Longitudinal Evaluation of Serum Symmetric Dimethylarginine (SDMA) and Serum Creatinine in Dogs Developing Chronic Kidney Disease

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ABSTRACT

Objective

To evaluate symmetric dimethylarginine (SDMA) and serum creatinine (sCr) for detection of early chronic kidney disease (CKD) in a prospective, longitudinal study of older dogs.

Sample Population

Forty three healthy dogs with an initial median age of 8.9 years.

Procedures

SDMA, serum and urine clinicopathologic tests were measured biannually for four years.

Results

CKD was documented in 22 (51.2%) dogs confirmed by one or more of the following: ultrasound (US) abnormalities (n = 13), decreased glomerular filtration rate (GFR> 40% reduction) (n = 11), renal proteinuria (UPC ≥ 0.5) (n = 6), or renal histology at necropsy (n = 7). Eight of 22 dogs had increased SDMA (≥ 14 µg/dl) concurrent or subsequent to CKD diagnosis. Conversely, only 2 of the 22 dogs had increased sCr (defined as > 1.8 mg/dl based on laboratory reference interval) at any point and both of these dogs had concurrent/prior SDMA increases. In the 11 dogs with decreased GFR, sCr and SDMA were increased in 1 and 5 dogs, respectively. There were no persistent increases in SDMA without CKD. SDMA

agreed with study defined CKD in 36.4 % of dogs whereas sCr agreed with study defined CKD in 9.1 % (P = 0.032).

Conclusions and Clinical Relevance

This study demonstrates that over a 4-year period in which otherwise healthy, senior dogs developed naturally occurring CKD. Serum SDMA concentration was a more sensitive marker for CKD than was sCr concentration. Assessment of SDMA is recommended as part of longitudinal screening programs in elderly dogs.

ABBREVIATIONS

CBC Complete blood count

CKD Chronic kidney disease

GFR Glomerular filtration rate

IRIS International Renal Interest Society

RI reference interval

sCr serum creatinine

SDMA Symmetric dimethylarginine

UPC urine protein: creatinine ratio

USG urine specific gravity

UTI urinary tract infection

INTRODUCTION

CKD is a common problem and major cause of morbidity and mortality in older dogs. Although CKD is irreversible and often progressive, feeding a diet formulated for long-term maintenance and nutrition for CKD patients has been shown to improve survival and/or improve renal function parameters and slow renal functional decline in dogs with CKD.[1-5] It is a logical, but unproven, hypothesis that early diagnosis of CKD and early initiation of reno-protective treatments (e.g., dietary change, phosphate binders, and possibly renin angiotensin aldosterone system antagonism) will be associated with improved outcomes.[1-3]

Early diagnosis of CKD is somewhat elusive, however, inasmuch as early clinical signs tend to be mild and non-specific, and standard clinicopathologic tests remain normal until greater than 67 – 75% of the nephron mass is lost.[1-5] Plasma clearance techniques to estimate GFR are more sensitive and available for early diagnosis of CKD, but remain cumbersome and underutilized in clinical practice.[1,5] Longitudinal assessment of sCr has been shown to have increased sensitivity for early diagnosis of CKD compared with a single sCr determination, but longitudinal data are not always available.[7] In addition, decreases in patient muscle mass can confound interpretation of sCr.[1-6]

SDMA has emerged as a biomarker for early detection of CKD in veterinary medicine.[5,7,8] SDMA is a byproduct of cellular protein metabolism, specifically the methylation of arginine residues and their subsequent release during proteolysis.[9] It appears to be a relative biologically inert molecule. However, SDMA may function as an indirect inhibitor of nitric oxide synthase.[10-13] SDMA is eliminated from the body primarily via renal excretion (>90%). It is not protein bound in plasma and freely filtered by the glomerulus, and it is not secreted or re-absorbed by the renal tubules.[10-12] Previous studies have demonstrated SDMA concentration correlates well with GFR and sCr in dogs.[3,5,14-17] The objective of the study reported here was to compare serum concentrations of SDMA and creatinine for detection of early CKD in a prospective, longitudinal study of older dogs.

Materials and Methods

Animals

Clinically healthy dogs, primarily owned by first-year veterinary students or faculty and staff from the College of Veterinary Medicine at Kansas State University, were recruited for this study. The Institutional Animal Care and Use Committee of Kansas State University approved the study and written owner consent was obtained prior to patient entry into the study.

Inclusion/Exclusion Criteria

Dogs > 7 years of age and apparently disease-free at the time of enrollment (e.g., no known or suspected diabetes mellitus, hyperadrenocorticism, neoplasia, and CKD) were eligible for inclusion into the study. Medications given at the attending clinician's discretion over the study course for

disorders that developed during the study included cephalexin, metronidazole, trazodone, zinc methionine, insulin, carprofen, amitriptyline, deracoxib, firocoxib, samE, sotolol, tramadol, diethylstilbesterol, amoxicillin/clavulanic acid, cyclosporine, enalapril, amlodipine, levothyroxine (two patients included in the study were previously diagnosed with hypothyroidism. However, both were supplemented with levothyroxine and both had total thyroid hormone concentrations within the reference interval throughout the study), aspirin, joint supplements, and routine heartworm and flea/tick preventatives. Dogs were excluded if a fractious temperament prevented frequent handling or multiple sample collections. Dogs were also excluded if the owners would not be available for periodic examination over the intended 4-year study period.

Study Design

A four-year, prospective, observational study was conducted (August, 2011 through September, 2015). Each dog was evaluated biannually, at approximately 6-month intervals (+/- 4 weeks). At each evaluation, the patient had a complete physical examination, and historical information was collected and recorded, including current diet, environment, past medical history, current medications, and any other pertinent information. Dogs were managed as needed for concurrent or related diseases or conditions at the discretion of the clinician.

At each evaluation, systolic blood pressure was measured and clinicopathologic tests including a CBC, biochemistry profile, serum total thyroxine, urinalysis with quantitative aerobic bacterial culture, UPC, and SDMA were measured. Over the course of the study, if the clinicopathologic data suggested a possible decrease in renal function (including increases in sCr or SDMA, increases in UPC, or decreases in USG compared to baseline values), a plasma clearance of iohexol and abdominal ultrasound examinations were added to the biannual evaluations. Ultrasound examinations were performed by a board-certified radiologist or by a radiology resident and reviewed by board-certified radiologist.

Blood and Urine Sample Collection

Blood samples were collected from the jugular or lateral or medial saphenous veins. Urine samples were collected by cystocentesis when possible. If samples were collected via urinary catheter or free catch, the collection method was considered and repeat urinalyses were performed if proteinuria or urinary tract infection were potential concerns. All laboratory evaluations were performed by IDEXX Reference Laboratories. Blood and urine samples were collected and serum was harvested Monday through Thursday and shipped overnight on dry ice to be analyzed within 24 hours of collection. A CBC was performed on blood that had been stored and shipped in ethylenediaminetetraacetic acid tubes.

Clinicopathologic Analyses

Symmetric dimethylarginine concentrations were determined by IDEXX Reference Laboratories using high-performance liquid chromatography mass spectrometry methodology, as previously reported.[8,15] The upper limit of the RI for SDMA in healthy adult dogs was previously determined to be <14 µg/dL.[5,7]

Each CBC was reviewed by a technical specialist for accuracy and presence of organisms or abnormal cells. Serum biochemical profiles were measured with an automated chemistry analyzer at Idexx Reference Laboratories. Normal RI for both CBC and biochemistry profiles were previously established for healthy dogs by Idexx Reference Laboratories.

USG was determined using a refractometer. Each urinalysis had a sediment examination that was microscopically reviewed by a laboratory technician. Urine protein and creatinine concentrations were determined using urine supernatant. Urine protein was quantified using the benzethonium reaction method and urine creatinine was quantified using the buffered Jaffe reaction, with an automated chemistry analyzer. The UPC was calculated from this data for each urine

sample with an inactive urine sediment, defined as a urine sample that had < 200 red blood cells/hpf, < 5 white blood cells/hpf, few epithelial cells, and no bacteria.

Approximately 0.5 mL of whole urine was submitted for aerobic quantitative bacterial culture and minimum inhibitory concentration sensitivity within 24 hours of collection.

Blood Pressure Measurement

Systolic blood pressure measurements were obtained via an ultrasonic Doppler monitora after the dogs were acclimated to the hospital environment but prior to physical examination and specimen collection. Dogs were placed in lateral recumbency and the up forelimb was prepared for pressure measurement by clipping the hair over the common digital branch of the radial artery. An inflatable cuff with a width approximately 35-40% the circumference of the leg was placed directly below the elbow and secured. The cuff was briefly inflated to a pressure of approximately 240mmHg to stop arterial flow and was then deflated gradually until an audible pulse signal, representing systolic pressure, returned. The systolic blood pressure recorded represented the mean of 3-4 separate determinations. At subsequent evaluations, blood pressure measurements were obtained using the same body position, limb, and cuff size.

Determination of GFR

A single intravenous injection of iohexol (300 mg/kg) was administered via a cephalic or saphenous vein. Three blood samples were collected at 2, 3, and 4 hours after injection from the jugular vein or opposite saphenous vein. Serum concentrations of iohexol were measured by a commercial laboratory using an Inductively Coupled Plasma-Mass Spectrometry focusing on the iodine components of iohexol.[b] Results were reported as clearance with units of ml/min/kg, as well as the percentage reduction in GFR relative to a cohort of normals from the same species.[b] GFR was estimated from calculations made using a one-compartment model A/α (mg/ml x min), then clearance was calculated using the total dose of iohexol administered (mg/AUC). Median (range) GFR for normal healthy dogs was determined by the laboratory and was 5.48 ml/min/kg (range, 2.89 – 8.07 ml/min/kg).

Histopathology

Complete necropsies were performed when possible when dogs died or were euthanized. Histopathologic samples were obtained from the kidney and any other grossly affected organs or organs of interest based on the cause of death and clinical signs/laboratory tests. Histopathologic analysis was performed with standard sample processing at the Kansas State University Veterinary Diagnostic Laboratory. Pathologists had access to the patient history and medical record.

CKD Definition

For the purposes of this study, CKD was defined/diagnosed in dogs by one or more of the following abnormalities:18

1. Chronic renal changes compatible with CKD observed by ultrasound examination (e.g., increased cortical echogenicity, loss of the corticomedullary junction, cortical infarcts, irregular renal contour, and nephrolithiasis).
2. Renal proteinuria (UPC > 0.5) that was present on two or more urine evaluations with normal urine sediment examinations.
3. A persistent or endpoint decrease in plasma iohexol clearance > 40% without evidence of dehydration.
4. Renal histologic changes compatible with CKD at necropsy examination.

Statistical Analysis

Statistical analysis was performed using standard statistical software.[c] Continuous data were reported as mean (median; IQR). Categorical data was represented as frequency (percent). The N-1 Chi-squared test was used to determine the difference in proportions between SDMA and sCr compared to CKD. The P-value for significance was set at <0.05.

Dogs that developed CKD were identified per the above criteria over the 4-year

period. Dogs that were prospectively enrolled in the study that did not develop CKD were used as an age-matched comparison group.

RESULTS

Dogs

A total of 43 dogs were enrolled in the study. At the time of enrollment, the average dog age was 8.9 years with a range of 7 – 14 years. Breeds represented included 11 mixed breed dogs, four Cocker Spaniels, four Labrador Retrievers, three German Shepherd dogs, three Border Collies, two Chihuahuas, two Golden Retrievers, two Boston Terriers, two Pembroke Welsh Corgis, and one each Siberian Husky, Bichon Frise, Parsons Jack Russell Terrier, Norwich Terrier, Boxer, English Springer Spaniel, Dachshund, German Shorthair Pointer, Australian Shepherd, and Basenji. Twelve patients died or were euthanized during (n = 9) or shortly after the study period (n = 3). Four or more longitudinal samples had been collected for patients that died during the study period. Necropsy was performed in 9 of 12 dogs that died or were euthanized. Causes of death were determined to be:

- neoplasia (hemangiosarcoma (n = 3)
- gastric adenocarcinoma (n = 2)
- hepatocellular carcinoma (n = 2)
- bronchoalveolar carcinoma
- mesothelioma
- chemodectoma
- pulmonary adenocarcinoma
- melanoma
- gastrointestinal stromal cell tumor
- nasal adenocarcinoma) in 11 dogs
- laryngeal paralysis
- cervical intervertebral disc disease in one dog, and
- non-suppurative encephalitis in one dog.

Some dogs had multiple neoplasms or causes for euthanasia. The reason for euthanasia for the 3 dogs that were euthanized without necropsy were:

- severe arthritis
- a bladder wall mass and medial iliac lymphadenopathy and inability to urinate,
- severe cervical disc disease.

CKD was documented in 22 dogs (51.2%) confirmed by ultrasound abnormalities (n = 13), decreased GFR (> 40% reduction) (n = 11), persistent renal proteinuria (UPC > 0.5) (n = 6), or renal histology (n = 7). Twelve dogs had multiple criteria of CKD (Table 1).

Eight of 22 CKD dogs had multiple (> 2), persistent or endpoint increases in SDMA (> 14µg/dl) prior to, concurrent, or after CKD diagnosis. Conversely, only 2 of the 22 dogs had increased sCr (> 1.8mg/dl upper end of IDEXX Reference Laboratories RI) at any point and both dogs had concurrent/prior increases in SDMA. SDMA had a sensitivity of 36.4 % of dogs whereas the sensitivity of sCr was only 9.1 % (P = 0.032). Both SDMA and sCr had specificities of 100%. In the 11 dogs with decreased GFR (40 - 80% reduction from median baseline), sCr and SDMA were increased in one and five dogs, respectively. There were no persistent increases in SDMA in dogs without CKD. There were also no instances when SDMA was within a normal range and sCr was increased in either group. There were no increases in SDMA or sCr in the control group at any time.

Of the dogs with CKD, 11 of 22 patients (50%) had persistent hypertension, classified as moderate (n = 6) with a persistent systolic blood pressure greater than or equal to 160 mmHg, or severe (n = 5) with a persistent systolic blood pressure of greater than or equal to 180 mmHg. This contrasts with only three 3 dogs with hypertension in the non-CKD control group. Hypertension was managed at the discretion of the attending clinician with amlodipine (for hypertension > 180 mmHg systolic on Doppler measurement) and/or enalapril (for moderate hypertension).

Diet was recorded as part of each visit. Of the 43 dogs enrolled in the study, all dogs were fed commercially available or veterinary prescription diets. Fourteen

were reported to be on diet formulated for senior dogs, 16 were fed diets formulated to support joint health, 10 were fed a maintenance diet, four were fed a diet formulated to support gastrointestinal health, three were fed weight loss diets, two were fed novel antigen diets, and one dog (who was not diabetic) was fed a diet formulated for diabetic dogs. Some dogs were fed multiple diets throughout the 4-year study period. Six dogs in the CKD group were transitioned to a prescription renal diet throughout the course of the study.

Seventeen of 43 (40%) dogs (n = 11 neutered males, n = 6 females) developed UTI based on quantitative urine culture at some point during the 4-year study period. Only four of these dogs were reported to have clinical signs consistent with UTI at the time of bacteria confirmation. The other 13 dogs were either asymptomatic or signs were not reported by the owners. Isolated organisms included *P. mirabilis, E. coli, Enterococcus* spp.*, P. aeruginosa, S. pseudointermedius,* and *K. pneumoniae*. Dogs were treated with oral antibiotics (based on minimum inhibition concentration sensitivity profile, as described above). Dogs were treated if they had greater than 100,000 cfu/ml in voided urine, >10,000 cfu/ml in catheterized urine, and greater than 1,000 cfu/ml in cystocentesis samples. All dogs treated with antibiotics had subsequent resolution of the bacterial growth on urine culture at the next time point, and were evaluated prior to the 6-month recheck for repeat culture and sensitivity to ensure their UTI had resolved. Of the 17 dogs that developed UTI, 13 also had CKD (76%).

Of the dogs in the CKD group (n = 22), 12 lost weight (54.5%) and 10 gained weight (45.5%). In the non-CKD group (n = 21), 14 dogs lost weight (66.7%) and seven dogs gained weight (33.3%). Of the dogs in the CKD group that lost weight, the average amount of weight lost was 3.26kg (median: 1.42kg, range: 0.67 - 14.4kg). The average percent body weight loss was 17.7% (median: 12.7%; range: 3.9% - 40.3%). The CKD dog that lost 14.4kg (40.3% body weight) was euthanized and a gastrointestinal stromal cell tumor and hepatocellular carcinoma was also found at necropsy in addition to the CKD. Of the dogs in the non-CKD group that lost weight, the average amount of weight lost was 1.9kg (median: 1.1kg, range: 0.3 - 6.8kg). The dog that lost 6.8kg had a gastric adenocarcinoma at necropsy. The percent of weight lost in the non-CKD group was an average of 7% (median: 5.8%; range: 1.3% - 15.3%). The actual number of kilograms lost and the number of dogs that lost weight were not statistically significant between the two groups. However, the percentage of weight loss was significantly greater in the CKD group than in the non-CKD group ($p < 0.05$). Of the dogs that had CKD and weight loss, 50% (n = 6) had neoplasia that was identified during the study and seven dogs (58.3%) had normal sCr in the face of persistently increased SDMA.

Some dogs developed concurrent chronic diseases that were diagnosed antemortem throughout the course of the study (Table 2). There was no significant difference between CKD and non-CKD groups regarding the number of dogs that developed concurrent disease ($p = 0.131$).

Six dogs had persistently increased UPC (two or more concurrent elevations in UPC), two of which had GFR analysis performed and both were reduced by greater than 40% of baseline. Seven dogs had histopathologic changes in the kidney (two of which had GFR measurements and both were reduced at greater than 40% of baseline). Thirteen dogs had ultrasonographic changes compatible with CKD; eight of these dogs had GFR studies performed and six of the eight had reduced GFR measurements.

DISCUSSION

The IRIS CKD Stage I criteria include:

- inadequate urinary concentrating ability without an identifiable non-renal cause
- abnormal renal palpation or renal imaging findings

- persistent proteinuria of renal origin,
- abnormal renal histopathology.[18]

Criteria 2-4, as well as a > 40% reduction in plasma clearance of iohexol, were used in this study as evidence of early CKD. Decreased urine concentration was not used as a defining criteria for CKD in this study because of the inability to definitively rule out extra-renal causes of decreased urine concentration. Persistent, multiple, or end-point increases in serum SDMA were more sensitive for detection of CKD using the above criteria than was sCr (36.4 % versus 9.1 %, respectively, p = 0.032).

Creatinine is an end-product of normal muscle metabolism. Therefore, in patients with decreased muscle mass, renal excretory function may be overestimated.[6,19] Serum SDMA concentrations do not appear to be impacted by lean body mass in healthy beagles.6 In this previous study, lean body mass was measured using dual-energy x-ray absorptiometry along with sCr and SDMA over a six-month period. Changes in sCr were significantly correlated with changes in lean body mass, whereas changes in lean body mass did not influence SDMA. In the present study, without benefit of an objective measure of lean body mass, 61% of the CKD dogs that lost weight had normal sCr with increased SDMA.

Increases in sCr over time, even if the sCr level remains within reference intervals may be compatible with early non-azotemic CKD.7,18 An increase in sCr of greater than 0.3mg/dL using the same laboratory methodology where dehydration and increases in muscle mass have been ruled out, is a potential marker of early CKD.18 Longitudinally increased sCr (>0.3mg/dl) was only valuable for documenting early CKD in this study in four dogs (17.4%), and three of these four had persistently increased SDMA. Because weight loss and loss of lean body mass is a common problem in dogs with CKD, longitudinal assessment of sCr may lack sensitivity for detection of decreased GFR.

Systemic hypertension is a commonly recognized consequence of CKD, and has been associated with significantly shorter survival times.[20,21] Hypertension, defined as persistent elevation in systolic blood pressure (>160mmHg for moderate hypertension and >180mmHg for severe hypertension) by Doppler technique, was documented in 50% of the CKD dogs in this study. This is consistent with previously reported prevalence rates for hypertension in CKD patients.[20] Only three dogs in the non-CKD group developed hypertension of undetermined cause.

Diet changes occurred during the study period in almost all the patients enrolled. This could be since most enrolled patients were owned by veterinary students and staff in a university and discounted or free pet food available through feeding programs may have influenced diet choices. Approximately half of the dogs in this study were fed a diet available by prescription only. Patients with CKD were transitioned to a renal diet if needed (persistent renal proteinuria or azotemia). Due to the wide variety of diets fed during this study, no conclusions could be drawn regarding the influence of diet.

In this study, 76% of the dogs that developed urinary tract infections had CKD and, of these individuals, only 35% were female. These results are higher than previous reports and the higher percentage of males vs. females was unexpected. Advancing age, decreasing body condition score, and CKD are potential risk factors for the development of UTI. It is possible that some of the dogs in this study that developed a UTI had additional, nondetected local or systemic diseases that further compromised their defense mechanism against UTI. Based on these results, routine urine culture may be an important diagnostic tool for both male and female dogs with CKD.

CKD was evaluated over the 4-year time period and was diagnosed based on plasma iohexol clearance and IRIS Stage 1 CKD criteria.[18] One limitation of this study is that plasma iohexol clearance, US, and renal histology were not performed in every patient at every time point. This may have

introduced some bias and some patients may have had unrecognized CKD that were not evaluated by plasma clearance of iohexol and US. This study, however, may more closely approximate clinical practice, where it would be uncommon to obtain GFR, abdominal imaging, and histopathology on an otherwise healthy patient with unremarkable clinicopathologic findings. An additional limitation was the inclusion of two patients diagnosed concurrently with hypothyroidism, which may have affected GFR and assessment of CKD. However, both of these patients were treated with levothyroxine and had normal thyroid hormone concentrations throughout the study period.

Another important consideration in the design of this study was that the diagnosis of CKD was based on a combination of imaging findings, proteinuria, and histology, which may not always be associated with decreases in GFR. This may have contributed to the relatively high percentage of dogs diagnosed with CKD over the course of the study. The design of this study made every attempt to screen for early stage 1 CKD dogs, which may go unrecognized in clinical practice where ultrasound, UPC, iohexal clearance testing, or renal histopathology are routinely employed. While SDMA appears to be more sensitive than sCr for early detection of CKD, it may not actually predict renal histologic or ultrasonographic changes or renal proteinuria that are compatible with CKD.

A criticism of previous studies regarding sensitivity and specificity of SDMA compared to sCr is that the laboratory reference intervals used for diagnosis of azotemia are variable. In our study, using a lower sCr value of >1.4 mg/dL for the cutoff (the sCr value that indicated IRIS Stage 2 CKD in dogs) would not alter the number of dogs diagnosed with CKD, nor would it affect the sensitivity and specificity of creatinine. Previous studies have demonstrated that longitudinal assessment of sCr over time increases the sensitivity of early CKD detection compared with evaluation of single sCr concentrations.[3,5,6]

CONCLUSION

This study demonstrates that over a four-year period in which otherwise healthy, senior dogs developed naturally occurring CKD, serum SDMA concentration was a more sensitive marker for CKD than was sCr concentration. Assessment of SDMA is recommended as part of longitudinal screening programs in elderly dogs.

FOOTNOTES

a Ultrasonographic Doppler Flow Detector, Parks Medical Electronics Inc., Aloha, OR.

b Michigan State University Diagnostic Center for Population and Animal Health, East Lansing, MI.

c Microsoft® Excel® for Mac 2011, version 14.4.9 ©2008

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