual variability in GFR was higher in azotaemic cats (28.94% vs. 19.98%). The within-population variability was greatest for USG (67.86% in azotaemic cats and 38.00% in non-azotaemic cats). There was a negative rate of change in creatinine concentration in azotaemic and non-azotaemic cats (-0.0265 and -0.0344 μmol/l/day respectively) and a positive rate of change of GFR in azotaemic and non-azotaemic cats (0.0062 and 0.0028 ml/min/day respectively).

Conclusions and relevance: The within-individual variability data suggests creatinine concentration to be the more useful marker for serial monitoring of renal function in azotaemic cats. In contrast, in non-azotaemic cats, GFR is a more useful marker for serial monitoring of renal function. The majority of cats with azotaemic CKD did not have an appreciable decline in renal function during the study.

Introduction

Important clinical applications of renal function testing include early detection of renal dysfunction and monitoring for progressive disease. Plasma or serum creatinine concentration is the most widely used renal function test in veterinary clinical practice and is a surrogate marker of glomerular filtration rate (GFR). There exists an exponential relationship between creatinine and GFR so that in early chronic kidney disease (CKD) there can be large changes in GFR with relatively small changes in creatinine concentration.[1] Therefore, creatinine is considered insensitive for detecting early CKD. In addition, factors other than GFR can influence creatinine concentration, most notably muscle mass. Reference intervals determined by individual laboratories for creatinine are variable.[2] This can lead to misclassification of patients as normal or abnormal depending on the laboratory to which the sample is submitted.[2]

GFR is considered the most sensitive and accurate measurement of functioning renal mass. Limited[3] and single[4] sampling plasma clearance techniques have been validated for cats facilitating measurement of GFR and making it practical and accessible for patients in clinical practice. However, reference intervals remain poorly defined.

It is recognized that better methods for early detection of CKD are required for cats. Considering the limitations of using reference intervals and specific cut-offs to define if a patient has normal or abnormal renal function and the insensitivity of single measurements of creatinine for early kidney disease, repeated measurements in which each patient serves as its own control may provide more clinically useful information when evaluating change in renal function. This requires knowledge of the normal variability in measurement between two time points. It also allows more dynamic rather than static assessment of renal function. Furthermore, an increase in creatinine concentration or decrease in GFR greater than the expected variability in cats with stable CKD, may suggest more progressive CKD and prompt the clinician to change the management plan or monitor the cat more closely.

The study objectives were twofold; firstly, to describe the variability in serum creatinine concentration, GFR and USG as markers of renal function in non-azotaemic and azotaemic patients and secondarily to describe the rate of change in the markers.

Materials and methods

Study population

Client-owned senior cats (>9 years) with varying renal function were identified through a senior cat wellness screening programme that was conducted at a London-based first opinion practice (Beaumont Sainsbury Animals' Hospital, Royal Veterinary College). Cats with evidence of concurrent medical disease such as hyperthyroidism were excluded. Informed consent was obtained from the owners and the study was conducted with approval from the Royal Veterinary College's Ethics and Welfare committee.

Measurement of renal function markers

GFR was determined using a previously described slope-intercept iohexol clearance method.[3] Briefly, a bolus dose of iohexol (Omnipaque^{TM} [647mg/ml; 300mg of iodine/ml]) was administered intravenously (1ml/kg). Blood samples were collected at 120, 180 and 240 min post-injection. Iohexol concentrations were determined at an external commercial laboratory using a HPLC method[i]. Clearance was determined as dose/AUC where AUC is area under the plasma concentration versus time curve determined using a one-compartment model. A previously validated cat specific correction formula for slope-intercept clearance was applied to correct for the one compartment assumption.[3] In addition, creatinine concentrations were determined from a sample collected at the same time as GFR measurement. USG was determined from a

urine sample collected by cystocentesis prior to the administration of iohexol. For statistical analysis, one was subtracted from USG.

Renal function testing, as described above, was performed at baseline and repeated approximately 6 months after the initial measurement. Measurements were therefore performed at two time-points in each cat.

Cats were classified as having azotaemic CKD if they had a persistently increased plasma creatinine concentration above the laboratory reference interval (> 2.0 mg/dl [177 μmol/l]) in association with decreased urine concentrating ability (USG < 1.035). Non-azotaemic cats did not receive any drugs or diet that might influence GFR during the study period. Azotaemic cats did not receive any drugs that may influence GFR, however, renal diet was offered to all azotaemic cats, the intake of which was variable.

Data analysis

Descriptive statistics only were performed due to the small numbers of cats included in the study and the high variability between cats that would limit the statistical power if performing inferential statistics.

Percent variation (CV;%) was calculated as (standard deviation [SD]/mean) x 100. Rate of change over time was calculated as (measurement time-point 2 – measurement time-

point 1)/ number of days between measurements. GFR unscaled to body weight (i.e. ml/min) was also included to ensure variations in weight were not influencing variation in GFR. Units for rate of change of the reciprocal of creatinine were converted to l/mmol/day.

Results

There were a total of 29 cats included in the study. Five of these cats had azotaemic CKD. The median (range) age was 12.1 (7.8 – 19.0) years. Of the 29 cats, 14 were female neutered and 15 were male neutered. Twenty cats were DSH/DLH and nine cats were pedigree (two Burmese, two Russian blue, two Persian, British short hair, Bengal and Ocicat). Repeated measurements of GFR were performed a mean number of 234 days following initial measurement. The within-population variability (CV%) for creatinine concentration, reciprocal of creatinine, USG and GFR was greater in both azotaemic and non-azotaemic cats compared to the within-individual variation (see Table 1) except for non-standardised GFR in azotaemic cats. Azotaemic cats had lower within-individual variability for creatinine concentration (6.81 vs 8.82%; see Table 1) and USG (13.19% vs 26.66%; see Table 1) compared to non-azotaemic cats. The within-individual variability in GFR was higher in azotaemic versus non-azotaemic cats (28.94% vs 19.98%). The mean within-individual body weight in azotaemic and non-azotaemic cats

was 4.62kg and 4.25kg respectively and the mean within-individual variability 4.65% and 5.44% respectively.

The rate of change of creatinine concentration was negative in both azotaemic (-0.0265 µmol/l/day; see Table 2) and non-azotaemic cats (-0.0344 µmol/l/day). There was a positive rate of change of GFR in both azotaemic and non-azotaemic cats (0.0062 ml/min/day and 0.0028 ml/min/day respectively). The mean ± SD rate of change of BW in azotaemic and non-azotaemic cats was 0.0009 ± 0.0008kg and -0.0005 ± 0.0017kg.

Discussion

Repeated measurements of renal function were performed in cats with varying renal function to investigate within-individual variability and changes in kidney function over time. The within-population variability in renal function markers is larger when compared to the within-individual variation. Therefore serial monitoring of renal markers in which each cat serves as its own baseline may prove to be more useful in the earlier detection of disease than evaluating a single static measurement using a defined cut-off with a dichotomous diagnosis (does the cat have or not have azotaemia).

The within-individual variability (CV%) in creatinine concentration was lower in azotaemic cats compared to non-azotaemic cats (6.81% vs 8.82%) whereas, the within-individual variability in GFR was higher in azotaemic cats (28.94% vs 19.98%). These values were similar to those reported in human patients with normal renal function in which the within-individual variation in creatinine concentration was 5.8% and GFR was 18.7%.[5] The results of the present study suggest that if performing serial monitoring in a patient that is azotaemic, creatinine may be the more useful marker as normal within-individual variability is lower and an increase in concentration is more likely to be clinically significant. In contrast, there is lower within-individual variability in GFR in non-azotaemic cats and a decline in GFR is more likely to be clinically significant. Considering the exponential relationship between creatinine concentration and GFR it is apparent that in early stages of disease there are large decreases in GFR with a correspondingly small increases in creatinine concentration but in later stages of disease, when the change in GFR is smaller, the increase in creatinine concentration is greater. This would also support the use of creatinine as a monitoring tool for patients with abnormal renal function and GFR as monitoring tool for patients with normal or borderline renal function. The reason as to the greater within-individual variability in GFR in azotaemic patients in unclear. GFR is biologically more variable due to the influence of renal haemodynamics and fluid volume status whereas the production of endogenous creatinine is relatively constant. The within-individual variability in GFR

does suggest that in cats with azotaemic CKD, there still remains functional renal reserve. However, the influence of feeding a renal diet cannot be completely excluded. All of the azotaemic cats in the present study were in IRIS stage 2 and 3 and none of the cats were in advanced stage (IRIS stage 4) CKD. It has been shown in cats with surgically induced models of kidney disease that following partial nephrectomy, the kidneys undergo renal hypertrophy and that this correlates with an increase in single nephron GFR.[6, 7] It is possible that the cats included in the study also underwent similar renal hypertrophy. Renal biopsies were not performed to explore this hypothesis further. It is possible that some of the variability in GFR could reflect poor assay repeatability, however, it is reported that the methodological imprecision associated with iohexol analysis is minor compared to biological variation in GFR.[8]

A further finding of interest in the present study is the positive slope for the rate of change of GFR in both azotaemic and non-azotaemic cats. This supports the suggestion that azotaemic cats do indeed have sufficient functional renal reserve to increase their GFR. This may be the result of hyperfiltration of remaining nephrons which is a presumed maladaptive process contributing to progressive CKD, however, further studies would be required to investigate this. There was a corresponding decrease in creatinine concentration in azotaemic cats over time. One may assume this is due to increased renal clearance of creatinine. It is possible that decreased endogenous production of creatinine due to reduced muscle mass in azotaemic cats may also

185 contribute to a reduced creatinine concentration over time, however, the positive rate of
186 change in body weight would not support this.

187 The within-population variation in USG was high in all cats but particularly in
188 azotaemic cats (67.86% in azotaemic cats vs 38.00% in non-azotaemic cats). This most
189 likely reflects the influence of non-renal factors such as water intake or diet on USG and
190 highlights the limitations of using a single static urine sample in interpretation of renal
191 function. USG can range from 1.001 to 1.080 in cats with normal renal function and
192 cats that have undergone surgical ablation of the kidneys have been shown to retain
193 significant urine concentrating ability. The within-individual variability in USG
194 (13.19% in azotaemic cats and 26.66% in non-azotaemic cats) was lower than the
195 within-population suggesting that serial monitoring of USG may prove more useful in
196 detecting change in renal function compared to a single static measurement. USG is a
197 simple clinical measurement that can be obtained from a urine sample perhaps collected
198 by an owner at home and further longitudinal studies evaluating this marker would be
199 an area for future study. A single USG measurement at baseline has not been found to
200 predict the development of azotaemic CKD in cats within a 12-month follow up period.[9]
201 However, rate of change has not been studied.

202 The reciprocal of creatinine has been suggested to be a useful marker of progression of
203 kidney disease. Serial measurement of GFR and the reciprocal of creatinine in canine
204 remnant kidney models found poor correlation.[6] In the present study, the correlation

between rate of change of the reciprocal of creatinine and GFR in cats was not significant in either azotaemic (r = -0.24, P = 0.695) or non-azotaemic (r = 0.21, P = 0.334) cats.

Longitudinal measurements in human patients with early CKD identified a severe decrease in eGFR (>4ml/min/year) in 24%, moderate decrease in eGFR (1-4ml/min/year) in 28%, mild decrease in eGFR (0-1ml/min/year) in 10% and no decrease in eGFR in 38% of patients.[10] In the present study, there were only a small number of cats included with azotaemic CKD (n=5) and of these cats only 1/5 (20%) had a decrease in GFR over time. It is possible that the remaining azotaemic cats belonged to a subset of diseased population in which there is no progressive decline in renal function or it may be that the repeated measurements were performed over an insufficient time period. A recent study that assessed renal function over a 6 month follow-up period also reported that in the dogs with IRIS stage 2 CKD, there was no change in GFR.[11]

The wide use of electronic clinical record systems in the majority of veterinary practices may facilitate monitoring of serial measurements of creatinine and/or GFR in clinical patients. Rate of decline of renal function could be incorporated into IRIS guidelines to help classify patients with early stage CKD or progressive disease. Furthermore, an increase in creatinine concentration variability (CV%) above that considered to be normal within-individual variation (e.g. 6.81% in azotaemic cats and 8.82% in non-

azotaemic cats) could be bought to the attention of the clinician prompting closer monitoring or a change in management for the patient.

It remains unclear how many cats with early stage CKD have intrinsic kidney damage that is likely to progress. Furthermore, there are no studies examining renal pathology in these early stages of naturally occurring disease. The fibrotic and inflammatory changes typically reported in cats with chronic kidney disease likely just reflect a chronic and irreversible disease process associated with late stage disease. By monitoring serial measurements and observing an increase in creatinine concentration or decrease in GFR above the expected norm suggesting declining renal function and potential on-going intrinsic renal damage, would be a strong argument for performing renal biopsy. This could provide valuable information regarding pathophysiology of disease.

There are a number of limitations to the present study not least the small number of cats particularly those with azotaemic CKD that were included. Only two repeated measurements were performed with a mean 234 day interval. This may not be a sufficient number of samples to detect a clinically significant measure of the rate of change in an individual patient and further longitudinal studies with additional measurements over a longer time course could provide further information. A further limitation is that the population of cats studied mainly included older cats. However, given that this is the population in which CKD is most commonly recognised and often present the greatest diagnostic challenge, the findings were considered to be

representative. In addition, the findings of the study cannot be extrapolated to cats with concurrent disease such as hyperthyroidism that may itself affect renal function, as cats with concurrent disease were excluded.

Conclusions

The within-individual variability in creatinine concentration is lower in azotaemic cats compared to non-azotaemic cats which, coupled with the insensitivity of creatinine as a marker of early renal dysfunction, suggests it is a more useful marker for serial monitoring of renal function in azotaemic cats. In contrast, the within-individual variability in GFR is lower in non-azotaemic cats and its sensitivity as a marker of early renal dysfunction suggests it is a more useful marker for serial monitoring of renal function in non-azotaemic cats. The majority of cats with azotaemic CKD included in the study did not have a decline in renal function defined by decreasing GFR which may suggest that there was sufficient adaptation of remaining functioning nephrons to increase GFR over the time period studied.

Footnotes

i. Epsom and St Helier University NHS Trust, Epsom, UK

1. Finch N. Measurement of glomerular filtration rate in cats: methods and advantages over routine markers of renal function. *Journal of feline medicine and surgery* 2014; 16: 736-748. DOI: 10.1177/1098612X14545274.
2. Ulleberg T, Robben J, Nordahl KM, et al. Plasma creatinine in dogs: intra- and inter-laboratory variation in 10 European veterinary laboratories. *Acta veterinaria Scandinavica* 2011; 53: 25. DOI: 10.1186/1751-0147-53-25.
3. Finch NC, Syme HM, Elliott J, et al. Glomerular filtration rate estimation by use of a correction formula for slope-intercept plasma iohexol clearance in cats. *Am J Vet Res* 2011; 72: 1652-1659. DOI: 10.2460/ajvr.72.12.1652.
4. Finch NC, Heiene R, Elliott J, et al. A single sample method for estimating glomerular filtration rate in cats. *J Vet Intern Med* 2013; 27: 782-790. DOI: 10.1111/jvim.12089.
5. Toffaletti JG and McDonnell EH. Variation of serum creatinine, cystatin C, and creatinine clearance tests in persons with normal renal function. *Clinica chimica acta; international journal of clinical chemistry* 2008; 395: 115-119. DOI: 10.1016/j.cca.2008.05.020.
6. Finco DR, Brown SA, Brown CA, et al. Progression of chronic renal disease in the dog. *J Vet Intern Med* 1999; 13: 516-528. 1999/12/10.
7. Brown SA and Brown CA. Single-nephron adaptations to partial renal ablation in cats. *Am J Physiol* 1995; 269: R1002-1008. 1995/11/01.
8. Krutzen E, Back SE, Nilsson-Ehle I, et al. Plasma clearance of a new contrast agent, iohexol: a method for the assessment of glomerular filtration rate. *The Journal of laboratory and clinical medicine* 1984; 104: 955-961.
9. Jepson RE, Brodbelt D, Vallance C, et al. Evaluation of predictors of the development of azotemia in cats. *J Vet Intern Med* 2009; 23: 806-813. 2009/07/02. DOI: JVIM339 [pii]
10.1111/j.1939-1676.2009.0339.x [doi].
10. Al-Aly Z, Zeringue A, Fu J, et al. Rate of kidney function decline associates with mortality. *Journal of the American Society of Nephrology : JASN* 2010; 21: 1961-1969. DOI: 10.1681/ASN.2009121210.
11. Cobrin AR, Blois SL, Abrams-Ogg AC, et al. Neutrophil gelatinase-associated lipocalin in dogs with chronic kidney disease, carcinoma, lymphoma and endotoxaemia. *The Journal of small animal practice* 2016; 57: 291-298. DOI: 10.1111/jsap.12481.

295 **Table 1: Within-population and within-individual mean, SD and CV for creatinine and reciprocal of**

296 **creatinine concentration, USG, and GFR in azotaemic and non-azotaemic cats.**

		Mean within-population (baseline)	SD within-population (baseline)	CV within-population (baseline)	Mean within-individual	Mean SD within-individual	Mean CV within-individual
Creatinine (µmol/l)	All cats	153.96	55.33	35.94%	150.79	12.29	8.47%
	Azotaemic cats	247.80	58.10	23.45%	244.22	15.81	6.81%
	Non-azotaemic cats	134.40	28.75	21.39%	131.32	11.55	8.82%
	All cats	0.007	0.002	28.57%	0.007	0.001	8.47%
	Azotaemic cats	0.004	0.001	25.00%	0.004	<0.001	6.81%

Reciprocal creatinine (l/µmol)	Non-azotaemic cats	0.008	0.002	25.00%	0.008	0.001	8.82%
USG	All cats	0.046	0.020	43.48%	0.042	0.011	23.85%
	Azotaemic cats	0.028	0.019	67.86%	0.025	0.004	13.19%
	Non-azotaemic cats	0.050	0.019	38.00%	0.047	0.012	26.66%
GFR (ml/min/kg)	All cats	1.63	0.63	38.65%	7.91	1.64	21.53%
	Azotaemic cats	0.84	0.37	44.05%	4.39	1.35	28.94%
	Non-azotaemic cats	1.80	0.54	30.00%	8.64	1.70	19.98%
GFR (ml/min)	All cats	6.80	2.73	40.15%	7.13	1.28	19.01%
	Azotaemic cats	3.51	0.79	22.51%	4.22	1.20	27.03%
	Non-azotaemic cats	7.49	2.48	33.11%	7.74	1.31	17.33%

297

298

299 **Table 2: Rate of change per day of creatinine and reciprocal of creatinine concentration, USG and GFR in**

300 **azotaemic and non-azotaemic cats.**

		Rate of change	
		Mean	SD
Creatinine (μmol/l/day)	All cats	-0.0331	0.1135
	Azotaemic cats	-0.0265	0.1065
	Non-azotaemic cats	-0.0344	0.1171
Reciprocal creatinine (l/mmol/day)	All cats	0.0014	0.0062
	Azotaemic cats	0.004	0.0023
	Non-azotaemic cats	0.0016	0.0068
USG (USG/day)	All cats	<-0.0001	0.0001

	Azotaemic cats	<-0.0001	<0.0001
	Non-azotaemic cats	<-0.0001	0.0001
GFR (ml/min/day)	All cats	0.0034	0.0105
	Azotaemic cats	0.0062	0.0060
	Non-azotaemic cats	0.0028	0.0113

301

302

303