

# Symmetric Dimethylarginine

## Improving the Diagnosis and Staging of Chronic Kidney Disease in Small Animals



Roberta Relford, DVM, MS, PhD, Jane Robertson, DVM, Celeste Clements, DVM\*

### KEYWORDS

- SDMA • Symmetric dimethylarginine • Renal biomarker • Chronic kidney disease
- CKD • IRIS • IDEXX SDMA test • GFR

### KEY POINTS

- Symmetric dimethylarginine (SDMA) is a new kidney biomarker that accurately reflects glomerular filtration rate (GFR).
- SDMA level increases earlier in chronic kidney disease (CKD), on average with 40% reduction of GFR, compared with up to 75% reduction needed to increase creatinine level.
- Unlike creatinine, SDMA is not affected by lean body mass so it is a more sensitive indicator of kidney function in patients with muscle loss.
- The validated immunoassay for SDMA, the IDEXX SDMA test, is a clinically relevant and reliable tool for diagnosing early CKD in small animals when creatinine level is still within the reference interval.
- SDMA was added to the International Renal Interest Society CKD guidelines to complement creatinine testing in staging early and advanced disease.

### INTRODUCTION

The diagnosis and management of chronic kidney disease (CKD) is a routine part of clinical small animal practice. CKD is a common problem seen throughout the lives of pets but increases in frequency as pets age. The prevalence of CKD has recently been identified to be greater than previously reported.<sup>1</sup> Patients diagnosed with CKD are often managed successfully for years by the partnership of a diligent veterinary staff and motivated pet owners. Use of the International Renal Interest Society

---

Disclosure: Drs R. Relford, J. Robertson, and C. Clements are currently employed by IDEXX. Companion Animal Group Medical Organization, IDEXX, 1 IDEXX Drive, Westbrook, ME 04092, USA

\* Corresponding author.

E-mail address: [celeste-clements@idexx.com](mailto:celeste-clements@idexx.com)

Vet Clin Small Anim 46 (2016) 941–960  
<http://dx.doi.org/10.1016/j.cvsm.2016.06.010>

[vetsmall.theclinics.com](http://vetsmall.theclinics.com)

0195-5616/16/© 2016 IDEXX Laboratories Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

(IRIS) CKD guidelines for staging and treatment of patients with CKD encourages standardized and informed management practices to address common complications.<sup>2</sup> Symmetric dimethylarginine (SDMA), a novel kidney biomarker, permits earlier diagnosis of kidney disease than traditional creatinine testing, and has been included provisionally as part of the IRIS CKD guidelines, as modified in 2015, for staging of both early and advanced CKD.<sup>2</sup> On diagnosis of CKD, veterinarians should investigate for underlying conditions and complications that could be treated. Staging of CKD allows customized patient management for the best possible outcome.

## DISCOVERY

SDMA was first identified in 1970<sup>3</sup> and later characterized as a molecule that is primarily cleared by the kidneys. SDMA emerged as a candidate kidney biomarker during investigations into the pathogenicity of a closely related compound, asymmetric dimethylarginine (ADMA), in people with advanced CKD.<sup>4</sup> Vallance and colleagues<sup>4</sup> found increased concentrations of both dimethylarginines, SDMA and ADMA, in a group of hemodialysis patients. They concluded that ADMA was a potent inhibitor of nitric oxide (NO) synthesis and proposed that ADMA might be contributing to the hypertension, immune dysfunction, and cardiovascular disease that complicate CKD.<sup>4</sup> They also recognized that significant metabolism of ADMA occurs before it reaches the kidney, whereas SDMA was primarily cleared by the kidney, which further differentiated the two molecules. SDMA's value as a kidney biomarker was not identified. Because no active role for SDMA was identified in the pathogenesis of CKD and their focus was more on hypertension and heart disease, SDMA was not the immediate target of additional research in methylated arginines.<sup>5</sup>

In 1997, Marescau and colleagues<sup>6</sup> reported a strong correlation between serum and urine concentrations of SDMA and kidney dysfunction by estimating glomerular filtration rate (GFR) with creatinine clearance ( $R$  of  $-0.916$ ;  $P < .0001$ ) in 135 people with CKD, and suggested serum SDMA as a good marker of kidney disease. Serum SDMA level increased as kidney function declined, as shown by GFR decline, in an inverse relationship, with negative correlation ( $R$  value). This work was later included as one of 18 studies in a powerful meta-analysis that showed highly significant correlations between SDMA and kidney function tests in people.<sup>7</sup>

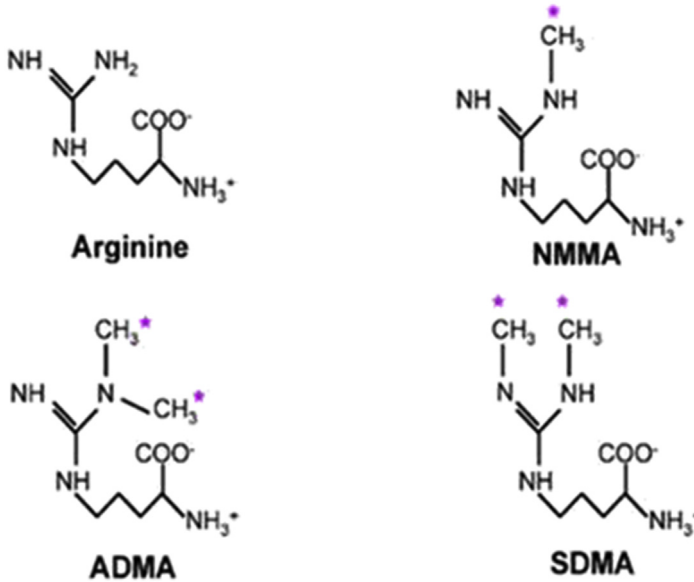
In the first clinical study of SDMA in veterinary patients with spontaneous kidney disease, Jepson and colleagues<sup>8</sup> reported that SDMA correlated well with creatinine ( $r = 0.741$ ;  $P < .001$ ) in 69 cats with CKD and hypertension.

## BIOCHEMISTRY

### *Methylarginine Synthesis*

---

SDMA is a stable molecule that originates from intracellular proteins that play an integral role in basic cellular metabolism. SDMA and related compounds are produced in the nucleus of all cells. Their formation occurs by obligate posttranslational modification and methylation of arginine residues of various proteins and subsequent proteolysis.<sup>7</sup> The molecular structures of arginine, SDMA, and other methylated products of protein metabolism are shown in [Fig. 1](#). The family of enzymes, arginine *N*-methyltransferases (PRMT), symmetrically methylate arginine residues of histones, spliceosomal Sm proteins, and receptor tyrosine kinases that generate SDMA and NG-monomethyl-L-arginine (NMMA) on their hydrolysis.<sup>9,10</sup> Other PRMT enzymes asymmetrically methylate histones and myelin basic protein,<sup>11</sup> which liberate ADMA and NMMA when degraded.<sup>12,13</sup> Note that although these substances are made



**Fig. 1.** Molecular structure for arginine and methylated arginines. NMMA, NG-monomethyl-L-arginine.

during a similar process, their mechanisms in the body and their clearance processes are very different.

### **Elimination**

SDMA's small molecular size (molecular weight [MW], 202 g/mol)<sup>14</sup> and positive charge allow it to be freely filtered by glomerular filtration. Because SDMA is largely excreted by the kidney, it is a good candidate biomarker for kidney function, whereas highly protein-bound ADMA undergoes extensive metabolism by the tissue-specific enzyme dimethylarginine dimethylaminohydrolase.<sup>7,13</sup> In a 2011 review, Schwedhelm and Böger<sup>5</sup> estimated the renal excretion of SDMA to be greater than or equal to 90%, with putative cleavage of the remainder by an unnamed enzyme. In contrast, only about 20% of ADMA is excreted into the urine.<sup>5</sup> The extensive renal clearance of SDMA explains its correlation with other kidney clearance markers and its potential suitability as an endogenous kidney biomarker.

## **CURRENT CHALLENGES WITH ASSESSING KIDNEY FUNCTION**

### **Glomerular Filtration Rate**

Direct measurement of GFR is the gold standard for quantitative assessment of kidney filtration but is not routinely performed on dogs and cats because of the need to administer a suitable filtration marker and obtain multiple timed blood samples and/or urine samples, which are both difficult for the pet and time consuming.<sup>15</sup> Although people might think that a gold standard is an agreed-on process, there are a variety of products, methods, and calculations to formulate GFR and there are perceived difficulties with each protocol. Renal clearance methods that measure the appearance of the marker substance in the urine were once preferred to plasma clearance methods, with inulin commonly regarded as the ideal marker substance because it

is safe and inert, and is eliminated solely via glomerular filtration, without tubular reabsorption or secretion.<sup>16</sup> However, the complicated nature of the inulin assay and the requirement for accurate and complete urine recovery have made inulin renal clearance testing unpopular outside the research setting.<sup>17</sup> Alternate markers validated for GFR measurement in dogs and cats include endogenous or exogenous creatinine, cystatin C, iohexol, and radiolabeled molecules,<sup>15</sup> including [<sup>125</sup>I] sodium iothalamate and [<sup>131</sup>I] sodium iodohippurate.<sup>17</sup>

Plasma clearance of iohexol and exogenous creatinine offer practical and accurate methods to estimate GFR in a clinical setting. Iohexol is a commonly available iodinated contrast agent that may be given by single intravenous bolus injection. Plasma samples are collected at predetermined, accurately recorded times, usually 2, 3, and 4 hours after injection, before assay of the iodine components by mass spectroscopy or other methods, which is available only at a select few locations, without the need for urine.<sup>18</sup> Plasma clearance results are calculated using pharmacodynamics algorithms from marker decline on successive samples and are reported as the volume of plasma that has been cleared of the marker substance over a given interval of time, in milliliters per minute per kilogram of patient body weight,<sup>17</sup> ideally compared with a cohort of normal animals of the same species, breed, and size.

GFR continues to be a valuable measure of kidney function, although the complicated nature of testing and associated expense reduces the clinical utility of clearance methods. To help solve this problem in human patients, clearance methods are often replaced by calculated (rather than measured) estimates of GFR using serum creatinine (sCr) level, body weight, and various correction factors based on the patient's gender, age, and race, alone or in conjunction with a timed urine collection for a urinary creatinine clearance.<sup>7</sup> The muscle mass and protein intake of the individual should also be considered. However, there is no standard agreement on which formula should be used and each formula can provide a different GFR estimate, again making estimating GFR difficult. Similar algorithms for estimating GFR from sCr level in dogs and cats are inaccurate because of greater individual gender and breed variation.<sup>19</sup> These challenges of determining GFR continue to fuel the investigation into endogenous biomarkers of kidney function.<sup>7</sup>

### ***Poor Sensitivity and Specificity of Serum Creatinine Testing***

Measurement of sCr level has been the most widely used indirect estimate of GFR in veterinary medicine because its small MW<sup>20</sup> and neutral charge allow it to be freely filtered by the glomeruli. SCr has an inverse but nonlinear relationship to GFR; that is, sCr level increases exponentially as GFR declines. The steep curvilinear relationship between sCr and GFR poses a significant limitation to the sensitivity of sCr level for detecting early kidney disease because significant changes in GFR are reflected by modest or minimally detectable changes in sCr, and early kidney disease might be missed.<sup>21</sup> The converse is also true: in advanced disease, small changes in GFR make a large impact on sCr level, but this may have fewer clinical implications. SCr level has been shown to not increase beyond laboratory reference intervals until up to 75% of functional renal mass has been lost. The sensitivity of sCr level can be improved by establishing a baseline for each individual pet while in good health and then trending sCr over time using a consistent laboratory and analytical method.<sup>21–23</sup> Increases and degree of magnitude in sCr level cannot determine reversibility of the kidney disease or localization to renal, prerenal, or postrenal sources.<sup>24</sup> By the time sCr level has increased in CKD, the nephron loss is often irreversible and long-term prognosis may be poor.

A major preanalytical limitation of sCr level is dependence on muscle mass. Although regarded as a specific marker of kidney filtration, sCr level may be significantly increased in heavily muscled dogs, or significantly decreased in dogs and cats with muscle loss.<sup>20</sup> Comprehensive descriptions of the breed variability of sCr are lacking, but the greyhound is often cited as a breed with higher than expected sCr levels in health.<sup>25</sup> Evaluating kidney function in patients with increased muscle mass requires careful consideration of the patient's general health status; complete urinalysis findings, including appropriateness of the urine specific gravity; and any history, physical findings, or imaging results that suggest kidney disease. Creatinine level can significantly underestimate the degree of kidney disease present when dogs or cats lose muscle mass because of aging or any chronic disease, especially protein wasting diseases, cancer, or advanced kidney disease. In these scenarios, sCr level overestimates the degree of remaining kidney function.<sup>26,27</sup> These common clinical conditions create the need for a more sensitive and specific kidney biomarker.

A commonly overlooked cause of increases in sCr level is diet. A study in humans shows that sCr level can increase by 20% after eating cooked meat.<sup>28</sup> Studies in dogs show a similar effect following ingestion of meat. Six dogs were each fed soft moist, raw, and boiled meat in a crossover feeding trial. Following ingestion, sCr concentrations increased in all dogs; and a persistent increase was noted for several hours in the dogs fed boiled meat. The raw and soft moist diets were associated with an initial increase that was followed by a decline in sCr level.<sup>29</sup> These findings support the recommendation that, when measuring sCr level, the patient should be fasted for an accurate determination of kidney function.

#### ***Analytical Variability of Serum Creatinine Measurements***

---

The fact that diagnostic reagents for measurement of sCr level are economical and widely available for reference laboratory and point-of-care testing has helped sCr to become the major predictor of kidney function. However, the commonly used Jaffe method is not specific for creatinine and, according to some estimates, noncreatinine compounds may contribute as much as 45% to 50% to reported sCr values.<sup>18,20</sup> The modification to the Jaffe method has addressed some of these challenges. What has remained problematic is that different laboratory reagents and methods of measurement continue to result in nonstandardized laboratory-dependent reference intervals for sCr level. Analytical variability within and between the creatinine assay is also a limitation. SDMA is a novel biomarker that can help to clarify the inaccuracies and non-kidney variables associated with sCr as a diagnostic.

#### ***Criteria for a Better Kidney Biomarker***

---

Concentrations of soluble serum and urinary compounds that change consistently with early kidney damage have been the focus of study in people with naturally occurring kidney disease and laboratory animal models of nephrotoxicity because the current testing has limitations. As discussed previously, clinical testing for kidney disease in most species relies on sCr measurement. In addition, although sCr seems to satisfy the definition of a biomarker proposed by Puntmann,<sup>30</sup> namely that "A biomarker is a characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention,"<sup>30</sup> it has limitations and the authors suggest criteria for a better biomarker than sCr.

In the clinical setting, sCr is primarily a diagnostic and staging test. A better kidney biomarker should be more sensitive and specific than sCr, compared with the gold standard, with the clear ability to predict or exclude disease in an individual patient

relative to a diverse population with many breeds, sizes, ages, and comorbidities.<sup>30</sup> Receiver operating curves generating  $R$  values assist with this comparison. Biomarkers for diagnosing acute conditions, like acute kidney injury, should appear early and in proportion to the magnitude of the insult, with analysis available in real time, especially at the point of care.<sup>30</sup>

For accurate monitoring of chronic conditions, such as CKD, a biomarker with narrow biological variability improves the assessment of longitudinal changes.<sup>30</sup> A good kidney biomarker should perform consistently in individual patients regardless of whether they are aged, underweight, with variable nutrition, or affected with multiple concurrent health concerns. The candidate biomarker for CKD should then be studied to show that it has a positive impact on patient outcome, with improved quality of life and survival times, compared with other methods.

Early studies of rodent and dog remnant kidney models of CKD support SDMA as an endogenous kidney biomarker. SDMA correlated well with creatinine clearance after partial nephrectomy in rats<sup>31</sup> and with creatinine and blood urea nitrogen (BUN) levels in rats after total nephrectomy.<sup>32</sup> In 10 dogs undergoing partial or complete nephrectomy, plasma SDMA concentrations increased with reductions in renal mass and correlated well with GFR by inulin clearance ( $r$  value of  $-0.851$ ;  $P < .0001$ ) and with sCr ( $r$  value of  $-0.749$ ;  $P = .0013$ ).<sup>33</sup>

Reviewing 18 early studies of 2136 human patients, Kielstein and colleagues<sup>7</sup> found that systemic SDMA concentrations correlated highly with GFR by inulin clearance ( $R = 0.85$ ; confidence interval,  $0.76-0.91$ ;  $P < .0001$ ), as well as with sCr ( $R = 0.75$ ; confidence interval,  $0.46-0.89$ ;  $P < .0001$ ), concluding that SDMA should be further investigated as a marker of renal function.<sup>7</sup> In recent GFR studies using continuous infusion of very-low-dose of iohexol, SDMA accurately and precisely estimated GFR in people, and was more sensitive than sCr.<sup>34</sup> In people with and without CKD, SDMA outperformed creatinine and creatinine-based equations in estimating kidney function compared with measured GFR.<sup>35</sup>

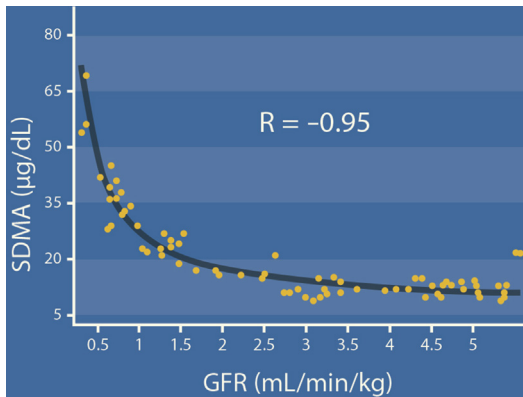
These cumulative data from animal experiments and studies in people supported the investigation of SDMA as a kidney biomarker for cats and dogs.

### **SYMMETRIC DIMETHYLARGININE CORRELATES WITH GLOMERULAR FILTRATION RATE** ***Dogs with X-linked Hereditary Nephropathy***

Nabity and colleagues<sup>22</sup> recently published a prospective foundational study evaluating SDMA as a marker for kidney disease in a colony of dogs with progressive x-linked hereditary nephropathy (XLHN). They validated the SDMA assay for dogs using liquid chromatography–mass spectroscopy (LC-MS), then compared serial SDMA measurements with sCr and GFR by exogenous plasma iohexol clearance in a cohort of 8 affected male dogs and 4 unaffected littermate controls.

Intra-assay precision coefficient of variability (CV) of the LC-MS was 1.5% to 2.8% (mean of 2.2%) and the inter-assay precision was 2.3% to 3.7% (mean of 2.7%), both with 98% accuracy or greater, consistent with excellent analytical performance.<sup>22</sup> Preanalytical factors such as added hemoglobin, lipids, bilirubin, arginine, monomethylarginine, ADMA, and homocitrulline did not interfere with SDMA measurement. SDMA was highly stable in canine serum or plasma, resisting significant change at room (20°C) and refrigerator (4°C) temperatures for 14 days.<sup>22</sup>

The subjects were studied over 37 weeks, with 6 of 8 affected dogs reaching the targeted end point of sCr level greater than or equal to 5 mg/dL. In affected dogs, SDMA level increased during progression from preclinical disease to end-stage



**Fig. 2.** Serum SDMA correlates with decreasing GFR by iohexol clearance in affected male dogs with XLHN ( $R = -0.95$ ). (From Nabity NB, Lees GE, Boggess MM, et al. Symmetric dimethylarginine assay validation, stability, and evaluation as a marker for the detection of chronic kidney disease in dogs. *J Vet Intern Med* 2015;29:1040; with permission.)

kidney disease, correlating strongly with an increase in sCr level ( $r = 0.95$ ), and with a decrease in GFR ( $r = -0.95$ ), as shown in **Fig. 2**. An SDMA cutoff of greater than or equal to 14 µg/dL identified, on average, a less than 20% decrease in GFR, which was earlier than sCr by any comparison method, including using the sCr cutoff for azotemia at greater than or equal to 1.2 mg/dL, serial trending of sCr levels, or in comparison with sCr levels of unaffected littermates.<sup>22</sup> **Fig. 3** compares the rapid clinical course of an affected dog with XLHN with an unaffected littermate control.

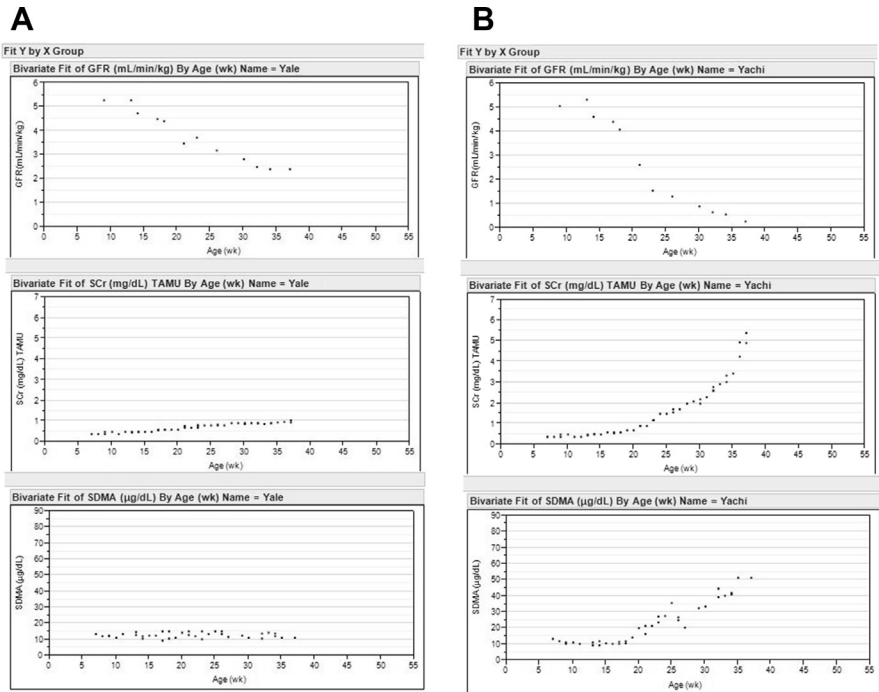
Affected male dogs with XLHN have mutations in the genes coding for glomerular type IV collagen and develop proteinuric end-stage kidney disease between 6 and 18 months of age, creating a convenient model of rapidly progressive CKD.<sup>36</sup> The heterozygous carrier females develop proteinuria as juveniles, but most have sufficient normal glomerular basement membrane to maintain structural and functional integrity, with clinical normalcy, adequate urine concentrating ability, and normal sCr levels until they are about 5 years old.<sup>37</sup> SDMA also correlated strongly with the stable GFR of the colony's female carriers ( $R^2 = 0.85$ ) (Mary Nabity, DVM, PhD, DACVP, College Station, TX, personal communication, 2013.).

### **Cats, Azotemic and Nonazotemic**

In a retrospective analysis, SDMA level was measured by LC-MS on previously frozen serum samples from 10 client-owned cats and compared with their plasma creatinine concentrations and GFRs by exogenous plasma iohexol clearance from the same date. Serum SDMA levels and GFR were correlated strongly ( $R^2 = 0.82$ ;  $P < .001$ ) across a range of GFRs from 0.54 to 2.37 mL/min/kg (**Fig. 4**), whether cats were azotemic or not.<sup>38</sup>

### **REFERENCE INTERVAL DEVELOPMENT**

Reference intervals for dogs and cats were established following Clinical Laboratory Standards Institute (CLSI) guidelines<sup>39</sup> to facilitate the continued development of SDMA as a clinical tool to measure kidney function.



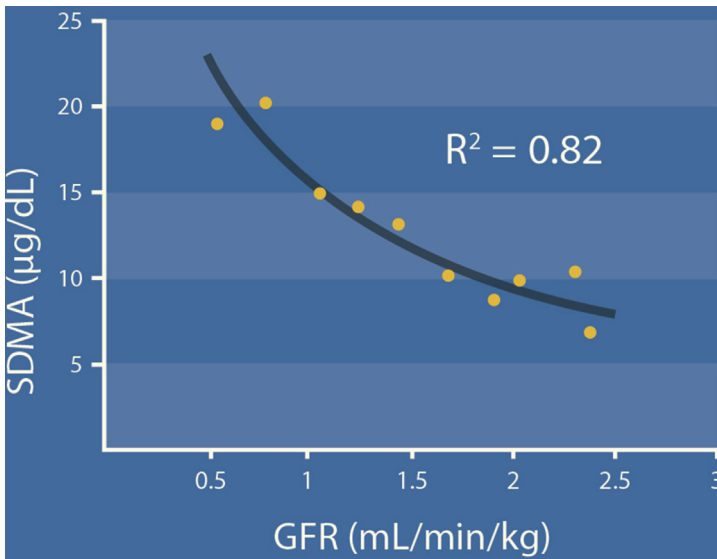
**Fig. 3.** Typical clinical course of progressive kidney disease over 55 weeks in a dog with XLHN compared with control litter mate, showing increasing SDMA and sCr levels as GFR declines. (A) The dog Yale represents an age-matched control from the litter. His results show an initial decline in GFR with maturation, seen in all the control pups, that plateaued after 30 weeks, with stable GFR, sCr level, and SDMA level. (B) In contrast, the affected dog Yachi's results show a progressive decline in kidney function beginning at approximately 15 weeks that reached the study end point of sCr level greater than or equal to 5 mg/dL at approximately 38 weeks of age. SDMA concentration increased as sCr level increased and GFR decreased. TAMU, Texas A&M University. (Courtesy of Mary Nabity, DVM, PhD, DACVP, College Station, TX, personal communication, 2013.)

### Dogs

Serum samples were collected from 122 clinically healthy, adult dogs (defined as  $\geq 1$  year of age) of varying gender, age, and breed attending a heartworm clinic. The health status of each dog was based on physical examination; lack of any history of constitutional signs or illness over the last 6 months; and prescription medication limited to chemoprophylaxis for heartworm, fleas, and ticks. For each dog the following information was recorded: diet, results of comprehensive complete blood count with slide review, chemistry panel with electrolytes and total thyroxine concentrations, and when available results from a complete urinalysis, urine culture with minimum inhibitory concentration and urine protein/creatinine ratio.

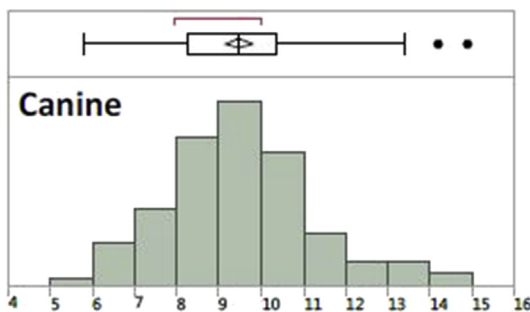
There were 151 dogs examined; 28 were excluded because of age (<1 year of age) or health reasons. One dog was removed because of immeasurable sCr level. Dogs ranged in age from 1 to 15 years, with a mean age of 4.7 years. Males and females were equally represented. Body weights varied between 2.7 and 60 kg, with a wide variety of body condition scores (data courtesy of IDEXX, Westbrook, ME.)



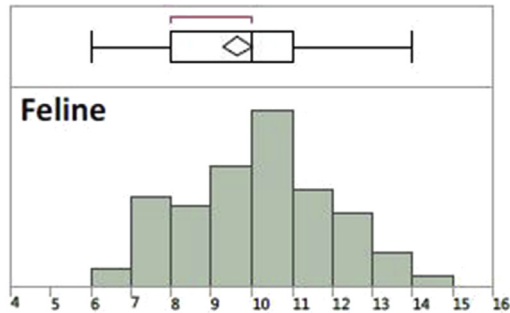


**Fig. 4.** The inverse relationship, with strong correlation ( $R^2 = 0.82$ ;  $P < .001$ ), between serum SDMA level (y axis) and GFR (x axis) in 10 client-owned cats with varied kidney function. (Data from Braff J, Obare E, Yerramilli M, et al. Relationship between serum symmetric dimethylarginine concentration and glomerular filtration rate in cats. *J Vet Intern Med* 2014;8:1699–701.)

Serum SDMA was measured by LC-MS as previously established and results analyzed with a nonparametric model, using a 2-sided 95% confidence interval. After exclusion of 2 outliers that were outside the mean, plus or minus 3 standard deviations, the reference interval for healthy adult dogs was established at less than 14  $\mu\text{g/dL}$ , as shown in [Fig. 5](#).<sup>40</sup>



**Fig. 5.** Canine serum SDMA concentration (LC-MS) ( $\mu\text{g/dL}$ ) plotted on the x axis against proportion of the reference population of healthy adult dogs ( $n = 122$ ) on the y axis. The reference interval was established at less than 14  $\mu\text{g/dL}$  with nonparametric analysis. The box and whiskers plot reflects the interquartile range, with outliers represented by dots. (From Rentko V, Nabity M, Yerramilli M, et al. Determination of serum symmetric dimethylarginine reference limit in clinically healthy dogs [ACVIM abstract P-7]. *J Vet Intern Med* 2013;27:750; with permission.)



**Fig. 6.** Feline serum SDMA results (LC-MS) ( $\mu\text{g/dL}$ ) plotted on the x axis against proportion of the reference population of healthy adult cats ( $n = 86$ ) on the y axis. The reference interval was established at less than  $14 \mu\text{g/dL}$  with nonparametric analysis. The box and whiskers plot reflects the interquartile range. (Courtesy of IDEXX, Westbrook, ME.)

### Cats

The reference interval for serum SDMA in cats was established similarly at less than  $14 \mu\text{g/dL}$  (Fig. 6). Data were collected from 86 clinically healthy, adult cats, aged 6 to 15 years, comprising domestic short hair (DSH), domestic longhair, Siamese, and ragdoll breeds. The cats were of both genders and weighed between 3.0 and 9.0 kg (data courtesy of IDEXX, Westbrook, ME).

### SYMMETRIC DIMETHYLARGININE LEVEL INCREASES EARLIER THAN CREATININE

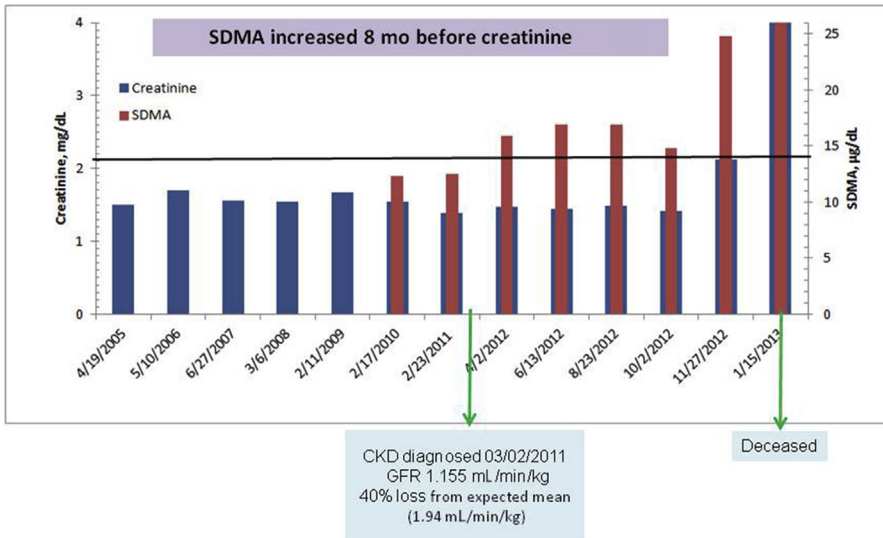
With normal SDMA concentrations established for healthy dogs and cats, researchers extended testing of its utility as a diagnostic tool for kidney disease.

### Cats

For 21 cats with CKD living at the Hill's Pet Nutrition colony, SDMA level was measured in banked frozen serum samples and compared with available documented sCr and GFR assessments at various time points throughout the cats' lives before and after a diagnosis of CKD. In this retrospective longitudinal study, SDMA level increased to greater than the reference interval on average 17 months before sCr, with a range of 1.5 to 48 months. SDMA level was found to be increased in the cats with CKD when there was on average a 40% reduction in their GFR from the median GFR of the healthy cats in the same colony. In 2 cats SDMA level was increased when there was only a 25% reduction in GFR from the designated normal for the colony. Serial results from a representative case are provided in Fig. 7, showing an increase in SDMA to greater than the reference interval 8 months before sCr. During this same interval, sCr level remained stable and did not trend upward as the kidney disease progressed.<sup>41</sup>

### Dogs

A similar study performed in 19 dogs with CKD showed that SDMA increased to greater than the reference interval before sCr, in 17 of 19 dogs, on average 9.8 months earlier, with a range of 2.2 to 27 months. SDMA level was significantly correlated with GFR ( $r = -0.80$ ;  $P < .001$ ).<sup>42</sup> The longitudinal laboratory data from an 11-year-old, male, castrated Beagle in the colony are presented in Fig. 8 to show that SDMA level increased to greater than the reference interval 19 months before sCr in this dog with CKD.<sup>42</sup> Postmortem renal histopathology confirmed lymphocytic/plasmacytic



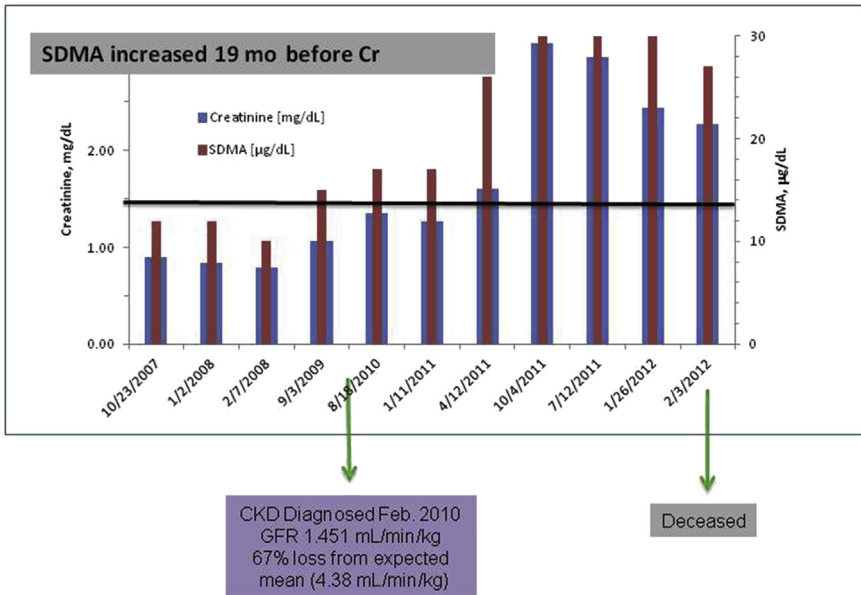
**Fig. 7.** Mystic, born 2001, a 12-year-old, neutered male DSH, was diagnosed with CKD in March of 2011 when GFR was 40% reduced from the expected mean of the colony. SCr level is on the left y axis and SDMA is on the right y axis. Time is on the x axis. The solid black line represents the upper end of the reference interval for both creatinine at 2.1 mg/dL and the upper end of the reference interval for SDMA at 14 µg/dL. In the bar graphs, creatinine is represented by the blue bars and SDMA is represented by the red bars. When a bar crosses over the black line, then the analyte result is increased. SDMA level increased 8 months before sCr, which was stable until a period of acute decompensation. (From Hall JA, Yerramilli M, Obare E, et al. Comparison of serum concentrations of symmetric dimethylarginine and creatinine as kidney function biomarkers in cats with chronic kidney disease. *J Vet Intern Med* 2014;28:1680; with permission.)

interstitial nephritis, with interstitial and periglomerular fibrosis, tubular ectasia, and glomerulosclerosis; changes that are consistent with CKD.<sup>43</sup>

### SPECIFICITY OF SYMMETRIC DIMETHYLARGININE

There is considerable evidence across species supporting SDMA as a specific endogenous renal biomarker that is not influenced by extrarenal factors. In people, SDMA level did not change with acute inflammatory response,<sup>44</sup> hepatic disease,<sup>45,46</sup> cardiovascular disease,<sup>47,48</sup> or diabetes,<sup>49</sup> unless there was concurrent kidney disease. SDMA concentrations did not change in preeclamptic women receiving oral arginine supplementation.<sup>50</sup>

SDMA level was not significantly increased after vigorous exercise in sled dogs with normal BUN values, and was not influenced by breed or gender in a cohort of dogs, unlike ADMA and nitric oxide metabolites, which are known markers of endothelial function.<sup>51</sup> In a group of Cavalier King Charles spaniels, SDMA level was not affected by age or asymptomatic mitral regurgitation.<sup>52</sup> Internal studies at IDEXX showed no correlation between SDMA and serum arginine levels measured in dogs and cats ( $R^2 = 0.002$ ). There was no correlation between the cardiac biomarker N-terminal pro-brain natriuretic peptide and SDMA in approximately 300 dogs over a wide range of results ( $R^2 = 0.0043$ ). Liver enzyme concentration, as a proxy for liver disease, and SDMA level were not correlated: alkaline phosphatase ( $R^2 = 0.01$ ), alanine transaminase ( $R^2 = 0.02$ ), or aspartate aminotransferase ( $R^2 = 0.05$ ) (data courtesy of IDEXX).



**Fig. 8.** Nicholas, an 11-year-old, neutered male Beagle, was diagnosed with CKD in February 2010 when his GFR was decreased by 67% of the expected mean of the healthy dogs in the same colony. Creatinine is on the left y axis and SDMA is on the right y axis. Time is on the x axis. The solid black line represents the upper end of the reference interval for both creatinine at 2.1 mg/dL and the upper end of the reference interval for SDMA at 14 µg/dL. In the bar graphs, creatinine is represented by the blue bars and SDMA is represented by the red bars. When a bar crosses over the black line, then the analyte result is increased. (From Hall JA, Yerramilli M, Obare E, et al. Serum concentrations of symmetric dimethylarginine and creatinine in dogs with naturally occurring chronic kidney disease. *J Vet Intern Med* 2016;30:799; with permission.)

Prospective veterinary studies have shown that, unlike sCr, SDMA level is independent of influences of lean body mass, so it is a more sensitive marker for kidney disease than sCr in patients with a wide variety of reasons for muscle loss. A study in older cats with age-related loss of muscle mass, as measured by dual energy x-ray absorptiometry, confirmed that creatinine level underestimates the loss of kidney function as GFR declines. In contrast, SDMA level showed no correlation with lean body mass. GFR declined with age, and serum SDMA level increased in concordance, better identifying the function loss. These results support the conclusion that SDMA is a more sensitive indicator of loss of kidney function.<sup>26</sup> A complementary study in healthy dogs showed comparable findings of a dependent correlation between lean body mass and creatinine level ( $r = 0.54$ ;  $P = .0003$ ), whereas SDMA level was not influenced by total lean body mass ( $r = -0.12$ ;  $P = .45$ ).<sup>27</sup>

## INTERNATIONAL RENAL INTEREST SOCIETY CHRONIC KIDNEY DISEASE GUIDELINES INCLUSION

IRIS was created in 1998 to help veterinary practitioners better understand, diagnose, and treat renal disease in dogs and cats. The board members created standardized staging guidelines to educate and encourage the best practices for managing kidney disease after diagnosis.

In 2015, SDMA was incorporated provisionally into the updates for IRIS CKD staging guidelines, acknowledging SDMA as a renal function test to complement sCr in evaluating patients with early kidney disease, because it may be a more sensitive biomarker of renal function than fasted blood creatinine concentrations.<sup>2</sup> Persistent increases in SDMA greater than 14 µg/dL suggest reduced kidney function and the possibility of IRIS CKD stage 1 in patients with sCr level less than the IRIS cutoff of 1.4 mg/dL for dogs and 1.6 mg/dL for cats. Thus SDMA can help to identify dogs and cats in IRIS stage 1 and early IRIS stage 2 in which clinical signs are absent or mild and creatinine level has not increased to greater than the reference interval.

The early recognition of kidney disease provides the opportunity to investigate for an underlying cause, manage associated diseases and the CKD, and plan for monitoring the patient accordingly. Investigating when kidney disease is in the early stages increases the likelihood of finding treatable causes such as upper urinary tract infection; vector borne diseases such as Lyme disease, ehrlichiosis, or leishmaniasis; obstructive urolithiasis; or chronic toxicities. Investigating for complications such as proteinuria and hypertension that may accelerate kidney disease is an important aspect of substaging CKD.<sup>2</sup> Even if no underlying cause or complications are identified, earlier diagnosis and treatment may slow the rate of progression of CKD and increase the pet's life span.<sup>53</sup>

Monitoring SDMA in patients with CKD can help to better identify progression of disease as dogs and cats lose weight, especially if it is associated with a loss of muscle condition.<sup>26,27</sup> According to the IRIS guidelines, patients with low body condition scores that are in stage 2 or stage 3 based on creatinine level and that also show an increased SDMA level greater than or equal to 25 µg/dL or 45 µg/dL, respectively, may be staged inappropriately low and the degree of renal dysfunction may be underestimated. Treatment of clinical signs or laboratory findings of a more advanced stage of CKD might be appropriate; that is, CKD stage 3 and stage 4.<sup>2</sup> Increased practitioner awareness and use of a muscle condition scoring (MCS) system as proposed by the World Small Animal Veterinary Association Global Nutrition Committee<sup>54</sup> assists with proper evaluation of patients with CKD. The MCS system is helpful in targeting muscle loss because it is possible for pets to be overweight and still have muscle loss.<sup>55</sup>

Enhanced evaluation and recognition of kidney disease with SDMA and IRIS staging may help veterinarians to better manage CKD and assist with objective patient monitoring and communication of measurable goals. Pet owners can be engaged to participate in this important team effort to optimize their pets' renal care for improved quality and length of life. IRIS recognizes that, compared with creatinine, SDMA may be a more sensitive biomarker of excretory renal function, and that SDMA can be a useful adjunct for the early diagnosis of CKD, as well as a guide for management of more advanced CKD. SDMA testing should be run alongside sCr, BUN, and a complete urinalysis to provide a comprehensive picture of kidney function.

## THE IDEXX SYMMETRIC DIMETHYLARGININE TEST

### *Validation*

The LC-MS analysis for SDMA, although extremely accurate and considered the gold standard, can be costly and time consuming, and thus inconvenient to add to the routine laboratory minimum data base for sick and well pets. The IDEXX SDMA test is a novel, high-throughput, competitive homogeneous immunoassay using a glucose-6-phosphate dehydrogenase conjugate and anti-SDMA monoclonal antibody to quantify SDMA in serum and plasma.<sup>56</sup> The technique is especially useful when developing an immunoassay for a small biomarker molecule such as SDMA,<sup>56</sup> which

is less immunogenic because of its small size.<sup>5</sup> The assay was validated following US Food and Drug Administration and CLSI standards for dogs and cats, using healthy and CKD populations.<sup>57</sup> Accuracy was confirmed by comparing the results with the LC-MS standard over a dynamic range of 5 to 100  $\mu\text{g}/\text{dL}$ . In the range of 10 to 20  $\mu\text{g}/\text{dL}$ , within-run precision was less than or equal to 7% CV, and total precision was less than or equal to 10% CV.<sup>56,57</sup>

The reference interval for the IDEXX SDMA test, established at less than or equal to 14  $\mu\text{g}/\text{dL}$ , is based on transference analysis of SDMA data by LC-MS from healthy dogs and cats (data courtesy of IDEXX<sup>40</sup>), considering mean bias, method precision, and whole-number rounding of the original data.

### **Clinical Use**

The SDMA test is available from IDEXX Reference Laboratories. The IDEXX SDMA test is performed along with all routine chemistries on a multichannel analyzer (Beckman Coulter, Inc, Brea, CA). SDMA results are provided at the same time as all other chemistry results without a delay in the reporting time. Serum is the preferred sample type; lithium heparin or EDTA plasma is also acceptable. The IDEXX SDMA test is not affected by mild to moderate hemolysis or any degree of lipemia or icterus. An interpretive comment is provided with all SDMA results to assist with interpretation along with creatinine results. For patient results with increased IDEXX SDMA and sCr within the reference interval, early kidney disease is likely and further investigation is indicated, as suggested in **Box 1**.

SDMA complements traditional tests for kidney disease. To diagnose kidney disease, the patient's clinical presentation, physical examination findings, and results of laboratory testing and imaging should be considered. The clinical presentation includes the signalment, consisting of age, breed, and gender; any relevant history, such as medication use, possible exposure to toxins, diet, and travel; with possible exposure to infectious diseases that may be risk factors for kidney disease, such as Lyme disease or leptospirosis.

In early CKD, clinical signs are often absent. With progression of CKD these signs are more common:

- Polyuria and polydipsia
- Decreased appetite
- Weight loss
- Lethargy

#### **Box 1**

##### **Interpretive criteria for the IDEXX SDMA test when SDMA level is increased and creatinine level is within the reference interval**

SDMA level is increased and creatinine level is within the reference interval, which indicates that early kidney disease is likely. Most animals with early kidney disease have an SDMA level between 15 and 20  $\mu\text{g}/\text{dL}$ . Because SDMA level increases as kidney function decreases, SDMA levels greater than 20  $\mu\text{g}/\text{dL}$  are typically seen in more advanced disease along with an increased creatinine level. SDMA is a more sensitive indicator of kidney function in poorly muscled animals. A complete urinalysis should be performed to evaluate for inappropriate specific gravity, proteinuria, and other evidence of kidney disease. SDMA results may be slightly higher ( $\sim 1 \mu\text{g}/\text{dL}$ ) in puppies, kittens, and greyhounds and results should be interpreted in light of other findings.

(Courtesy of IDEXX, Westbrook, ME.)

- Vomiting
- Bad breath

Early physical examination changes are often subtle or absent, but typically progress with advancing disease. Common physical examination findings in CKD include:

- Palpable kidney abnormalities
- Evidence of weight loss
- Dehydration
- Pallor
- Oral ulcers
- Hypertensive retinopathy

Because signs and physical examination findings are inconsistent or absent in early disease, relevant laboratory or imaging findings are necessary to assess kidney health. Urinalysis results vary in early kidney disease, with progressive loss of concentrating ability expected as the condition progresses. Inadequate concentrating ability is defined for cats as urine with specific gravity less than 1.035 and for dogs less than 1.030.<sup>58</sup> Making an early diagnosis of CKD commonly requires finding 1 or more of the following results:

- sCr level increasing within the reference interval
- Persistently increased SDMA level greater than 14  $\mu\text{g}/\text{dL}$
- Abnormal kidney imaging
- Persistent renal proteinuria, especially over weeks to months

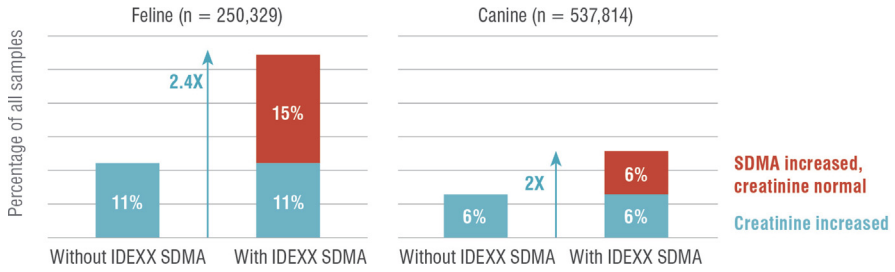
With the exception of changes in kidney size, shape, or architecture that may be detected by examination or kidney imaging on the first visit (eg, finding small, irregular kidneys), these diagnostic criteria for CKD must persist over time in stable patients. Patients changing rapidly, with increasing SDMA level and the development of azotemia, should be suspected of acute kidney injury or an active primary disease and be evaluated aggressively.

With more advanced CKD, levels of both sCr and SDMA are chronically increased, and urine becomes progressively more dilute. In advanced cases of renal disease, SDMA can provide insight into the severity of disease because sCr level may be influenced by loss of muscle mass and under-represent the severity of the disease. Rarely do these patients present a diagnostic challenge to identify that kidney disease is present; instead the focus turns to managing a known problem.

## PATIENT DATA AND IMPACT

SDMA data collected to date support that kidney disease is more prevalent than was previously reported, and increases with increasing pet age. In the first 750,000 or more IDEXX SDMA tests performed in the United States, dog samples outnumbered cat samples approximately 2 to 1. These samples showed that 11% of feline samples and 6% of canine samples had an increase in creatinine level to greater than the reference interval. However, there was an additional 15% of cats and 6% of dogs identified to have increased SDMA levels, whereas the creatinine level remained within the reference interval. These findings suggest that, by using SDMA, which is a more sensitive biomarker, veterinarians have the potential opportunity to diagnose kidney disease 2.4 times more often in cats and 2.0 times more often in dogs, compared with the traditional use of sCr (**Fig. 9**) (data courtesy of IDEXX).

IDEXX data gathered also highlight that the prevalence of CKD increases with increasing age and might exceed historical estimates that 1 in 3 cats<sup>59</sup> and 1 in 10



**Fig. 9.** Patient results for the IDEXX SDMA test and paired creatinine tests expressed as a percentage of feline and canine samples tested suggest that kidney disease may be more prevalent than estimates based on creatinine testing alone. (Courtesy of IDEXX, Westbrook, ME.)

dogs<sup>60</sup> develop CKD in their lifetimes based on creatinine alone. More than 50% of cats more than 15 years of age had increased SDMA and normal or increased Cr levels. In comparison, among dogs 15 years of age or older, 40% had increased SDMA levels and possible kidney disease (data courtesy of IDEXX). The IDEXX SDMA test data support Marino and colleagues<sup>1</sup> recent report that the prevalence of CKD in cats older than 15 years was 86.2% (primarily IRIS CKD stage 1 and 2).

## SUMMARY

SDMA may affect how veterinarians diagnose and manage kidney disease in dogs and cats. SDMA, a product of intranuclear protein metabolism, is freely filtered by the kidneys, and serum levels of SDMA correlate inversely with measurements of GFR in people, rats, mice, dogs, and cats. SDMA was investigated as a potential clinical renal biomarker for almost a decade, and a reference interval for SDMA has been established in healthy dogs and cats, as measured by LC-MS.

SDMA is a sensitive and specific renal biomarker. Longitudinal studies of SDMA in dogs and cats with CKD showed that SDMA level increased months earlier than sCr, when there was on average a 40% reduction in GFR, whereas sCr level increases late, when there is up to 75% reduction of GFR. Unlike sCr, SDMA is independent of lean body mass, so it is a more sensitive marker for kidney disease than sCr in patients with muscle loss. As a result of vigorous analysis and clinical review, SDMA has been included in the updated IRIS guidelines to complement sCr in the diagnostic evaluation and monitoring of CKD.

Early experience with the use of the IDEXX SDMA test, which is a new immunoassay for SDMA, in a large patient population suggests that kidney disease is more prevalent than was previously predicted by increased sCr levels alone. Kidney disease and SDMA increase more frequently as dogs and cats progress in age, supporting historical data. Earlier diagnosis of kidney disease provides an opportunity for intervention by investigation for underlying causes and complications associated with kidney disease. This, in turn, leads to more effective treatment and management.

## REFERENCES

1. Marino CL, Lascelles BD, Vaden SL, et al. The 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; 16:465–7.



2. International Renal Interest Society guidelines. 2016. Available at: <http://www.iris-kidney.com/pdf/staging-of-ckd.pdf>. Accessed March 18, 2016.
3. Kakimoto Y, Akazawa S. Isolation and identification of N-G,N-G- and N-G,N'-G-dimethyl-arginine, N-epsilon-mono-, di-, and trimethyllysine, and glucosylgalactosyl- and galactosyl-delta-hydroxylysine from human urine. *J Biol Chem* 1970;245:5751–8.
4. Vallance P, Leone A, Calver A, et al. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 1992;339:572–5.
5. Schwedhelm E, Böger RH. The role of asymmetric and symmetric dimethylarginines in renal disease. *Nat Rev Nephrol* 2011;7:275–85.
6. Marescau B, Nagels G, Possemiers I, et al. Guanidino compounds in serum and urine of nondialyzed patients with chronic renal insufficiency. *Metabolism* 1997;46:1024–31.
7. Kielstein JT, Salpeter SR, Bode-Böger SM, et al. Symmetric dimethylarginine (SDMA) as endogenous marker of renal function – a meta analysis. *Nephrol Dial Transplant* 2006;21:2445–51.
8. 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;22:317–24.
9. Sanchez SE, Petrillo E, Beckwith EJ, et al. A methyl transferase links the circadian clock to the regulation of alternative splicing. *Nature* 2010;468:112–6.
10. Hsu JM, Chen CT, Chou CK, et al. Crosstalk between Arg 1175 methylation and Tyr 1173 phosphorylation negatively modulates EGFR-mediated ERK activation. *Nat Cell Biol* 2011;13:174–81.
11. Nicholson TB, Chen T, Richard S. The physiological and pathophysiological role of PRMT1-mediated protein arginine methylation. *Pharmacol Res* 2009;60:466–74.
12. Ogawa T, Kimoto M, Sasaoka K. Occurrence of a new enzyme catalysing the direct conversion of NG,NG-dimethyl-l-arginine to l-citrulline in rats. *Biochem Biophys Res Commun* 1987;148:671–7.
13. Achan V, Broadhead M, Malaki M, et al. Asymmetric dimethylarginine causes hypertension and cardiac dysfunction and is actively metabolized by dimethylarginine dimethylaminohydrolase. *Arterioscler Thromb Vasc Biol* 2003;23:1455–9.
14. Glorieux G, Neirynek N, Pletnick A, et al. Uraemic toxins: overview. In: Turner N, Lamiere N, Goldsmith DJ, et al, editors. *Oxford textbook of clinical nephrology*. 4th edition. Oxford (United Kingdom): Oxford University Press; 2016. p. 2161–73.
15. Pressler BM. Clinical approach to advanced renal function testing in dogs and cats. *Vet Clin North Am Small Anim Pract* 2013;43:1193–208.
16. Haller M, Müller W, Binder H, et al. Single-injection inulin clearance—a simple method for measuring glomerular filtration rate in dogs. *Res Vet Sci* 1998;64:151–6.
17. Von Hendy-Willson VE, Pressler BM. An overview of glomerular filtration rate testing in dogs and cats. *Vet J* 2011;188:156–65.
18. Sanderson SL. Measuring glomerular filtration rate: practical use of clearance tests. In: Bonagura JD, Twedt DC, editors. *Kirk's veterinary therapy XIV*. St Louis (MO): Saunders Elsevier; 2009. p. 868–71.
19. Lefebvre HP, Craig AJ, Braun JP. GFR in the dog: breed effect. In: *Proceedings of the 16th ECVIM-CA Congress*. Amsterdam (Holland): European College of Veterinary Internal Medicine—Companion Animals; 2006. p. 261.
20. Braun JP, Lefebvre HP, Watson ADJ. Creatinine in the dog: a review. *Vet Clin Pathol* 2003;32:162–79.

21. Grauer G. Early diagnosis of chronic kidney disease in dogs & cats: use of serum creatinine & symmetric dimethylarginine. *Today's Vet Pract* 2016;6:68–72. Available at: <http://todaysveterinarypractice.navc.com/early-diagnosis-of-chronic-kidney-disease-in-dogs-cats-use-of-serum-creatinine-symmetric-dimethylarginine/>. Accessed March 20, 2016.
22. Nability NB, Lees GE, Boggess MM, et al. Symmetric dimethylarginine assay validation, stability, and evaluation as a marker for the detection of chronic kidney disease in dogs. *J Vet Intern Med* 2015;29:1036–44.
23. Nability MB, Lees GE, Boggess M, et al. Week-to-week variability of iohexal clearance, serum creatinine, and symmetric dimethylarginine in dogs with stable chronic renal disease [ACVIM abstract NU-14]. *J Vet Intern Med* 2013;27(3):604–756.
24. Dibartola SP. Renal disease: clinical approach and laboratory evaluation. In: Ettinger SC, Feldman EC, editors. *Textbook of veterinary internal medicine*. 6th edition. St Louis (MO): Elsevier Saunders; 2005. p. 1716–30.
25. Feeman WE, Couto CG, Gray TL. Serum creatinine concentrations in retired racing greyhounds. *Vet Clin Pathol* 2003;32:40–2.
26. Hall JA, Yerramilli M, Obare E, et al. 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;202:588–96.
27. Hall JA, Yerramilli M, Obare E, et al. Relationship between lean body mass and serum renal biomarkers in healthy dogs. *J Vet Intern Med* 2015;3:808–14.
28. Preiss DJ, Godber IM, Lamb EJ, et al. The influence of a cooked-meat meal on estimated glomerular filtration rate. *Ann Clin Biochem* 2007;44:35–42.
29. Watson AD, Church DB, Fairburn AJ. Postprandial changes in plasma urea and creatinine concentrations in dogs. *Am J Vet Res* 1981;42:1878–80.
30. Puntmann VO. How-to guide on biomarkers: biomarker definitions, validation and applications with examples from cardiovascular disease. *Postgrad Med J* 2009;85:538–45.
31. Al Banachaabouchi M, Marescau B, Possemiers I, et al. NG, NG-dimethylarginine and NG, NG-dimethylarginine in renal insufficiency. *Pflug Arch* 2000;439:524–31.
32. Carello KA, Whitesall SE, Lloyd MC, et al. Asymmetrical dimethylarginine plasma clearance persists after acute total nephrectomy in rats. *Am J Physiol Heart Circ Physiol* 2006;290:H209–16.
33. Tatematsu S, Wakino S, Kanda T, et al. Role of nitric oxide-producing and -degrading pathways in coronary endothelial dysfunction in chronic kidney disease. *J Am Soc Nephrol* 2007;18:741–9.
34. Dixon JJ, Lane K, Dalton RN, et al. Symmetrical dimethylarginine is a more sensitive biomarker of renal dysfunction than creatinine. *Crit Care* 2013;17(Suppl. 2):423.
35. Payto DA, El-Khoury JM, Bunch DR, et al. SDMA outperforms serum creatinine-based equations in estimating kidney function compared with measured GFR [AACC Annual Meeting & Clinical Lab Expo Abstract 105]. *Clin Chem* 2014;60:S22.
36. Lees GE, Helman G, Kashtan CE, et al. New form of X linked dominant hereditary nephritis in dogs. *Am J Vet Res* 1999;60:373–83.
37. Lees GE. Kidney diseases caused by glomerular basement membrane type IV collagen defects in dogs. *J Vet Emerg Crit Care* 2013;23:184–93.
38. Braff J, Obare E, Yerramilli M, et al. Relationship between serum symmetric dimethylarginine concentration and glomerular filtration rate in cats. *J Vet Intern Med* 2014;8:1699–701.

39. Clinical and Laboratory Standards Institute. Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guidelines. 3rd edition. Wayne (PA): CLSI; 2010.
40. Rentko V, Nabity M, Yerramilli M, et al. Determination of serum symmetric dimethylarginine reference limit in clinically healthy dogs [ACVIM abstract P-7]. *J Vet Intern Med* 2013;27:750.
41. Hall JA, Yerramilli M, Obare E, et al. Comparison of serum concentrations of symmetric dimethylarginine and creatinine as kidney function biomarkers in cats with chronic kidney disease. *J Vet Intern Med* 2014;28:1676–83.
42. Hall JA, Yerramilli M, Obare E, et al. Serum concentrations of symmetric dimethylarginine and creatinine in dogs with naturally occurring chronic kidney disease. *J Vet Intern Med* 2016;30:794–802.
43. Yee J, Yu C, Kim J, et al. Histopathological study of canine renal disease in Korea, 2003-2008. *J Vet Sci* 2010;11:277–83.
44. Blackwell S, O'Reilly DS, Reid D, et al. Plasma dimethylarginines during the acute inflammatory response. *Eur J Clin Invest* 2011;4:635–41.
45. Mookerjee RP, Malaki M, Davies NA, et al. Increasing dimethylarginine levels are associated with adverse clinical outcome in severe alcoholic hepatitis. *Hepatology* 2007;45:62–71.
46. Lluch P, Mauricio MD, Vila JM, et al. Accumulation of symmetric dimethylarginine in hepatorenal syndrome. *Exp Biol Med (Maywood)* 2006;23:70–5.
47. Meinitzer A, Kielstein JT, Pilz S, et al. Symmetrical and asymmetrical dimethylarginine as predictors for mortality in patients referred for coronary angiography: the Ludwigshafen risk and cardiovascular health study. *Clin Chem* 2011;57:112–21.
48. Cavalca V, Veglia F, Squellerio I, et al. Circulating levels of dimethylarginines, chronic kidney disease and long-term clinical outcome in non-ST-elevation myocardial infarction. *PLoS One* 2012;7:e48499.
49. Krzyzanowska K, Mittermayer F, Shnawa N, et al. Asymmetrical dimethylarginine is related to renal function, chronic inflammation and macroangiopathy in patients with type 2 diabetes and albuminuria. *Diabet Med* 2007;24:81–6.
50. Rytlewski K, Olszanecki R, Korbut R, et al. Effects of prolonged oral supplementation with L-arginine on blood pressure and nitric oxide synthesis in preeclampsia. *Eur J Clin Invest* 2005;35:32–7.
51. Moesgaard SG, Holte AV, Mogensen T, et al. Effects of breed, gender, exercise and white-coat effect on markers of endothelial function in dogs. *Res Vet Sci* 2007;82:409–15.
52. Pedersen LG, Tarnow I, Olsen LH, et al. Body size, but neither age nor asymptomatic mitral regurgitation, influences plasma concentrations of dimethylarginines in dogs. *Res Vet Sci* 2006;80:336–42.
53. Grauer GF. Early detection of renal damage and disease in dogs and cats. *Vet Clin North Am Small Anim Pract* 2005;35:581–96.
54. WSAVA Nutritional Assessment Guidelines Task Force Members, Freeman L, Becarova I, Cave N, et al. WSAVA Nutritional Assessment Guidelines. *J Small Anim Pract* 2011;52:385–96.
55. Chandler ML, Takishima G. Nutritional concepts for the veterinary practitioner. *Vet Clin North Am Small Anim Pract* 2014;44:645–66.
56. Prusevich P, Patch D, Obare E, et al. Validation of a novel high throughput immunoassay for the quantitation of symmetric dimethylarginine (SDMA) [AACC 2015 abstract B-048]. *Clin Chem* 2015;16:S135 (supplement 2015).

57. Patch D, Obare E, Prusevich P, et al. High throughput immunoassay for kidney function biomarker symmetric dimethylarginine (SDMA) [AACC 2015 abstract B-047]. *Clin Chem* 2015;16:S135 (supplement 2015).
58. Watson ADJ, Lefebvre HP, Elliot J. Using urine specific gravity. International Renal Interest Society Guidelines. 2015. Available at: [http://www.iris-kidney.com/education/urine\\_specific\\_gravity.html](http://www.iris-kidney.com/education/urine_specific_gravity.html). Accessed March 18, 2016.
59. Lulich JP, Osborne CA, O'Brien TD, et al. Feline renal failure: questions, answers, questions. *Compend Contin Educ Pract* 1992;14:127–53.
60. Brown SA. Renal dysfunction in small animals. The Merck Veterinary Manual Web site. 2013. Available at: [https://www.merckmanuals.com/vet/urinary\\_system/noninfectious\\_diseases\\_of\\_the\\_urinary\\_system\\_in\\_small\\_animals/renal\\_dysfunction\\_in\\_small\\_animals.html](https://www.merckmanuals.com/vet/urinary_system/noninfectious_diseases_of_the_urinary_system_in_small_animals/renal_dysfunction_in_small_animals.html). Accessed March 14, 2016.