

Relationship between Serum Symmetric Dimethylarginine Concentration and Glomerular Filtration Rate in Cats

J. Braff, E. Obare, M. Yerramilli, J. Elliott, and M. Yerramilli

Background: Direct measurement of glomerular filtration rate (GFR) is the preferred method to assess renal function in cats, but it is not widely used in the diagnosis of chronic kidney disease (CKD). In cats with CKD, symmetric dimethylarginine (SDMA) has been shown to increase and to correlate with plasma creatinine concentrations.

Hypothesis: In cats, reduced GFR corresponds with increased serum SDMA concentration.

Animals: The study group consisted of ten client-owned cats whose GFR had been measured previously. Cats ranged in age from 11.1 to 16.9 years; both azotemic and nonazotemic animals were included.

Methods: Glomerular filtration rate was determined for each cat by plasma iohexol clearance using the three sample slope-intercept method, and serum SDMA concentration was measured by liquid chromatography-mass spectrometry.

Results: A linear relationship was observed between GFR and the reciprocal of serum SDMA concentration ($R^2 = 0.82$, $P < .001$). A similar relationship was found between GFR and the reciprocal of plasma creatinine concentration ($R^2 = 0.81$, $P < .001$).

Conclusions and Clinical Importance: Increased serum SDMA concentrations were observed in cats with reduced renal function as determined by direct measurement of GFR. This finding indicates that SDMA could have clinical applications in the diagnosis of CKD in cats.

Key words: Creatinine; Feline; Kidney; Renal.

Although direct measurement of glomerular filtration rate (GFR) is widely accepted as the gold standard for measuring renal function in cats, it is infrequently used in veterinary practice. The requirement for a bolus injection of contrast agent and the need for multiple, precisely timed blood samples make the testing costly and inconvenient to perform. Because of the infrequent use of direct GFR measurement in veterinary practice and the limitations of available markers for renal function, alternate methods that approximate GFR have utility in the diagnosis of chronic kidney disease (CKD) in cats. Measurements of plasma creatinine concentrations are commonly used to assist veterinarians in the diagnosis of CKD in cats, and plasma creatinine concentrations increase when GFR reduces.¹ However, plasma creatinine concentration is impacted by a number of extrarenal factors such as muscle mass that can confound accurate diagnosis,² and significant kidney function has already been lost by the time creatinine concentrations are outside of the reference range for normal cats.³ Urine specific gravity can be helpful in assessing kidney function, but urine samples are often not collected in routine clinical practice despite their diagnostic utility.

Abbreviations:

ADMA	asymmetric dimethylarginine
CKD	chronic kidney disease
DDAH	dimethylarginine dimethylaminohydrolase
GFR	glomerular filtration rate
HPLC	high performance liquid chromatography
LC-MS	liquid chromatography-mass spectrometry
PRMT	protein-arginine methyltransferase
SDMA	symmetric dimethylarginine

Dimethylated derivatives of arginine, symmetric dimethylarginine (SDMA) and asymmetric dimethylarginine (ADMA) derive from intranuclear methylation of L-arginine residuals by protein-arginine methyltransferase and are released into circulation after proteolysis. While ADMA is metabolized enzymatically by dimethylarginine dimethylaminohydrolase, SDMA is mostly eliminated by renal excretion suggesting SDMA to be an ideal candidate for a surrogate endogenous marker of GFR. A meta-analysis of 18 studies involving 2,136 human patients found high correlation of SDMA to both GFR and serum creatinine and concluded that SDMA holds considerable potential as a marker of renal function.⁴

Plasma SDMA is increased among cats with CKD and correlates with plasma creatinine concentration.⁵ This suggests that SDMA could provide a reliable alternative to creatinine for estimating renal function in cats. The observed correlation with plasma creatinine points to an increase in SDMA concentration among cats with reduced GFR, but the relationship between SDMA and GFR in cats is yet to be directly evaluated. The analysis presented in this pilot study is intended to assess the potential of SDMA as a surrogate marker for GFR in cats.

From the IDEXX Laboratories, Inc., Westbrook, ME (Braff, Obare, Yerramilli, Yerramilli); and the Department of Comparative Biomedical Sciences, Royal Veterinary College, London, UK (Elliott).

Corresponding author: M. Yerramilli, IDEXX Biotechnology Group, IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, ME 04092; e-mail: yerramilli-murthy@idexx.com.

Submitted February 24, 2014; Revised May 1, 2014; Accepted July 30, 2014.

Copyright © 2014 by the American College of Veterinary Internal Medicine

DOI: 10.1111/jvim.12446

Materials and Methods

Ten client-owned cats were included in this study retrospectively from a group of 89 cats in which GFR had been previously measured and the data published.⁶ Cats were selected in order to represent a range of GFR and creatinine values from the group of 89 cats included in the original study. Four of the cats selected had plasma creatinine concentrations above the laboratory reference range, but no concurrent medical disorders were identified based on the recorded history, physical examination or clinicopathologic data, which included routine plasma biochemistry, urinalysis and packed cell volume determination. The remaining six cats had plasma creatinine concentrations within the laboratory reference range but varied from well within to bordering on the upper limit of the reference range (2 mg/dL). These cats were all deemed healthy based on their recorded history, physical examination and clinicopathologic data.

Glomerular filtration rate had been determined by plasma iohexol clearance in each cat using the three sample slope-intercept method as previously described.^{6,7} Serum SDMA concentrations were measured on previously frozen samples (stored at -80°C) collected during the iohexol clearance test using liquid chromatography-mass spectrometry as described below.

Sample analysis for SDMA was performed using API4000 coupled with Shimadzu Nexera high performance liquid chromatography (HPLC) system. Acetonitrile extracts of serum (10 μL) were injected into HPLC equipped with a Waters XBridge C18 5 μm 4.6 \times 30 mm column and eluted using 0.1% formic acid and 0.5 mM perfluoroheptanoic acid in water (Mobile phase A) and acetonitrile with 0.1% formic acid (Mobile phase B) with a flow rate of 1 mL/min and column temperature of 20°C .

The estimation of SDMA concentration was accomplished using the standard curve obtained from 9 calibrator solutions ranging from 1.56 to 100 $\mu\text{g/dL}$. Calibrators were derived from a synthetic SDMA stock solution (1 mg/mL in water) and diluted in charcoal stripped serum. The calibrator samples were prepared using the same protocol described for the feline serum samples. The response of the MS/MS detection for all of the samples is linear and quantitation is within the calibration curve with a correlation coefficient greater than 0.999.

Results from the analyses described above are presented as median values with associated ranges. Initial data analysis was conducted using Excel software, and additional statistical analysis was performed using SAS software.^{a,b} Linear regression analyses were used to assess the relationships between GFR and 1/SDMA, GFR and 1/creatinine, and creatinine and SDMA. Coefficients of determination (R^2) were calculated to evaluate fit, and P values

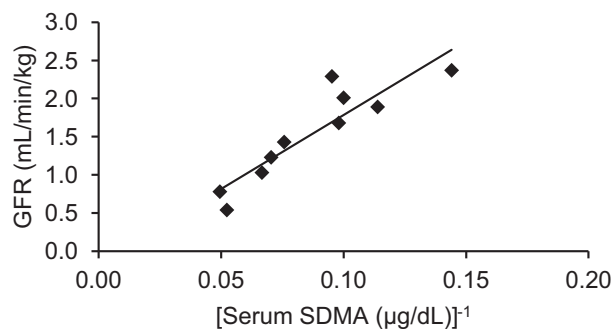


Fig 1. Relationship between glomerular filtration rate (GFR) and the reciprocal of serum symmetric dimethylarginine (SDMA) for 10 cats selected to represent a range of GFR and plasma creatinine values ($R^2 = 0.82$, $P < .001$).

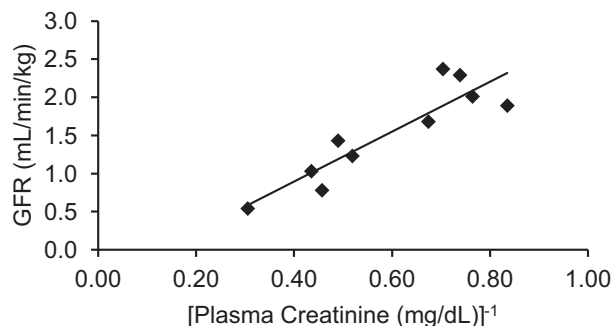


Fig 2. Relationship between glomerular filtration rate (GFR) and the reciprocal of plasma creatinine for 10 cats selected to represent a range of GFR and plasma creatinine values ($R^2 = 0.81$, $P < .001$).

$\leq .05$ were considered significant. Although good linear fits were found for GFR with SDMA and GFR with creatinine ($R^2 = 0.88$ and $R^2 = 0.79$, respectively), the reciprocals of SDMA and creatinine (1/SDMA and 1/creatinine) were selected because the reciprocal of creatinine has a better linear relationship with GFR across a broad range of plasma creatinine concentrations.^c

Results

The 10 cats included in this study, three males and seven females, ranged in age from 11.1 to 16.9 years with a median age of 13.2 years. All were domestic shorthairs except for one Persian cross and one Tiffany. GFR for these cats ranged from 0.54 to 2.37 mL/min/kg with a median value of 1.56 mL/min/kg. The median serum SDMA concentration was 11.9 $\mu\text{g/dL}$ with concentrations ranging from 6.9 to 20.2 $\mu\text{g/dL}$, whereas the median plasma creatinine concentration was determined to be 1.71 mg/dL with concentrations ranging from 1.20 to 3.27 mg/dL. Urine specific gravity for these cats was between 1.018 and 1.080 with a median value of 1.050.

Glomerular filtration rate and the reciprocal of serum SDMA concentration among these cats was found to exhibit a linear relationship ($R^2 = 0.82$, $P < .001$). The highest SDMA values occurred in cats with GFRs of

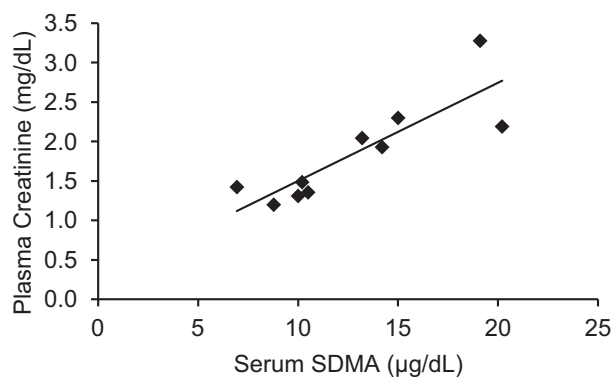


Fig 3. Relationship between creatinine and serum symmetric dimethylarginine (SDMA) for 10 cats selected to represent a range of glomerular filtration rate (GFR) and plasma creatinine values ($R^2 = 0.73$, $P = .0017$).

less than 1 mL/min/kg (see Fig 1). The relationship between GFR and the reciprocal of plasma creatinine concentration was observed to be similar ($R^2 = 0.81$, $P < .001$) (see Fig 2). Consistent with previous findings,⁵ a linear correlation existed between SDMA and plasma creatinine ($R^2 = 0.73$, $P = .0017$) (see Fig 3).

Discussion

Results from this analysis are consistent with our hypothesis that serum SDMA concentration is increased in cats with reduced GFR. In this group of cats, SDMA performs as well as creatinine in terms of its relationship with GFR with the reciprocal of both exhibiting linear relationships with GFR over a range of GFR values (see Figs 1, 2).

Increased serum SDMA concentration among cats with compromised renal function, as determined by direct measurement of GFR, indicates that SDMA could have utility in assessing renal function and as a surrogate endogenous marker for GFR in cats. This finding, taken in combination with the correlation between SDMA and creatinine,⁵ underscores the need to examine a larger number of cats across the full range of GFRs to fully evaluate the relationship between SDMA and GFR. Given the known limitations of creatinine, particularly its sensitivity to muscle mass and body composition,² more reliable markers of renal function in cats would greatly facilitate early and accurate diagnosis of CKD. Future studies are needed in order to identify clinical factors that might influence the relationship between GFR and SDMA and to assess the potential utility of SDMA in diagnosing CKD in cats.

Acknowledgment

Conflict of Interest Declaration: J. Braff, E. Obare, Maha Yerramilli, and Murthy Yerramilli are currently employed by IDEXX Laboratories, Inc. J. Elliott has served on an advisory board and received travel reimbursement from IDEXX Laboratories, Inc. within the past 3 years.

References

1. Sox EM, Chiotti R, Goldstein RE. Use of gadolinium diethylene triamine penta-acetic acid, as measured by ELISA, in the determination of glomerular filtration rates in cats. *J Feline Med Surg* 2010;12:738–745.
2. Miyagawa Y, Takemura N, Hirose H. Assessments of factors that affect glomerular filtration rate and indirect markers of renal function in dogs and cats. *J Vet Med Sci* 2010;72:1129–1136.
3. Von Hendy-Willson VE, Pressler BM. An overview of glomerular filtration rate testing in dogs and cats. *Vet J* 2011;188:156–165.
4. Kielstein JT, Salpeter SR, Bode-Boeger SM, et al. Symmetric dimethylarginine (SDMA) as endogenous marker of renal function—A meta-analysis. *Nephrol Dial Transplant* 2006;21:2446–2451.
5. Jepson RE, Syme HM, Vallance C, et al. Plasma asymmetric dimethylarginine, symmetric dimethylarginine, L-arginine, and nitrite/nitrate concentrations in cats with chronic kidney disease and hypertension. *J Vet Intern Med* 2008;22:317–324.
6. 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.
7. 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.

Footnotes

^a Microsoft Office Excel 2007; Microsoft Corporation, Redmond, WA

^b SAS 9.3; SAS Institute Inc., Cary, NC

^c Finch NC, Brodbelt D, Syme H, Elliott J. Prediction Formulae for Fat Free Mass (FFM) and Estimated Glomerular Filtration Rate (eGFR) In Cats. Royal Veterinary College, London, UK (ECVIM abstract 2010)
