Big or small?  
One MRI fits all.

A revolutionary MRI built specifically for animals...  
no matter what their size.

Learn more at info.hallmarq.net/jvim-samri

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

Marleen Brans1 | Sylvie Daminet1 | Femke Mortier1 | Luc Duchateau2 | Hervé P. Lefebvre3 | Dominique Paepe1

1Small Animal Department, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium
2Department of Nutrition, Genetics and Ethology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium
3Department of Physiology and Therapeutics, National Veterinary School of Toulouse, Toulouse, France

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 SDMAplasma (26.7 ± 9.9 μg/dL) and sCr (249.7 ± 71.6 μmol/L [2.8 ± 0.8 mg/dL]; both P < .001) values. SDMAplasma (τB = −0.57; P < .001) and sCr (τB = −0.56; P < .001) were significantly correlated with GFR. SDMAplasma (τB = 0.52; P < .001) had a significant relationship with sCr. SDMAplasma and sCr had similar sensitivity (76%-94% and 71%-88%, respectively) in detecting reduced renal function. Creatinine had higher specificity (94%-96%) than SDMAplasma (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.
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. 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. 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.

Assessing early renal function loss by measuring glomerular filtration rate (GFR) is the gold standard but is impractical. 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. Presence of renal azotemia reflects irreversible loss of 50% to 75% of functional nephrons, correlating to 50% to 60% decrease in kidney function. 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. However, 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). Additionally, extra-renal factors may interfere with the results of routine biomarkers, sCr being influenced by diet, age, sex, and muscle mass. Moreover, sCr has high interindividual variability, hence the RI is usually wide. A consequence of the high interindividual variability is that sCr is not a sensitive biomarker to identify early CKD using population-based RI. 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. Its excretion is mainly renal (>90%), via glomerular filtration and active secretion. Tubular reabsorption is absent. In small animals, SDMA is not affected by muscle mass or sex, 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 and GFR (rit 0.82; P < .001), similar to the relationship between sCr and GFR (rit 0.81; P < .001).

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 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. 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). These cats were mainly recruited among staff and students of the faculty of veterinary medicine for participation in the mentioned prospective research projects.

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. 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). 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. 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. 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. A borderline GFR cut-off value, indicating mildly impaired renal function, was set at 1.7 mL/[min kg]. The low GFR cut-off value indicating CKD was set at 1.2 mL/[min kg].

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). 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

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 (SDM$_{\text{plasma}}$, 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 ($\tau_B$) were calculated to investigate the relationship between GFR and SDM$_{\text{plasma}}$ and sCr. Subsequently, we calculated the specificities and sensitivities (and 95% confidence interval) for SDM$_{\text{plasma}}$ 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) 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, 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
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\textsubscript{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\textsubscript{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\textsubscript{B}) revealed for the 49 cats that the correlation between SDMA\textsubscript{plasma} and GFR (τ\textsubscript{B} = −0.57; P < .001) was moderate; however, the correlation between sCr and GFR was of the same magnitude (τ\textsubscript{B} = −0.56; P < .001). High concentrations of both biomarkers were associated with reduced filtration rate. The correlation between the 2 renal biomarkers SDMA\textsubscript{plasma} and sCr was also moderate (τ\textsubscript{B} = 0.52; P < .001).

## 3.3 | Relationship between SDMA and sCr

SDMA\textsubscript{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\textsubscript{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\textsubscript{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 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)\textsuperscript{21,22} |
|---|---|---|---|---|
| **Breed** | **CKD n = 17** | **DM n = 15** | **Control n = 17** | **Total n = 49** |
| Eur SH | n = 11 (65%) | n = 12 (80%) | n = 17 (100%) | n = 40 (82%) |
| British SH | n = 2 | Eastern SH | n = 1 | British SH n = 3 |
| Siamese | n = 1 | British SH n = 1 | Burmese n = 2 |
| Persian | n = 1 | Burmese n = 1 |
| Ragdoll | n = 1 | Persian n = 1 |
| Burmese | n = 1 | Ragdoll n = 1 |
| Eastern SH | n = 1 | Siamese n = 1 |
| | | Eastern SH n = 1 |

| **Sex** | **CKD n = 17** | **DM n = 15** | **Control n = 17** | **Total n = 49** |
|---|---|---|---|---|
| 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 |
|---|---|---|---|---|
| **Group** | **GFR (mL/[min kg])** | **<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.
The associated sensitivities and specificities are also presented in Table 4.

Receiver operating characteristic curves (Figure 2) illustrate that the AUC of SDMAplasma 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]), SDMAplasma 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).
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.\textsuperscript{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\textsubscript{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.\textsuperscript{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.\textsuperscript{24} Therefore, we excluded hyperthyroidism based on a number of diagnostic tools.

### TABLE 4

| Cut-off GFR 1.7 mL/(min kg) | Cut-off GFR 1.2 mL/(min kg) |
|-----------------------------|-----------------------------|
| Sensitivity | Specificity | Sensitivity | Specificity |
| SDMA\textsubscript{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) |
| sCr | 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) |
| 161.8 μmol/L (1.83 mg/dL) | 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) | 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\textsubscript{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. 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 = 7) 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 al27 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 cross-
sectional 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 post-natal 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.

ACKNOWLEDGMENT

No funding was received for this study.

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.
REFERENCES

1. Polzin DJ. Chronic kidney disease in small animals. Vet Clin North Am Small Anim Pract. 2011;41:15-30.
2. Lund EM, Armstrong PJ, Kirk CA, Kolar LM, Klausner JS. Health status and population characteristics of dogs and cats examined at private veterinary practices in the United States. J Am Vet Med Assoc. 1999; 214:1336-1341.
3. Watson ADJ. Indicators of renal insufficiency in dogs and cats presented at a veterinary teaching hospital. Aust Vet Pract. 2001;31: 54-59.
4. Marino CL, Lascelles BDX, Vaden SL, Gruen ME, Marks SL. Prevalence and classification of chronic kidney disease in cats randomly selected from four age groups and in cats recruited for degenerative joint disease studies. J Feline Med Surg. 2014;16:465-472.
5. FAB (Feline Advisory Bureau). WeilCat for life. A guide to engaging your clients in a lifelong partnership. Weilcat Veterinary Handbook. 1st ed. Tisbury, UK: International Cat Care; 2008:1-30.
6. Pape D, Daminet S, Feline CKD. Diagnosis, staging and screening - what is recommended? J Feline Med Surg. 2013;15:15-27.
7. Cannon M. Diagnosis and investigation of chronic kidney disease in cats. In: Pract. 2016;38:2-9.
8. Von Hendy-Willson VE, Pressler BM. An overview of glomerular filtration rate testing in dogs and cats. Vet J. 2011;188:156-165.
9. Braun JP, Lefebvre HP, Watson AD. Creatinine in the dog: a review. Vet Clin Pathol. 2003;32:162-179.
10. Hokamp JA, Nabity MB. Renal biomarkers in domestic species. Vet Clin Pathol. 2016;45:28-56.
11. Forrester SD, Adams LG, Allen TA. Chronic kidney disease. In: Hand MS, Thatcher CD, Remillard RL, et al., eds. Small Animal Clinical Nutrition. 5th ed. Topeka, KS: Mark Morris Institute; 2010:765-810.
12. Lees GE. Early diagnosis of renal disease and renal failure. Vet Clin North Am Small Anim Pract. 2004;34:867-885.
13. Braff J, Obare E, Yerramilli M, Elliott J, Yerramilli M. Relationship between serum symmetric dimethylarginine concentration and glomerular filtration rate in cats. J Vet Intern Med. 2014;28:1699-1701.
14. El-Khoury JM, Bunch DR, Hu B, et al. Comparison of symmetric dimethylarginine with creatinine, cystatin C and their eGFR equations as markers of kidney function. Clin Biochem. 2016;49:1140-1143.
15. Kielstein JT, Fliesser D, Veldink H. Asymmetric dimethylarginine and symmetric dimethylarginine: axis of evil or useful alliance? Semin Dial. 2009;22:346-350.
16. Kopke MA, Burchell RK, Ruach CG, Burton SE, Lopez-Villalobos N, Gal A. Variability of symmetric dimethylarginine in apparently healthy dogs. J Vet Intern Med. 2018;32:736-742.
17. Yerramilli M, Farace G, Quinn J, Yerramilli M. Kidney disease and the nexus of chronic kidney disease and acute kidney injury; the role of novel biomarkers as early and accurate diagnostics. Vet Clin North Am Small Anim Pract. 2016;46:961-993.
18. Hall JA, Yerramilli M, Obare E, Yerramilli M, Yu S, Jewell DE. Comparison of serum concentrations of symmetric dimethylarginine and creatinine as kidney function biomarkers in healthy geriatric cats fed reduced protein foods enriched with fish oil, L-carnitine, and medium-chain triglycerides. Vet J. 2014;202:588-596.
19. Hall JA, Yerramilli M, Obare E, Yerramilli M, Melendez LD, Jewell DE. Relationship between lean body mass and serum renal biomarkers in healthy dogs. J Vet Intern Med. 2015;29:808-814.
20. Hall JA, Yerramilli M, Obare E, Yerramilli M, Jewell DE. Comparison of serum concentrations of symmetric dimethylarginine and creatinine as kidney function biomarkers in cats with chronic kidney disease. J Vet Intern Med. 2014;28:1676-1683.
21. Pape D, Ghys LFE, Smets P, Lefebvre HP, Croubels S, Daminet S. Routine kidney variables, glomerular filtration rate and urinary creatinin C in cats with diabetes mellitus, cats with chronic kidney disease and healthy cats. J Feline Med Surg. 2015;17:880-888.
22. Ghys LFE, Pape D, Lefebvre HP, et al. Evaluation of creatinin C for the detection of chronic kidney disease in cats. J Vet Intern Med. 2016;30:1074-1082.
23. Pape D, Lefebvre HP, Concordet D, van Hoek I, Croubels S, Daminet S. Simplified methods for estimating glomerular filtration rate in cats and for detection of cats with low or borderline glomerular filtration rate. J Feline Med Surg. 2015;17:889-900.
24. Buresova E, Stock E, Pape D, et al. Assessment of symmetric dimethylarginine as a biomarker of renal function in hyperthyroid cats treated with radioiodine. J Vet Intern Med. 2019;33:516-522.
25. van Hoek I, Vandermeulen E, Ducheateau L, et al. Comparison and reproducibility of plasma clearance of exogenous creatinine, exo-lohexol, endo-lohexol, and 51Cr-EDTA in young adult and aged healthy cats. J Vet Intern Med. 2007;21:950-958.
26. Watson ADJ, Lefebvre HP, Concordet D, et al. Plasma exogenous creatinin clearance test in dogs: comparison with other methods and proposed limited sampling strategy. J Vet Intern Med. 2002;16:22-23.
27. Ghys LFE, Pape D, Ducheateau L, et al. Biological validation of feline serum creatinin C: the effect of breed, age and sex and establishment of a reference interval. Vet J. 2015;204:168-173.
28. NCSS. Confidence Intervals for the Area under an ROC Curve. https://ncss-wpengine.netdna-ssl.com/wp-content/themes/ncss/pdf/Procedures/PASS/Confidence_Intervals_for_the_Area_Under_an_ROC_Curve.pdf. Accessed July 21, 2020.
29. IRIS. Kidney Guidelines - IRIS Staging of CKD (modified 2019). http://www.iris-kidney.com/guideline/staging.html. Accessed January 12, 2020.
30. IDEXX. SDMA Frequently Asked Questions. https://www.idexx.com/en/veterinary/reference-laboratories/sdma/sdma-faqs/. Accessed January 12, 2020.
31. Langhorn R, Kieler IN, Koch J, Christiansen LB, Jessen LR. Symmetric dimethylarginine in cats with hypertrophic cardiomyopathy and diabetes mellitus. J Vet Intern Med. 2018;32:57-63.
32. Relford R, Robertson J, Clements C. Symmetric dimethylarginine: improving the diagnosis and staging of chronic kidney disease in small animals. Vet Clin North Am Small Anim Pract. 2016;46:941-960.
33. Pelander L, Häggström J, Larsson A, et al. Comparison of the diagnostic value of symmetric dimethylarginine, cystatin C, and creatinine for detection of decreased glomerular filtration rate in dogs. J Vet Intern Med. 2019;33:630-639.
34. Nabity MB, Lees GE, Boggess MM, et al. Symmetric dimethylarginine assay validation, stability, and evaluation as a marker for the early detection of chronic kidney disease in dogs. J Vet Intern Med. 2015; 29:1036-1044.
35. Ernst R, Ogeer J, McCrann D, et al. Comparative performance of IDEXX SDMA test and the DLD SDMA ELISA for the measurement of SDMA in canine and feline serum. PLoS One. 2018;13:e0205030.