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Plasma symmetric dimethylarginine and creatinine concentrations and glomerular filtration rate in cats with normal and decreased renal function

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Abstract

Background: Glomerular filtration rate (GFR) is the gold standard in assessing renal function but is impractical. Serum creatinine (sCr) has limited sensitivity in identifying early chronic kidney disease (CKD), whereas symmetric dimethylarginine (SDMA) has been commercialized as more accurate biomarker. Studies comparing SDMA and sCr with GFR in cats are limited.

Objectives: To further investigate the diagnostic performance of SDMA in non-azotemic and azotemic cats.

Animals: Forty-nine client-owned cats: 17 cats with CKD, 15 cats with diabetes mellitus (DM), and 17 healthy cats.

Methods: Retrospective study using spare blood samples from cats with documented sCr and GFR results for SDMA analysis. Diagnostic performances of SDMA and sCr were evaluated using correlation coefficients, sensitivities, specificities, and receiver operator characteristic curves.

Results: Compared to healthy cats and cats with DM, CKD cats had significantly higher $SDMA_{plasma}$ (26.7 ± 9.9 μg/dL) and sCr (249.7 ± 71.6 μmol/L [2.8 ± 0.8 mg/dL]; both $P < .001$) values. $SDMA_{plasma}$ (τ_B = −0.57; $P < .001$) and sCr (τ_B = −0.56; $P < .001$) were significantly correlated with GFR. $SDMA_{plasma}$ (τ_B = 0.52; $P < .001$) had a significant relationship with sCr. $SDMA_{plasma}$ and sCr had similar sensitivity (76%-94% and 71%-88%, respectively) in detecting reduced renal function. Creatinine had higher specificity (94%-96%) than $SDMA_{plasma}$ (75%-76%) ($P < .05$).

Conclusion and Clinical Importance: In this study of azotemic and nonazotemic cats, SDMA was a reliable marker to identify decreased GFR. However, superiority of SDMA over sCr could not be confirmed.

Abbreviations: AUC, area under the curve; CKD, chronic kidney disease; DM, diabetes mellitus; GFR, glomerular filtration rate; IRIS, International Renal Interest Society; LC-MS, liquid chromatography-mass spectrometry; RI, reference interval; ROC, receiver operator characteristic; sCr, serum creatinine; SDMA, symmetric dimethylarginine; USG, urine-specific gravity.

KEYWORDS
chronic kidney disease, diabetes mellitus, feline, healthy, renal biomarker

1 | INTRODUCTION

Chronic kidney disease (CKD) is characterized by loss of structure and function of 1 or both kidneys over a time span of 3 months or longer.[1] Between 1.6% and 20% of all cats develop CKD and the disease mostly affects elderly cats, the prevalence of CKD reaching up to 80% in the population of geriatric cats.[2-4] It is hoped that early treatment will delay the natural progressive course of the disease, making early diagnosis important and regular health screening of senior (11-14 years) and geriatric (≥15 years) cats essential.[5-7]

Assessing early renal function loss by measuring glomerular filtration rate (GFR) is the gold standard but is impractical.[8] Chronic kidney disease is routinely diagnosed using a combination of thorough physical examination, extensive laboratory testing (including complete blood counts, serum biochemistry profile, and urinalyses), diagnostic imaging, and blood pressure measurement. Compatible clinical signs, persistent azotemia, and decreased urine concentrating ability (urine-specific gravity [USG] < 1.035) indicate onset of azotemic CKD.[6] Presence of renal azotemia reflects irreversible loss of 50% to 75% of functional nephrons, correlating to 50% to 60% decrease in kidney function.[9,10] This discrepancy is attributable to compensatory hyperfiltration of remaining nephrons. With the goal of maintaining the homeostasis, reduction of nephron mass leads to increase in perfusion and filtration of surviving nephrons. In the short term, the compensatory hyperfiltration is beneficial as the GFR is partly maintained, but in the long term this might be detrimental as it promotes intraglomerular hypertension and ultimately progression of CKD.[11] Although GFR decline can already be substantial in early stages of CKD, serum creatinine (sCr) will not necessarily exceed the upper limit of the reference interval (RI).[12] Additionally, extra-renal factors may interfere with the results of routine biomarkers, sCr being influenced by diet, age, sex, and muscle mass.[13-15] Moreover, sCr has high interindividual variability, hence the RI is usually wide.[16] A consequence of the high interindividual variability is that sCr is not a sensitive biomarker to identify early CKD using population-based RI.[16] These limitations create an urgent need for sensitive markers to identify impaired renal function before azotemia arises.

Symmetric dimethylarginine (SDMA) is a by-product of protein methylation that enters the circulation after proteolysis.[13] Its excretion is mainly renal (>90%), via glomerular filtration and active secretion.[17] Tubular reabsorption is absent.[14] In small animals, SDMA is not affected by muscle mass[18,19] or sex,[18] creating an advantage over sCr. It has been claimed that SDMA has superior sensitivity to detect renal dysfunction compared to sCr. This is mainly based on a retrospective study containing 42 CKD and healthy geriatric cats in which SDMA (cut-off 14 μg/dL) showed a higher sensitivity than sCr (cut-off value 185 μmol/L [2.1 mg/dL]) to identify a >30% decrease in GFR.[20] The correlation between SDMA and GFR (r = −0.79; P < .001) proved equivalent to the correlation between sCr and GFR (r = −0.77; P < .001), indicating that both renal markers are closely related to the GFR.[20] This finding was also confirmed by other studies which obtained a coefficient of determination between SDMA and GFR (R^2 = 0.82; P < .001), similar to the relationship between sCr and GFR (R^2 = 0.81; P < .001).[13]

Since research on the diagnostic performance of SDMA in cats is limited, this study aimed to verify whether SDMA has added value over sCr to detect impaired GFR. Our first objective was to compare the strength of the correlation between the biomarkers and GFR. The second goal was to evaluate sensitivities, specificities, and optimal cut-off values for SDMA and sCr.

2 | MATERIAL AND METHODS

2.1 | Study sample and design

This retrospective study was performed at the Small Animal Department of the Faculty of Veterinary Medicine. Frozen blood samples (−80°C) from adult, privately owned cats that had undergone GFR estimation and general health screening as part of previously published prospective studies[21,22] were used. The cats had been recruited in a 5-year period between 2009 and 2014.

Based on the results of an extensive physical examination and routine laboratory analysis, all animals had been categorized into 1 of 3 predefined groups: CKD, diabetes mellitus (DM), and the healthy control group. The presence of CKD had been determined by a sCr value higher than the RI (>161.8 μmol/L [>1.83 mg/dL]) in combination with USG <1.035 (renal azotemia) together with a compatible history and associated clinical signs.[23] The diagnosis of DM had been based on the combination of hyperglycemia, glucosuria, increased serum fructosamine concentration, and representative clinical complaints. Cats within the healthy control group did not show any significant abnormalities on physical examination, blood examination (complete blood count, serum biochemistry profile, and total thyroxine concentration), and urinalysis (urinary protein : creatinine ratio and bacterial culture included).[21] These cats were mainly recruited among staff and students of the faculty of veterinary medicine for participation in the mentioned prospective research projects.[21,22]

Signalment data, USG, total thyroxine values, sCr concentration, and GFR results determined by clearance of exo-iohexol were retrospectively retrieved from the medical file of all cats. Symmetric dimethylarginine concentrations were retrospectively measured in spare plasma samples. Animals with unknown sCr or exo-iohexol GFR results and cats with insufficient plasma samples were excluded from the study. Recently, a nonsignificant relationship was found between SDMA and GFR in a sample of hyperthyroid cats, possibly because both production and metabolism of SDMA may be altered due to thyroxine changes in the blood, independent of the GFR.[24] In the present study, hyperthyroidism was diagnosed based on thyroid gland palpation, compatible clinical signs and a serum total thyroxine concentration (with RI: 14.19-45.15 nmol/L and measured in the majority of the study cats). Hyperthyroid cats were excluded from the current study.

2.2 | Analyses

2.2.1 | Glomerular filtration rate

Glomerular filtration rate (GFR) had been measured using a combined plasma exogenous creatinine Iohexol clearance test (PEC-ICT).[21,22,25] Briefly, 64.7 mg/kg exo-iohexol and 40 mg/kg creatinine had been injected intravenously. Ethylenediaminetetraacetic acid (EDTA) plasma samples had been collected before and 5, 15, 30, 60, 90, 120, 180, 360, and 600 minutes after injection. For our study, only values of the exo-iohexol clearance were used. The plasma levels of exo-iohexol had been analyzed using high-performance liquid chromatography with ultraviolet detection.[23] Pharmacokinetic analyses had been performed using WinNonlin (WinNonlin Version 4.0.1, Scientific Consulting Inc, Apex, NC). The plasma data had been subjected to noncompartmental analysis using a statistical moment approach. The area under the curve (AUC) of plasma concentration-vs-time had been calculated using the trapezoidal rule with extrapolation to infinity, as described by Watson et al.[26] Plasma exo-iohexol clearance had been determined by dividing dose administered by AUC and indexed to bodyweight (mL/[min kg]).

Evaluation of sensitivity and specificity of indirect markers, SDMA and sCr, was done by 2 different GFR cut-off values using the same clearance technique as described by Paepe et al.[23] A borderline GFR cut-off value, indicating mildly impaired renal function, was set at 1.7 mL/(min kg).[23] The low GFR cut-off value indicating CKD was set at 1.2 mL/(min kg).[23]

2.2.2 | Serum creatinine

The sCr concentration had been determined using a modified Jaffe method with an RI of 64.5 to 161.8 μmol/L (0.73-1.83 mg/dL).[27]

2.2.3 | Symmetric dimethylarginine

Residual plasma samples (−80°C) from the clearance test were thawed to 20°C and sent in batches to IDEXX Laboratories GmbH, Leipzig, Germany for analysis. Quantification of SDMA concentration was performed using the validated immunoassay IDEXX SDMA Test (IDEXX Laboratories Inc, Westbrook, Maine). The upper limit of the RI was 14 μg/dL, meaning that values >14 μg/dL were indicative of impaired renal function. Symmetric dimethylarginine analysis was performed preferably on the basal (T0) sample that was collected just before the start of the GFR measurement. When plasma samples of T0 were missing, samples obtained 5 minutes after injection (T5) of the exo-iohexol marker were used. Storage time of the samples was at least 3 years and a maximum of 8 years. Plasma samples that underwent 1 freeze-thaw cycle were only used in cases where there was no alternative.

2.3 | Statistical analysis

SAS (Statistical Analysis Software Version 9.4, SAS Institute Inc, Cary, North Carolina) was used for all statistical analyses using a global significance level of 5%.

An ANOVA *F*-test was used to check for the presence of a group effect on each variable ($SDMA_{plasma}$, GFR, and sCr) and was followed by Dunnett's multiple comparisons where diseased animals (DM/CKD) were compared pairwise with healthy controls. *P* values were adjusted for multiple comparisons. Kendall's Tau correlation coefficients (τ_B) were calculated to investigate the relationship between GFR and, $SDMA_{plasma}$, and sCr. Subsequently, we calculated the specificities and sensitivities (and 95% confidence interval) for $SDMA_{plasma}$ and sCr at their predefined cut-off values and at 2 different levels of GFR impairment since the renal clearance is the gold standard for CKD diagnosis. With the 2 different threshold values for GFR in mind, we calculated sensitivities and specificities corresponding to a wide range of alternative cut-off values of SDMA and sCr. Sensitivities were plotted against 1-specificities ultimately resulting in 4 receiver operator characteristic (ROC) curves. For each plot, the AUC was calculated (with the 95% confidence interval included[28]) as an evaluation of the diagnostic accuracy or distinctiveness of the biomarker to detect a decreased GFR.

3 | RESULTS

3.1 | Study sample and descriptive statistics

A total of 49 cats were included in this study. Seventeen animals were diagnosed with CKD. Based on the current International Renal Interest Society (IRIS) guidelines,[29] 11 had IRIS stage 2 (sCr: 140-250 μmol/L [1.6-2.8 mg/dL]) and 6 had stage 3 CKD (sCr: 251-440 μmol/L [2.9-5.0 mg/dL]). Fifteen cats had DM and 17 cats were considered healthy control animals. The majority of the animals with DM were not sufficiently controlled for DM despite therapy, had fructosamine levels > 600 μmol/L and presence of glucosuria (blood glucose > 15 mmol/L [270 mg/dL]).

Further characteristics of the study sample are shown in Table 1.

Spare plasma samples were available for all cats. Samples of T0 were missing in 4 cats (all healthy cats), but spare plasma collected at T5 during the GFR procedure were available for SDMA analysis in these animals. Five plasma samples underwent 1 freeze-thaw cycle before SDMA measurement.

As shown in Table 2, a GFR < borderline cut-off value of 1.7 mL/(min kg) was present in 21/49 cats. Sixteen of them belonged to the CKD group while 2 were diagnosed with DM, and the remaining 3 cats belonged to the healthy group. Fifteen of these animals obtained a GFR < the low cut-off value of 1.2 mL/(min kg). One

TABLE 1 Overview of the different breeds and sexes within the CKD group, DM, and healthy control group presented in absolute numbers and (percentage ratio between brackets)[21,22]

		CKD n = 17	DM n = 15	Control n = 17	Total n = 49
Breed	Eur SH	n = 11 (65%)	n = 12 (80%)	n = 17 (100%)	n = 40 (82%)
	Other breeds	British SH n = 2 Siamese n = 1 Persian n = 1 Ragdoll n = 1 Burmese n = 1	Eastern SH n = 1 British SH n = 1 Burmese n = 1		British SH n = 3 Burmese n = 2 Persian n = 1 Ragdoll n = 1 Siamese n = 1 Eastern SH n = 1
Sex	M	n = 0	n = 1 (6.5%)	n = 0	n = 1 (2%)
	F	n = 1 (6%)	n = 1 (6.5%)	n = 0	n = 2 (45%)
	Mc	n = 10 (59%)	n = 12 (80%)	n = 5 (29.5%)	n = 27 (55%)
	Fc	n = 6 (35%)	n = 1 (6.5%)	n = 12 (70.5%)	n = 19 (39%)

Abbreviations: British SH, British Shorthair; CKD, chronic kidney disease; DM, diabetes mellitus; Eastern SH, Eastern Shorthair; Eur SH, European Shorthair; F, female; FC, female castrated; M, male; MC, male castrated.

TABLE 2 Classification of the cat sample of 49 cats based on estimation of the GFR by exo-iohexol clearance test

	GFR (mL/[min kg])		
Group	<1.2	1.2 ≤GFR < 1.7	≥1.7
Chronic kidney disease	15	1	1
Diabetes mellitus	0	2	13
Healthy	1	2	14
Total	16	5	28

Note: Within each group (CKD, DM, and healthy control group), the number of cats with a normal renal clearance (GFR ≥ 1.7 mL/[min kg]), mild renal impairment (GFR ≥ 1.2 mL/[min kg] and <1.7 mL/[min kg]), and severe renal dysfunction (GFR < 1.2 mL/[min kg]) are displayed.
Abbreviations: CKD, chronic kidney disease; DM, diabetes mellitus; GFR, glomerular filtration rate.

of these was recruited as a healthy cat and had sCr of 104 μmol/L and USG of 1.038. All other cats with a GFR < 1.2 mL/(min kg) belonged to the CKD group.

Mean results of exo-iohexol GFR, sCr, $SDMA_{plasma}$, and USG for the complete study sample and the different subgroups were available and presented in Table 3. As total thyroxine serum values were missing in 7/49 cats (subgroup CKD: n = 2; DM: n = 3; control: n = 3), mean values were calculated based on the available results.

$SDMA_{plasma}$, sCr, and GFR differed significantly among the groups ($P < .001$ for each variable). Cats with CKD possessed significantly lower GFR results and significantly higher sCr and SDMA concentrations compared to healthy individuals (all $P < .001$). No significant differences in GFR, sCr, and SDMA were found between DM and healthy animals.

3.2 | Correlation between kidney function tests

Kendall's Tau correlation coefficients (τ_B) revealed for the 49 cats that the correlation between $SDMA_{plasma}$ and GFR (τ_B = –0.57; $P < .001$) was moderate; however, the correlation between sCr and GFR was of the same magnitude (τ_B = –0.56; $P < .001$). High concentrations of both biomarkers were associated with reduced filtration rate. The correlation between the 2 renal biomarkers $SDMA_{plasma}$ and sCr was also moderate (τ_B = 0.52; $P < .001$).

3.3 | Relationship between SDMA and sCr

$SDMA_{plasma}$ and sCr concentrations of the 49 animals were plotted against each other in Figure 1. Concordant results between both renal biomarkers were confirmed in 39/49 cats. 24/49 had SDMA and sCr results within RI in quadrant I and 15/49 had SDMA and sCr results above the upper reference limit in quadrant IV. Discordant results were present in 10 animals. Five healthy control cats and 3 DM cats had normal sCr concentrations but increased SDMA values (quadrant II). However, 6 of them had a normal GFR (>1.7 mL/[min kg]). Within reference $SDMA_{plasma}$ and increased sCr levels were represented in quadrant III and contained 2 cats that were earlier assigned to the CKD group. The first cat had a GFR measurement <1.7 mL/(min kg), while the renal clearance of the second was <1.2 mL/(min kg), which indicated that in this cat SDMA obviously failed to identify CKD in contrast to sCr.

3.4 | Diagnostic value of sCr and SDMA as renal biomarkers

Sensitivities and specificities for $SDMA_{plasma}$ and sCr to detect GFR exceeding the borderline (1.7 mL/[min kg]) and low (1.2 mL/[min kg]) GFR cut-off values are shown in Table 4.

We determined the threshold concentrations for SDMA and sCr that resulted in the ideal combination of sensitivity and specificity to optimize the diagnostic force for both biomarkers. To identify any cat with a GFR result <1.7 mL/(min kg), a SDMA cut-off value of 18 μg/dL and sCr threshold value of 155.6 μmol/L (1.76 mg/dL) was more

TABLE 3 Overview of the mean values and (SDs between brackets) for GFR, sCr, $SDMA_{plasma}$, USG, bodyweight and age for 17 CKD cats, 15 DM cats, 17 healthy cats, and the complete study sample of 49 cats[21,22]

	CKD n = 17	DM n = 15	Control n = 17	Total n = 49
Exo-iohexol GFR	0.9 (0.4)[a]	2.1 (0.6)	2.1 (0.5)[a]	1.7 (0.8)
sCr	249.7 (71.6)[b] [2.8 (0.8)]	111.6 (24.8) [1.3 (0.3)]	104.1 (25.9)[b] [1.2 (0.3)]	156.9 (82.4) [1.8 (0.9)]
$SDMA_{plasma}$	26.7 (9.9)[c]	12 (2.4)	12.5 (4.6)[c]	17.3 (9.5)
USG	1.020 (0.009)	1.036 (0.010)	1.045 (0.009)	1.033 (0.014)
TT4	24.3 (10.1)	14.7 (8.2)	30.2 (6.3)	23.7 (10.3)
Bodyweight	4.2 (1.2)	4.9 (1.2)	4.5 (1.3)	4.5 (1.2)
Age	10.3 (4.9)	9.5 (2.9)	10.5 (3.1)	10.1 (3.7)

Note: Mean values and (SDs between brackets) for TT4 were available for 15 CKD cats, 12 DM cats, 15 healthy cats, and a total of 42 study cats.[21,22] GFR in mL/(min kg), sCr in µmol/L and [mg/dL], $SDMA_{plasma}$ in µg/dL, TT4 in nmol/L, bodyweight in kg, and age in years were available for the 49 cats. Means sharing the same letter differed significantly from each other ($P < .05$).
Abbreviations: CKD, chronic kidney disease; DM, diabetes mellitus; GFR, glomerular filtration rate; sCr, serum creatinine; SDMA, symmetric dimethylarginine; TT4, total thyroxine; USG, urine-specific gravity.

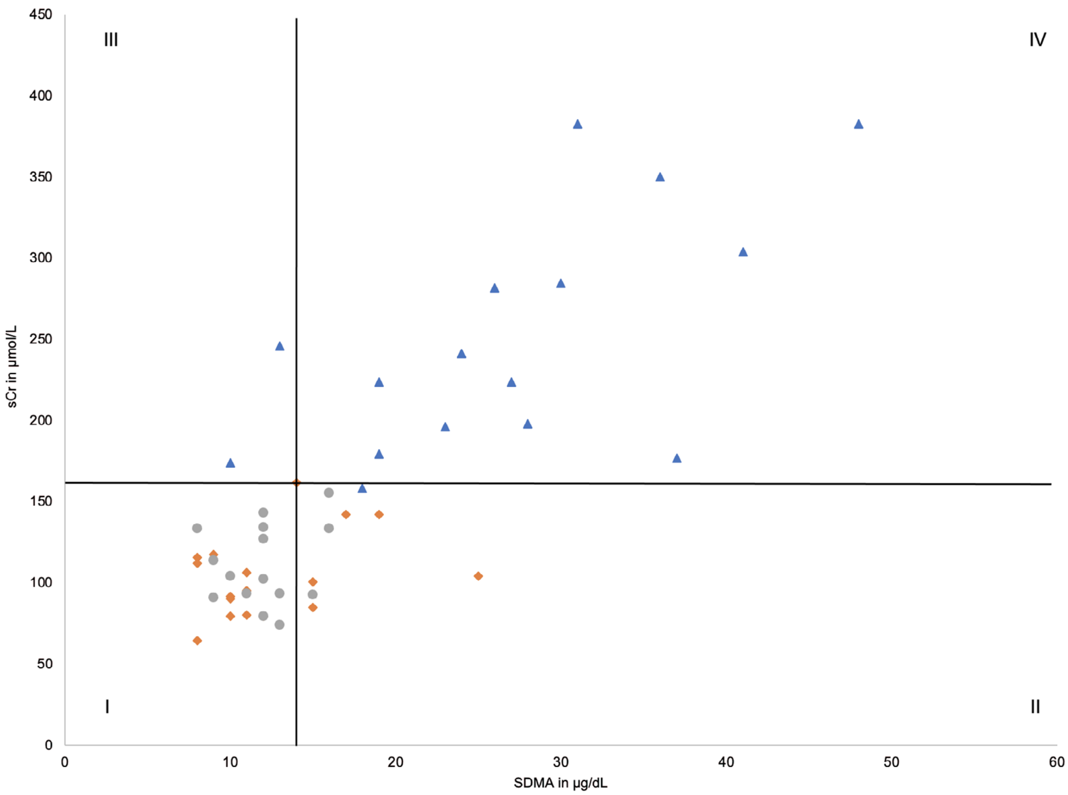

FIGURE 1 Scatter plot illustrating the relationship between SDMA (on the x-axis) and sCr (on the y-axis) concentrations measured in 17 healthy animals, 17 CKD cats, and 15 cats with DM. Chronic kidney disease animals are visualized as blue triangles; DM and control cats obtain the green circle and orange rhombus shape, respectively. Horizontal and vertical lines refer to the threshold values of sCr (161.8 µmol/L [1.83 mg/dL]) and SDMA (14 µg/dL), respectively. Quadrant I and IV represent the cats with concordant results between SDMA and sCr. Quadrant II represents the cats with increased SDMA and normal sCr values. Quadrant III represents the cats with normal SDMA and increased sCr values. The graph demonstrates the positive relationship between both kidney markers ($\tau_B = 0.52$; $P < .001$). CKD, chronic kidney disease; DM, diabetes mellitus; sCr, serum creatinine; SDMA, symmetric dimethylarginine

appropriate. The associated sensitivities and specificities are also presented in Table 4.

Receiver operating characteristic curves (Figure 2) illustrate that the AUC of $SDMA_{plasma}$ and sCr was 0.86 (95% CI = 0.79-0.93) and 0.90 (95% CI = 0.84-0.96), respectively, to detect mild kidney dysfunction (GFR < borderline GFR cut-off of 1.7 mL/[min kg]). To detect obvious kidney dysfunction (GFR < low GFR cut-off of 1.2 mL/[min kg]), $SDMA_{plasma}$ had an AUC of 0.95 (95% CI = 0.91-0.99) while sCr achieved an AUC of 0.93 (95% CI = 0.89-0.98) (Figure 3).

TABLE 4 Sensitivity and specificity for SDMA and sCr with upper reference limits 14 μg/dL and 161.8 μmol/L (1.83 mg/dL), respectively, and upper reference limits 18 μg/dL and 155.6 μmol/L (1.76 mg/dL), respectively

		Cut-off GFR 1.7 mL/(min kg)		Cut-off GFR 1.2 mL/(min kg)	
	Cut-off	Sensitivity	Specificity	Sensitivity	Specificity
$SDMA_{plasma}$	14 μg/dL	76.2 (52.8-91.8)	75 (55.1-89.3)	93.7 (69.8-99.8)	75.7 (57.7-88.9)
	18 μg/dL	71.4 (47.8-88.7)	96.4 (81.6-99.9)	87.5 (61.6-98.5)	93.9 (79.8-99.3)
sCr	161.8 μmol/L (1.83 mg/dL)	71.4 (47.8-88.7)	96.4(81.6-99.9)	87.5 (61.7-98.4)	93.9 (79.8-99.3)
	155.6 μmol/L (1.76 mg/dL)	76.2 (52.8-91.8)	92.9 (76.5-99.1)	93.8 (69.8-99.8)	90.9 (75.7-98.1)

Note: 95% CIs are added between the brackets. Test positive and negative results are objectively evaluated by means of exceeding the borderline GFR cut-off and the low GFR cut-off.
Abbreviations: GFR, glomerular filtration rate; sCr, serum creatinine; SDMA, symmetric dimethylarginine.

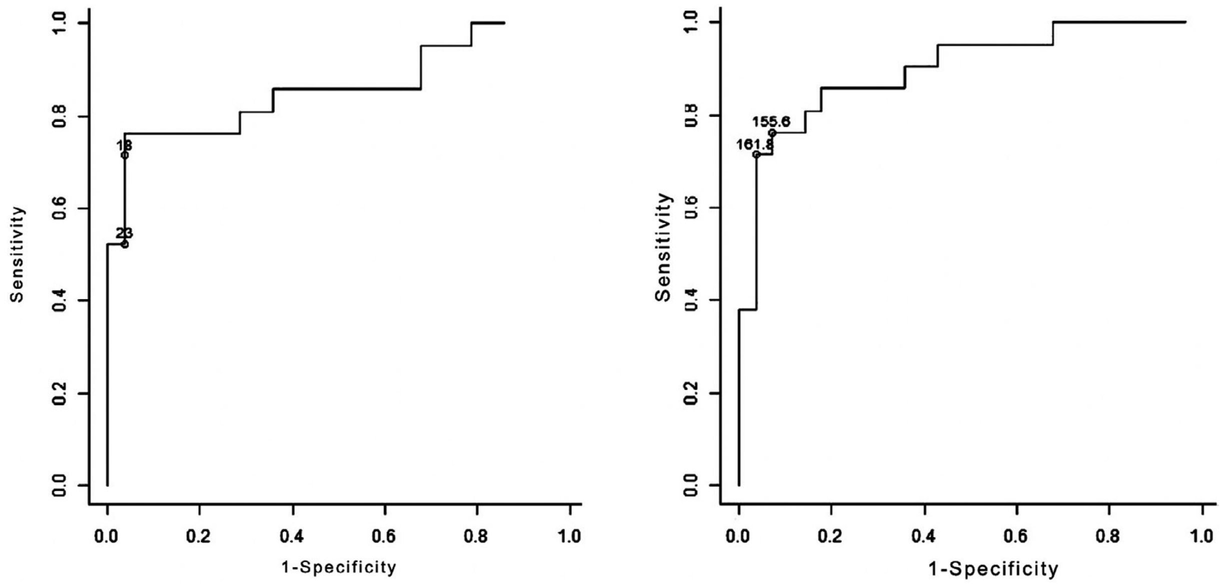

FIGURE 2 Receiver operator characteristic curves of SDMA (left) and sCr (right) showing the diagnostic ability of the biomarkers to detect GFR < borderline GFR cut-off (1.7 mL/[min kg]). On the x-axis, 1-specificity is shown for a range of possible cut-off values. The y-axis demonstrates the sensitivity of the renal biomarker for a range of possible cut-off values. The figure demonstrates a similar AUC of 0.86 (with a 95% CI of 0.79-0.93) and 0.90 (with a 95% CI of 0.84-0.96) for $SDMA_{plasma}$ and sCr, respectively. AUC, area under the curve; GFR, glomerular filtration rate; sCr, serum creatinine; SDMA, symmetric dimethylarginine

4 | DISCUSSION

The present study aimed to investigate the benefit of SDMA as an indirect renal biomarker in 49 nonazotemic and azotemic cats. By using an exo-iohexol clearance test, renal function was objectively established and diagnostic results of SDMA and sCr were evaluated in light of this gold standard. Using retrospective plasma samples, we recognized that both biomarkers were equally correlated with the GFR and we confirmed a mild and clinically nonrelevant difference in sensitivity between SDMA and sCr in the detection of renal function loss. In conclusion, the diagnostic results of both tests were comparable.

Unlike sCr, SDMA is only slightly subjected to extra renal influence.[18,19] Based on the claim that SDMA has superior sensitivity than sCr to detect real dysfunction, a stronger correlation between SDMA and GFR compared to the correlation between sCr and GFR could be anticipated. In contrast, we observed both $SDMA_{plasma}$ and sCr were equally correlated with the renal clearance. Both biomarkers are worthy as a surrogate marker for GFR measurement to objectively evaluate renal function. Plasma SDMA increased with decreasing renal clearance but both variables were not perfectly correlated. Variations in SDMA results cannot be completely attributed to changes in the filtration capacity of the kidney and additional factors in the metabolism or elimination of the SDMA molecule must be considered.

Until now, the influence of systemic conditions or treatment on SDMA concentration has been poorly studied in small animals.[30] Recent research with hyperthyroid cats showed a poor correlation between SDMA and GFR values, probably due to changes in protein metabolism by the hyperthyroid state of the cats.[24] Therefore, we excluded hyperthyroidism based on a number of diagnostic tools

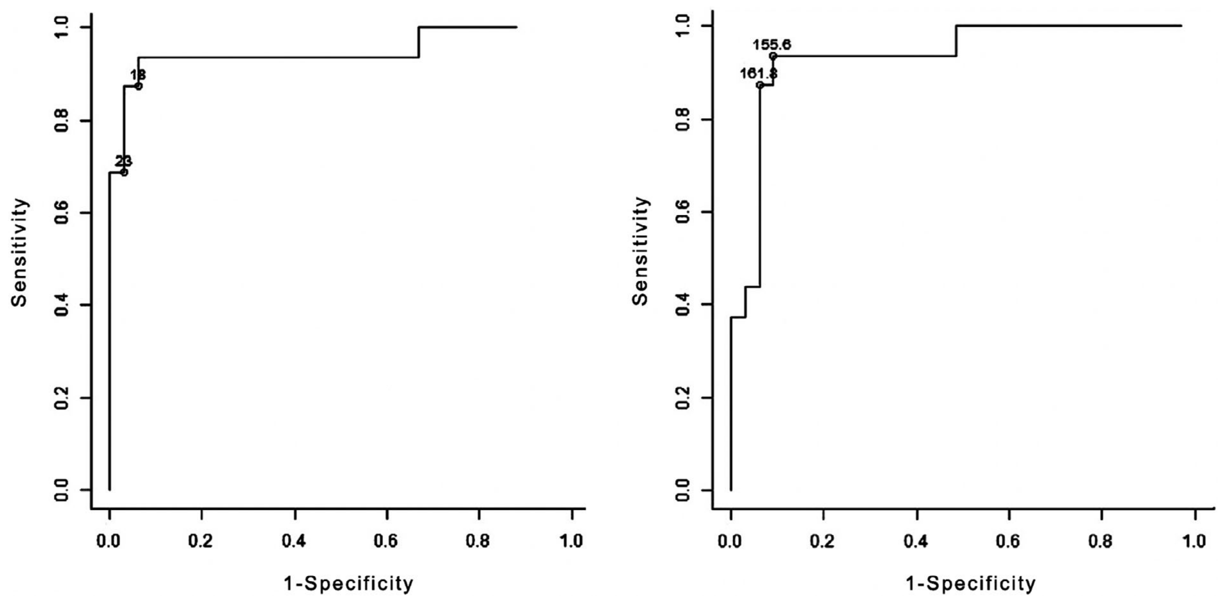

FIGURE 3 Receiver operator characteristic curves of SDMA (left) and sCr (right) showing the diagnostic ability of the biomarkers to detect GFR < low GFR cut-off (1.2 mL/[min kg]). On the x-axis, 1-specificity is shown for a range of possible cut-off values. The y-axis demonstrates the sensitivity of the renal biomarker for a range of possible cut-off values. The figure demonstrates a similar AUC of 0.95 (with a 95% CI of 0.91-0.99) and 0.93 (with a 95%CI of 0.89-0.98) for $SDMA_{plasma}$ and sCr, respectively. AUC, area under the curve; GFR, glomerular filtration rate; sCr, serum creatinine; SDMA, symmetric dimethylarginine

including the determination of serum total thyroxine. For a limited number of cats, thyroxine values were missing but based on their young age, the absence of compatible clinical signs, and a normal thyroid palpation; hyperthyroidism was an unlikely diagnosis. In the present study, a large number of animals with normal GFR suffered from DM so an extra-renal influence of this endocrine disorder on plasma SDMA could not be ruled out. Nevertheless, we could not prove significant difference in SDMA blood concentration between the healthy control group and nonazotemic cats with DM. The influence of (insufficiently controlled) DM on SDMA concentration with significantly lower SDMA blood levels in DM cats (without comorbidities) compared to healthy control animals has been reported, probably due to hyperfiltration and osmotic diuresis.[31] Since GFR was not measured in that study, these findings are hard to interpret. Further investigation to determine if DM can influence SDMA concentrations in cats is warranted.

The scatter plot illustrating all SDMA and sCr results indicated several discordant results. The GFR values indicated that the majority of cats with conflictingly high SDMA and normal sCr concentrations showed a false positive SDMA result, reflected in a somewhat lower specificity of SDMA (76%) compared to SCr (94%-96%). Using the upper limit of the RI (161.8 μmol/L [1.83 mg/dL] for sCr and 14 μg/dL for SDMA), sCr only generated a minimum number of false positive test results (n = 1) while SDMA incorrectly suspected more healthy cats with deteriorated kidney function (n = 7). As expected, sensitivity and specificity of both kidney biomarkers was partly influenced by the GFR cut-off value. The borderline GFR cut-off value generally led to fewer false positive test results for SDMA and sCr compared to the low GFR cut-off value. Increased sCr despite normal SDMA results was recorded in 2 cats. Both cats had a decreased GFR value indicating false negative SDMA results. However, the perceived differences in sensitivity between sCr and SDMA in the present study were not clinically relevant. Both SDMA and sCr correctly identified cats in advanced stages of renal function loss (<1.2 mL/[min kg]). Overall, it is important to note that the mild differences in sensitivity and specificity of sCr and SDMA are probably attributable to their RI as both biomarkers show equal performance in the ROC curve analysis.

According to our data, SDMA offered little added diagnostic value compared to the long-implemented sCr. The accuracy in detecting a GFR < 1.7 mL/(min kg) and a GFR < 1.2 mL/(min kg) was investigated, and diagnostic performance of both markers improved as renal impairment progressed. However, SDMA is commercially promoted as a highly sensitive diagnostic tool providing extra value in detecting cats with early renal function loss who are missed with the use of the traditional renal marker sCr.[10,32]

Our findings are not completely in line with previous research due to multiple reasons. Across studies the SDMA cut-off value stays fixed, but the sCr RI widely varies due to interlaboratory differences in samples selected for the establishment of the RI. This affects sensitivity of sCr. The higher the upper reference limit, the more false negative test results are generated which will ultimately result in a lower sensitivity for this biomarker. Our RI of sCr was determined by Ghys et al[27] and had the important advantage of being laboratory-specific, but compared to other studies, the upper reference limit for sCr was rather low. In addition, analytical variation can arise by the usage of different laboratory quantification techniques for measuring sCr (enzymatic vs colorimetric) as well as GFR (marker and sampling strategy). This makes comparison between studies challenging. The crosssectional retrospective nature of our research did not allow us to follow-up the animals over time. This led us to the additional disadvantage that we did not possess sufficient data of cats going through IRIS stage I before the presence of azotemia so it was not possible to assess the biomarkers for this purpose.

Using ROC curves, we evaluated the threshold values for SDMA (14 μg/dL) and sCr (161.8 μmol/L [1.83 mg/dL]), which were not ideal for our data set containing CKD and non-CKD cats. A cut-off for SDMA yielding a more optimal combination of sensitivity and specificity was 18 μg/dL. Symmetric dimethylarginine with the predetermined cut-off 14 μg/dL generated many false positives. Since sensitivity and specificity are strongly intertwined, a cut-off of 18 μg/dL inherently led to a slight loss in sensitivity, but this disadvantage was limited and in favor of a higher specificity. After the CKD diagnosis is confirmed in a clinically stable, hydrated animal (with exclusion of pre- or postrenal problems), recently updated IRIS guidelines allow staging of the chronic disease by at least 2 measurements of SDMA and sCr in a fasted animal. Symmetric dimethylarginine repeatedly exceeding 18 μg/dL (even in combination with normal sCr levels) suggests that the animal suffers from at least CKD stage 2.[29] Since our study did not include serial monitoring of SDMA and sCr, a direct comparison of our results with the IRIS staging system is not indicated.

For sCr, a slightly lower threshold value of 155.6 μmol/L (1.76 mg/dL) was preferred. Since the specificity of sCr was high in our study sample of cats, it was desirable to improve sensitivity to the disadvantage of a minor loss in specificity.

4.1 | Limitations

Although the present study is retrospective in nature, the majority of our data (sCr and GFR) originated from cats recruited in the context of previous prospective research studies.[21,22] All the cats included were subjected to the same protocol: a general physical examination, blood and urine testing, and a GFR clearance test. This minimized the limitations of the retrospective nature of the present study. The most important inclusion criterion for our study was the availability of sufficient residual blood samples in order to perform SDMA analysis, implying that the 49 cats were not randomly selected. This was disadvantageous in obtaining a representative study sample.

We aimed to test the accuracy of SDMA and sCr in a sample of nonazotemic and azotemic cats. Performance of a kidney marker is better with advanced renal failure (IRIS stage III/IV) while the challenge and added value of a biomarker is mainly based on its capacity to identify animals with minimal GFR loss.[33] Five cats out of all achieved a renal clearance between 1.2 and 1.7 mL/(min kg), indicating the presence of mild renal impairment. Furthermore, the small number of cats with early CKD could explain the reason for no added diagnostic value of SDMA that could be established in our data set and further studies are needed.

The allocation of the cats to the different subgroups was based on physical examination and routine laboratory analysis. But when the GFR result was taken into account, 3 cats initially considered as healthy and 2 cats with DM were found to have mildly impaired renal function. On the other hand, 1 cat of the CKD group had a GFR value >1.7 mL/(min kg).

Residual frozen blood samples stored for maximum 8 years were used for SDMA analysis. Short-term stability of the molecule in blood has already been reported.[34] The effect of long-term preservation on accurate analysis of SDMA is a relevant issue that has not been clarified. In addition, several plasma samples (n = 5) were subjected to 1 freeze-thaw cycle before SDMA analysis. Multiple freeze-thaw cycles do not generate significant changes in the SDMA concentration.[34] Furthermore, IDEXX claims the molecule remains stable for several years if the sample is frozen.[30]

Symmetric dimethylarginine concentrations were quantified using the immunoassay "SDMA IDEXX test" and not the gold standard liquid chromatography-mass spectrometry (LC-MS). The SDMA IDEXX test uses glucose-6-phosphate dehydrogenase conjugate and monoclonal anti-SDMA antibodies, is less expensive and time-consuming than the LC-MS, and is currently widely used in commercial veterinary laboratories. This means that the results of our study have the advantage of being clinically applicable to veterinary practice. The IDEXX SDMA test is an accurate technique. Within the 10 to 45 μg/dL range, the maximum measurement error is estimated to be 1 to 3 μg/dL.[35] In addition, the test is not sensitive to lipemia, icterus, or mild-to-moderate hemolysis, which usually arises during blood sampling.[32]

5 | CONCLUSIONS

In this retrospective study, SDMA behaved as an accurate biomarker for detecting impaired renal function defined by exo-iohexol clearance in cats. However, in the present sample of adult nonazotemic and azotemic cats, we could not prove that SDMA offered prominent added value compared to the conventional sCr biomarker. The diagnostic value of both molecules was approximately equivalent and improved in the advanced phases of renal dysfunction.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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