NOTE *Internal Medicine*

Relationship of glomerular filtration rate based on serum iodixanol clearance to IRIS staging in cats with chronic kidney disease

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ABSTRACT. We examined the correlation between the glomerular filtration rate (GFR) estimated from an equation based on the serum iodixanol clearance technique and International Renal Interest Society (IRIS) stages of chronic kidney disease (CKD) in cats. The equation included the injection dose, sampling time, serum concentration and estimated volume of distribution (Vd) of the isotonic, nonionic, contrast medium iodixanol as a test tracer. The percent changes in the median basal GFR values calculated from the equation in CKD cats resembled those of IRIS stages 1–3. These data validate the association between the GFR derived from the simplified equation and IRIS stages based on the serum creatinine concentration in cats with CKD. They describe the GFR ranges determined using single-sample iodixanol clearance for healthy cats and cats with various IRIS stages of CKD.

KEY WORDS: chronic kidney disease, feline, glomerular filtration rate, iodixanol, staging

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The primary factor in classification of chronic kidney disease (CKD) into International Renal Interest Society (IRIS) stages is the creatinine concentration in plasma or serum, because it is presently the most readily available index of kidney function in feline medicine [11]. Generally, the level of serum creatinine alone is affected by certain factors, such as age (juvenile, adult and elderly animals) and nourishment status (body condition score or lean body mass) [10]. Additionally, the analytical methods for measurement of creatinine from the manufacturers of automated chemistry analyzers and clinical laboratories are not standardized, thus leading to variations within and across veterinary facilities.

We recently reported a simplified equation based on Jacobsson's formula [3] with a new tracer, iodixanol, to estimate feline glomerular filtration rate (GFR), instead of the conventional multisample method with inulin [7]. Inulin is the standard trace for urinary clearance measurement, and the multisample method is an alternative procedure for measurement of this clearance. The equation was derived from a 1-compartment model combined with the volume of distribution (Vd) and optimum time for taking blood to accurately determine the GFR [3, 6, 8]. Therefore, this equation can be applied with a single blood sample in cats as an expedient procedure in a clinically relevant situation [6, 8]. Iodixanol is an isotonic, nonionic, dimeric, radiographic contrast medium, physiologically inert, stable in serum; and freely filtered at the glomerulus. It is not secreted, reabsorbed, synthesized; or metabolized in the kidney of animals [2, 4] or humans [4, 13]. Thus, the amount of iodixanol filtered at the glomerulus is considered equal to the amount excreted in urine.

The aim of the present study was to examine the correlation between the GFR estimated by the equation [6, 8] as discussed above and IRIS stages 1 to 4 using cats with CKD.

Forty-six clinically healthy cats and 154 cats with CKD defined by IRIS stages [11] were used. Although 21 healthy cats and 12 cats with CKD used in previous studies [6, 7] were enrolled again, 25 new healthy cats (including a juvenile cat) and 142 cats with CKD were added to this investigation (Table 1). Because no sex difference in the occurrence of CKD was identified in our previous studies [6–8], the present work included data from both males and females including castrated and spayed animals. Cats were regarded as "healthy" based on the results of clinical observations, hematology, serum chemistry (serum creatinine concentration, <1.0 mg/d*l*) and urinalysis (protein, blood, glucose, ketones and specific gravity). Cats in IRIS stage 1 demonstrated persistent proteinuria (UP:C >0.5) and serum creatinine of 1.00–1.57 mg/d*l* over 3 months without evidence of heart disease or other organ failure [11]. All cats in IRIS stages 3 and 4 had evidence of renal changes on ultrasonographic examination, such as enlargement and/or irregular surface of the kidneys. Cats with heart disease or other organ injury were identified based on findings from ultrasonography and results of serum biochemical tests including measurement of the activities of ALT, AST and ALP and concentrations of albumin, cholesterol, calcium, phosphorus and electrolytes. The enzymes imply nothing about organ function; they only indicate cholestasis (ALP) and liver (ALT and AST) and muscle (AST) injury. Healthy or abnormal ultrasonographic status was judged based on reference data from a standard

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Table 1. Profiles of healthy cats and cats with CKD used in the present study

Items	Healthy cats	IRIS stages in cats with CKD			
		1	2	3	4
n	46	72	50	17	15
Body weight (kg)	3.99 (1.1–5.1)	4.63 (2.7–7.0)	4.56 (2.9–8.8)	4.36 (2.3–7.2)	3.33 (2.5–4.9)
Age (years)	4.9 (1.5–12.9)	8.3 (0.7–14.7)	8.5 (1.0–18.6)	6.8 (4.0–9.5)	9.9 (4.0–12.9)
BUN (mg/d*l*)	21.6 (15.1–31.9)	25.9 (9.8–52.1)	33.0 (19.3–61.5)	52.1 (32.3–80.0)	133.8 (88.2–182.7)
Serum creatinine (mg/d*l*)	0.83 (0.54–0.98)	1.25 (1.00–1.57)	1.74 (1.60–2.70)	3.41 (2.80–4.30)	8.43 (5.16–15.7)

Values represent the median (ranges).

textbook [11]. Cats were allowed free access to food and water *ad libitum* before all of the tests were performed. Cats showing severe depression clinically or with a body condition score <2.5/5 were excluded from this trial. Fifteen out of 169 (8.9%) cats with CKD enrolled in the study were excluded owing to the above reasons. The cats were enrolled after obtaining owner consent for their participation in this investigation. All procedures were performed in accordance with the Guidelines for Animal Experimentation issued by the Japanese Association for Laboratory Animal Science [5] and approved by the Animal Experimental Ethics Committee of Iwate University (A201139).

Iodixanol (Visipaque 320; 320 mg I/m*l*, 290 mOsm/kg H_2O) was purchased from Daiichi Sankyo (Tokyo, Japan). The units used for the dose and serum concentrations of iodixanol were milligrams of iodine/kg of body weight (mg I/kg) and micrograms of iodine/m*l* (μg I/m*l*), respectively. Iodixanol was administered as a bolus injection of 40 mg I/kg of body weight into the cephalic vein of cats via a 24-G indwelling catheter (Nipro, Osaka, Japan). The blood sample (1 m*l*) was collected using a 2.5-m*l* syringe (Nipro) attached to a 25-G needle from the contralateral cephalic vein before and 90 min [7] after iodixanol administration. The preadministration sample was used as the blank specimen for iodixanol measurement. The blood obtained was moved to a serum tube (BD Vacutainer SST II, Nippon Becton Dickinson Co., Ltd., Fukushima, Japan), placed at room temperature until clotted; and centrifuged at 1,200 g for 15 min at 4°C. Sera were stored at −80°C until assayed.

The serum iodixanol concentration was measured by reversed-phase high-performance liquid chromatography according to a procedure reported previously [7]. The GFR was estimated using Jacobsson's formula [3].

$GFR=1/(t/Vd + 0.0016) \times \ln (Dose/Vd \times C_t)$,

where t, Vd, Dose and C_t represent the sampling time (90 min), estimated Vd ($=647.6e^{-0.023Ct}$), dose level (40 mg I/kg) injected and serum concentration (μg I/m*l*) of iodixanol. The GFR is shown in m*l*/min/kg. Serum biochemical items including urea nitrogen (BUN, urease-GLDH method) and creatinine (enzymatic method, Cre-III plus MOD-P, Roche Diagnostics, Tokyo, Japan) concentrations and urinary albumin (bromocresol green method) and creatinine (enzymatic method) levels were measured with an automated chemistry analyzer (Toshiba Medical Systems, Ootawara, Japan).

Results for the CKD cats were reported as the median and range, because the data were not normally distributed. Differences in GFR values among the groups were evaluated by Steel-Dwass' test with Bonferroni correction, because of large differences (n=15–72) in the sample size of cats used in the different IRIS stages. A P value of <0.05 indicated statistical significance. The change in GFR and serum creatinine in each IRIS stage is presented as the percent change relative to the median value in healthy cats.

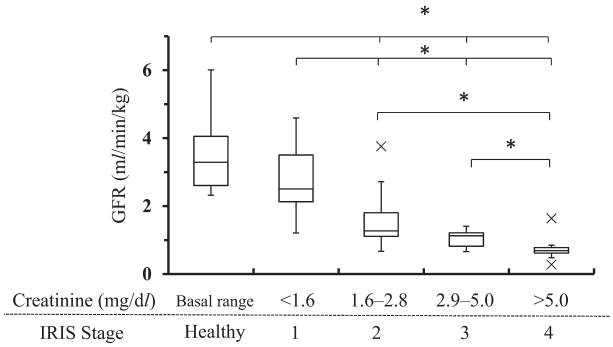

Fig. 1. Box and whisker plots of GFR values in healthy cats and cats with CKD used in this study. Median and quartile values are displayed in the box. Upper and lower bars represent maximum and minimum values, respectively. The × markers indicate outliers. *P<0.05 (Steel-Dwass' test). Alterations of GFR and serum creatinine in the respective IRIS stages are presented as percent changes in the median basal values of healthy cats.

The median GFR value (range) was 3.47 (2.18–6.01) m*l*/min/kg in the healthy group, 2.67 (1.21–4.60) m*l*/min/kg in IRIS 1 group, 1.71 (0.68–2.72) m*l*/min/kg in IRIS 2 group, 1.16 (0.66–2.13) m*l*/min/kg in IRIS 3 group and 0.78 (0.28–1.64) m*l*/min/kg in IRIS 4 group (Fig. 1). The GFR values obtained in the healthy group were in agreement with basal reference GFR data reported previously [12, 14]. Significant differences (P<0.05, Bonferroni correction: 0.0125) in GFR values were noted between the healthy group and the IRIS stage 2, 3; or 4 group, between the IRIS stage 1 and stage 2, 3; or 4 groups, between the IRIS stage 2 and 4 groups; and between IRIS stage 3 and 4 groups (Fig. 1). The GFR decreased by 0.4–39.5%, 48.7–68.4%, 65.5–76.7%; and 77.8–82.4% in IRIS stages 1, 2, 3; and 4, respectively, compared with the median GFR of healthy cats (3.47 m*l*/min/kg). In other words, their percentages corresponded to remaining GFR percentages of 99.6–60.5%, 51.3–31.6%, 34.5–23.3% and 22.2–17.6% for IRIS stages 1, 2, 3 and 4,

respectively. These remaining GFR percentages were almost within those (100–33%, 33–25%, 25–10% and ≤10%, respectively) calculated from IRIS stages [11] based on serum creatinine values, with the exception of stage 4. The difference between the remaining GFR percentages (Fig. 1) and those calculated in IRIS stages 1–4 [11] may be explained by 1) a vague assignment of marginal animals among IRIS stages, 2) a small sample size in IRIS stage 3; and 3) imprecision of the procedure for serum creatinine determination. The discrepancy relative to stage 4 was considered to be related to the characteristics of the equation [3], in which the serum iodixanol concentration did not increase in a linear manner over the maximum level (160 μg I/m*l*: below 0.3 m*l*/min/kg in GFR). The merit of the remaining percentage in GFR calculated was that it was possible to explain the current patient situation quantitatively to its owner, because the serum creatinine concentrations in the IRIS stages had somewhat wide ranges.

In our previous study [7] using Bland and Altman bias presentation, the mean bias (3.35 m*l*/min/m^2) between the multisample method with inulin and single sample method with iodixanol was relatively high compared with that (0.05 m*l*/min/m^2) between the multisample and single sample methods with iodixanol, suggesting that inulin may be eliminated via the bile to a small extent. According to a recent report from Finch *et al.* [1], the GFR estimated by a modified Jacobsson's formula using iohexol was in agreement with that by the multisample method with iohexol. However, the tracer (iohexol vs. iodixanol) used and modified approach (extracellular fluid volume vs. estimated Vd) were different between the two studies. In mouse, rat, dog and monkey studies, there was no difference in pharmacokinetics between iohexol [9] and iodixanol [2].

According to a previous report [7], serum creatinine concentrations began to increase when GFR was decreased by 70% or more. In contrast, the serum creatinine concentrations used to define IRIS stages were based on clinical experience and longitudinal studies [11]. Therefore, because Jacobsson's formula [3] was based on many assumptions to predict the true GFR, further studies are necessary to collect much more background data corresponding to each IRIS stage used in the present study.

The cause of the outlier GFR values in IRIS stages 2 and 4 (Fig. 1) remains unclear. Therefore, further studies are necessary to collect cumulative background data including GFR data for healthy cats and cats with various types of CKD, such as glomerular and tubulointerstitial lesions.

As with previous reports [6–8], no adverse reactions were observed in any of the cats during or after administration of iodixanol, as determined by physical examination and serum biochemical analyses. Although the patients in this investigation were allowed free access to food and water *ad libitum,* the effects of feeding and drinking on GFR estimates still remain to be clarified.

In conclusion, our data describe the GFR ranges determined using single-sample iodixanol clearance for healthy cats and cats with various IRIS stages of CKD.

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