Journal of Feline Medicine and Surgery (2009) **11**, 1028–1030
doi:10.1016/j.jfms.2009.07.005

ELSEVIER

jfms

SHORT COMMUNICATION
Comparison of plasma clearance of exogenous creatinine, exo-iohexol, and endo-iohexol over a range of glomerular filtration rates expected in cats

Ingrid M van Hoek DVM[1]*, **Hervé P Lefebvre** DVM, PhD, Dipl ECVPT[2], **Dominique Paepe** DVM[1], **Siska Croubels** PhD[3], **Vincent Biourge** DVM, PhD, Dipl ACVN[4], **Sylvie Daminet** DVM, PhD, Dipl ACVIM, Dipl ECVIM-CA[1]

[1]*Department of Medicine and Clinical Biology of Small Animals, Faculty of Veterinary Medicine, University of Ghent, Salisburylaan 133, 9820 Merelbeke, Belgium*
[2]*Department of Clinical Sciences and UMR181 de Physiopathologie et Toxicologie Expérimentales INRA, ENVT, École Nationale Vétérinaire de Toulouse, 23 Chemin des Capelles, BP 87614, 31076 Toulouse Cedex 3, France*
[3]*Department of Pharmacology, Toxicology, Biochemistry and Organ Physiology, Faculty of Veterinary Medicine, University of Ghent, Salisburylaan 133, 9820 Merelbeke, Belgium*
[4]*Royal Canin, Centre de Recherche, 650 Avenue de la Petite Camargue, 30470 Aimargues, France*

The study investigated plasma clearance of exogenous creatinine (PECCT), exo-iohexol (PexICT) and endo-iohexol (PenICT) in six healthy cats, four cats with chronic kidney disease (CKD) and six hyperthyroid (HT) cats to assess potential differences in glomerular filtration rate (GFR) measurement over a wide range of GFR values. The PECCT, PexICT and PenICT were performed in a combined protocol. There was a significant difference between PexICT and PenICT and PECCT in healthy cats. Differences between clearance techniques are suggested to be correlated to range in GFRs and should be taken into account when GFR is measured.

Date accepted: 3 July 2009

Glomerular filtration rate (GFR) measurement is a precise and direct evaluation of glomerular function, which is more sensitive in detecting a decreased kidney function before insufficiency or chronic kidney disease (CKD) develops.[1,2] Plasma clearance methods are less laborious and easier to apply in a clinical setting compared to urinary clearance techniques. Plasma iohexol concentration can be assayed with high-performance liquid chromatography (HPLC), which measures both exo- and endo-iohexol stereo-isomers. This way, two measures of GFR are provided after iohexol administration: plasma clearance of exo-iohexol (PexICT) and of endo-iohexol (PenICT).[3–5] The plasma clearance of exogenous creatinine test (PECCT) has been suggested to be a promising alternative for GFR measurement in cats.[4,5] Combined use of creatinine and iohexol in a plasma exogenous creatinine-iohexol clearance test (PEC-ICT) has been described in healthy cats, moderately azotaemic cats and hyperthyroid (HT) cats before and after treatment with radioiodine (^{131}I).[4–6] The combined use of different markers allows minimal time- and space related variation between the methods. Discrepancies within healthy cats, HT cats or cats with CKD exist when GFR is measured using two or three different clearance techniques due to external and internal factors.[4–10] The objectives of this study were to compare PexICT,

*Corresponding author. Present address: Royal Canin Research Center, 650 Avenue de la Petite Camargue, 30470, Aimargues, France. Tel: +33-(0)-466-8066; Fax: +33-(0)-466-730-709. E-mail: ivanhoek@royal-canin.fr

PenICT and PECCT within groups of healthy cats, HT cats and cats with CKD.

The study was conducted according to guidelines for animal care, with consent of the Ethical Committee of the Faculty of Veterinary Medicine from Ghent University, Belgium and with informed owner consent. The study included 16 cats divided into three groups: cats with CKD (International Renal Interest Society (IRIS) stage II, $n = 3$; IRIS stage III, $n = 1$, with an age of 10–13 years and bodyweight (BW) range of 4.5–6.6 kg (5.6 ± 0.9 kg)), healthy cats ($n = 6$, age of 7–12 months and BW range of 4.3–5.6 kg (4.7 ± 0.5 kg)) and HT cats ($n = 6$, age of 8–16 years and BW range of 2.6–6.2 kg (4.0 ± 1.2 kg)). Cats were screened using physical and routine laboratory examinations (complete blood count, biochemistry) and urinalysis after cystocentesis. Healthy cats were obtained from the population of laboratory animals of Ghent University and included if there were no clinically significant abnormalities. HT cats were included when clinical signs compatible with hyperthyroidism were observed: increased serum total thyroxin (TT_4) concentration and increased thyroidal uptake of $^{99m}TcO_4^-$ on a scintigraphic scan. Antithyroid drugs had to be discontinued at least 3 weeks prior to inclusion. Cats with CKD were included based on compatible clinical signs and azotaemia compatible with IRIS stage II or III (www.IRIS-kidney.com). The combined clearance of exogenous creatinine, exo- and endo-iohexol was performed as previously described.[4–6] A general linear model (Systat version 8.0, SPSS, Chicago, IL) was used to test for differences between GFR techniques in cats with CKD, HT cats and healthy cats at a global significance level of 0.05. Any effect of the three techniques in cats with CKD, healthy or HT cats was analysed with Analysis of Variance (ANOVA). When a significant effect of technique was observed, pair wise comparison of the techniques at a Bonferroni-adjusted comparison-wise significance level of 0.017 (=0.05/3) was performed. Results are expressed as range and mean ± SD.

Sixteen GFR assessments (each of them including the three markers) were performed. The values for the PECCT, PexICT and PenICT are described in Fig 1. The ratio between plasma exo- and endo-iohexol concentrations in the analysed samples was 5.8 ± 2.4. The part of the area under the curve (AUC) extrapolated to infinity expressed as % of the total AUC, was >25% in 1/16 (one cat with CKD) with creatinine clearance (range 1–57%), but was <25% in all 16 cats with exo-iohexol and endo-iohexol clearance (range 1.5–20% and 2.8–19%, respectively). The part of the AUC extrapolated to infinity for the three markers in cats with CKD, healthy and HT cats is described in Table 1. Plasma creatinine concentration did not return to pre-dosing level before the end of the sampling period in 7/16 cats (CKD $n = 4$, healthy $n = 1$, HT $n = 2$), whereas plasma exo- and endo-iohexol did not return to pre-dosing level in 3/16 cats (CKD $n = 3$) before the end of the sampling period. The GFR methods globally resulted in significant different GFR values ($P < 0.001$) but there was a statistically significant interaction between GFR method and the different groups ($P = 0.004$). A statistically significant difference between mean values of PECCT and PexICT (average difference 0.95 ml/min/kg, $P < 0.001$) and PexICT and PenICT (average difference 1.3 ml/min/kg, $P < 0.001$), though not between PECCT and PenICT (average difference 0.3 ml/min/kg, $P = 0.21$) was observed in healthy cats. There was no statistically significant difference between GFR values obtained with PexICT, PenICT or PECCT in cats with CKD ($P = 0.386$) or HT ($P = 0.185$) cats.

The present study is the first to report the comparison of three different GFR techniques in three groups of cats

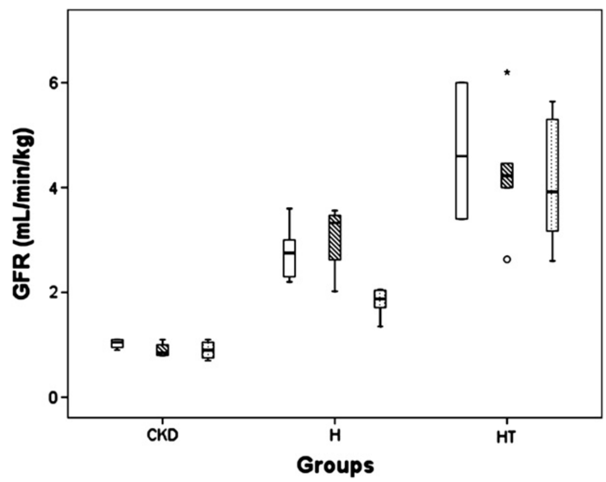

Fig 1. Box-plots of GFR measurement in the cats with CKD, healthy (H) and HT cats. Blank box: creatinine clearance; striped box: endo-iohexol clearance; dotted box: exo-iohexol clearance. Horizontal line: median; box: interquartile range (IQR); ○: outlier value larger than 1.5* (IQR); *, extreme value larger than 3* (IQR). CKD cats: exo-iohexol clearance median 0.9, IQR 0.3; endo-iohexol clearance median 0.9, IQR 0.2; creatinine clearance median 1.1, IQR 0.15. H cats: exo-iohexol clearance median 1.9, IQR 0.3; endo-iohexol clearance median 3.3, IQR 0.9; creatinine clearance median 2.8, IQR 0.7. HT cats: exo-iohexol clearance median 3.9, IQR 2.1; endo-iohexol clearance median 4.2, IQR 0.5; creatinine clearance median 4.6, IQR 2.6.

Table 1. Mean ± SD (range) of AUC extrapolated to infinity expressed as % of the total AUC.

Health status	Cats (n)	PexICT	PenICT	PECCT
Healthy	6	10.7 ± 5.9 (3.1–16.4)	10.3 ± 5.0 (5.6–18.2)	5.0 ± 4.1 (0.9–11.8)
CKD	4	8.0 ± 8.1 (3.8–20.1)	8.4 ± 7.3 (2.8–19.0)	26.9 ± 20.8 (12.4–57.1)
HT	6	3.9 ± 2.2 (1.5–6.8)	5.7 ± 2.9 (2.9–9.5)	3.1 ± 2.9 (1.0–8.7)

expected to have low (cats with CKD), normal (healthy) or high (HT) GFR values, thereby evaluating the techniques over a wide range of GFR values. Overall, there were significant differences between the three techniques, with PexICT values being significantly different from PenICT and PECCT values. These differences were apparent in healthy cats, but were not significant in HT and CKD cats, although the numbers in these groups was small. Differences between GFR methods are in accordance with studies described in the literature which compare different clearance techniques.[4,6–8] Several studies have compared two GFR techniques in cats with a declined kidney function and described significant differences, albeit other studies found no significant differences in healthy cats nor in cats with a decreased kidney function.[6–10] Recently, our group described the comparison between PexICT, PenICT and PECCT in HT cats before and after radioiodine (^{131}I) treatment.[5] The differences in GFR values according to the technique can be explained by external (marker and method related) and internal (cat and disease status related) factors. Storage time and temperature of plasma samples were similar for PECCT, PexICT and PenICT. Creatinine and iohexol were assayed in different laboratories using different assays, though both assays have been previously validated.[4] Because handling of exo- and endo-iohexol does not seem to be affected by azotaemia in cats, it is unlikely that an interference between creatinine, exo- and endo-iohexol, when these are used in a combined manner, exists.[6] In only 1/48 analyses performed, in a cat with CKD using the PECCT technique, was the AUC extrapolated to infinity higher than 25% of the whole AUC, which suggests the sampling strategy could be considered appropriate in healthy, HT and moderately azotaemic cats. Possibly, in cats with CKD the sampling period might have to be prolonged but this needs further research.

Because a combined PEC-ICT was used, factors related to the cats themselves cannot explain the difference in plasma clearance using creatinine, exo- and endo-iohexol. The difference between clearance methods, therefore, must relate to the techniques themselves.

Despite its tedious, time-consuming, stressful and potentially harmful nature, urinary clearance of inulin is considered the gold standard method for assessing GFR.[8,11,12] Nonetheless, use of a gold standard method in this study would have been useful to compare with the other GFR techniques used, and to determine which was most accurate.

We can conclude from this study that differences between clearance techniques themselves are significant and our study stresses the importance of using the same technique for measurement of GFR in the follow-up of kidney function in a cat.

Acknowledgements

The authors thank Idexx Laboratories Europe BV, Amsterdam, The Netherlands for supplying the Vettest Analyzer, and P Wassink for assistance in analysis of iohexol in the samples. This work was funded by a Bijzonder Onderzoeksfonds (BOF)-grant from Ghent University, Belgium.

References

1. DiBartola SP. Clinical approach and laboratory evaluation of renal disease. In: Ettinger SJ, Feldman EC, eds. Textbook of veterinary internal medicine. Philadelphia: WB Saunders, 2000: 1600−14.
2. Polzin DJ, Osborne CA, Ross S. Chronic kidney disease. In: Ettinger SJ, Feldman E, eds. Textbook of veterinary internal medicine. St Louis: Elsevier Saunders, 2005: 1756−85.
3. Laroute V, Lefebvre HP, Costes G, Toutain PL. Measurement of glomerular filtration rate and effective renal plasma flow in the conscious beagle dog by single intravenous bolus of iohexol and p-aminohippuric acid. *J Pharmacol Toxicol Methods* 1999; **41**: 17−25.
4. van Hoek I, Vandermeulen E, Duchateau L, et al. Comparison and reproducibility of plasma clearance of exogenous creatinine, exo-iohexol, endo-iohexol, and 51Cr-EDTA in young adult and aged healthy cats. *J Vet Intern Med* 2007; **21**: 950−8.
5. van Hoek I, Lefebvre H, Kooistra H, et al. Plasma clearance of exogenous creatinine, exo-iohexol and endo-iohexol in hyperthyroid cats before and after treatment with radioiodine. *J Vet Intern Med* 2008; **22**: 879−85.
6. Le Garrérès A, Laroute V, De La Farge F, Boudet KG, Lefebvre HP. Disposition of plasma creatinine in non-azotaemic and moderately azotaemic cats. *J Feline Med Surg* 2007; **9**: 89−96.
7. Brown SA, Finco DR, Boudinot FD, Wright J, Taver SL, Cooper T. Evaluation of a single injection method, using iohexol, for estimating glomerular filtration rate in cats and dogs. *Am J Vet Res* 1996; **57**: 105−10.
8. Brown SA, Haberman C, Finco DR. Use of plasma clearance of inulin for estimating glomerular filtration rate in cats. *Am J Vet Res* 1996; **57**: 1702−5.
9. Miyamoto K. Evaluation of plasma clearance of inulin in clinically normal and partially nephrectomized cats. *Am J Vet Res* 2001; **62**: 1332−5.
10. Miyamoto K. Use of plasma clearance of iohexol for estimating glomerular filtration rate in cats. *Am J Vet Res* 2001; **62**: 572−5.
11. Ross LA, Finco DR. Relationship of selected clinical renal function tests to glomerular filtration rate and renal blood flow in cats. *Am J Vet Res* 1981; **42**: 1704−10.
12. McClellan JM, Goldstein RE, Erb HN, Dykes NL, Cowgill LD. Effects of administration of fluids and diuretics on glomerular filtration rate, renal blood flow, and urine output in healthy awake cats. *Am J Vet Res* 2006; **67**: 715−22.