Title: The relationship between glomerular filtration rate, serum SDMA concentration and serum creatinine concentration in dogs

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Running Head: Relationship between GFR, SDMA and creatinine in dogs

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Abbreviations: CKD, chronic kidney disease; GFR, glomerular filtration rate; SDMA, Symmetric dimethylarginine; IRIS, international renal interest society; HPCE, high-performance capillary electrophoresis; RVC, Royal Veterinary College; CI, confidence interval; C, Celsius; PPV, positive predictive value; NPV, negative predictive value.

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Institutional Animal Care and Use Committee (IACUC) or other approval declaration: This project was reviewed and approved by the Royal Veterinary College (RVC) Social Sciences Research Ethical Review Board (reference URN SR2017-1223) who granted approval for access to joint iohexol clearance test submission forms held by deltaDOT Ltd. and the RVC and for contact with the veterinarians for access to the clinical records of the patients under investigation and for completion of a short questionnaire regarding patient outcomes.

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Abstract

Background: Glomerular filtration rate (GFR) estimation is considered the gold standard for assessment of renal function given its direct proportionality to renal mass. Despite this, serum creatinine, and increasingly symmetric dimethylarginine (SDMA), are more commonly used as surrogate markers of GFR in clinical practice. Data pertaining to the correlations between GFR, serum SDMA concentration and serum creatinine concentration in client-owned dogs are limited.

Objectives: to describe the relationship between GFR, serum SDMA concentration and serum creatinine concentration in a population of client-owned dogs, and to compare the clinical utility of serum SDMA to the gold standard of GFR estimation via serum iohexol clearance for the detection of pre-azotemic chronic kidney disease (CKD).

Animals: Medical records of 132 dogs who had GFR estimation performed between 2012 and 2017.

Methods: The medical records of 132 client-owned dogs having GFR estimation performed via iohexol clearance between 2012 and 2017 were assessed. Simultaneous serum creatinine and SDMA were available for 110 and 115 dogs respectively. All dogs included in the study population were non-azotemic. Regression analysis was performed to determine the associations between GFR, SDMA and serum creatinine concentrations. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of different cut-off points for SDMA and serum creatinine concentrations for detecting decreases in GFR were calculated, using a 95% confidence interval. ROC curve analysis was performed to determine the optimal cut-off for SDMA and serum creatinine concentration to detect a GFR decrease ≥40% below the mean GFR of the patient's bodyweight category.

Results: Serum creatinine and SDMA were moderately correlated with GFR (R^2=0.46 and 0.27 respectively, P<0.0001) and with each other (R^2=0.23, P<0.0001). Increased SDMA above the reference interval of 14 µg/dl was sensitive (85.7%) but relatively non-specific (52.8%) for detection of a ≥ 40% GFR decrease below the mean GFR of the patient's body weight category. The optimal SDMA concentration for assessing for a GFR decrease of ≥40% was 18 µg/dl (sensitivity 80%, specificity 90%). The optimal serum creatinine concentration cut-off detection of a ≥ 40% GFR decrease was ≥1.37mg/dL (sensitivity 64%, specificity 93%).

Conclusions and clinical importance: SDMA can be a sensitive and specific marker for detecting decreased renal function depending on the cut-off used. Using a cut-off of >18µg/dl rather than the traditional cut-off of >14µg/dl increases the specificity of SDMA for detecting decreased renal function as assessed by estimation of GFR.

Glomerular filtration rate (GFR) estimation is regarded as the gold standard method for assessing renal function, as it is directly proportional to renal mass.[1] While direct measurement of GFR is not possible, it can be estimated by assessing the clearance of a marker of GFR.[2] Measuring the plasma clearance of iohexol has become a widely used means of estimating GFR due to its availability, cost and ease of use.[3-12] Measuring the plasma clearance of iohexol using a limited sampling technique has been previously described in dogs.[13]

Although GFR estimation is the gold standard for assessing renal function, measurement of serum creatinine, a surrogate marker of GFR, remains the main means of assessing renal function in dogs in clinical practice.[14] However, using serum creatinine as a marker of GFR has significant limitations. The relationship between serum creatinine and GFR is exponential, such that serum creatinine has limited sensitivity for the early detection of declining renal function.[15] In addition, lean body mass has a significant effect on serum creatinine concentrations,[16] making assessment of GFR in well-muscled or cachexic animals challenging. Furthermore, false increases in serum creatinine concentrations are possible with certain assays,[17] and in male dogs a small amount of creatinine is secreted in the renal tubules.[18]

Recent studies indicate that symmetric dimethylarginine (SDMA) may be a promising marker of GFR in dogs.[19,20] SDMA is produced by the breakdown of proteins, the arginine residues of which have been post-translationally methylated, and is excreted primarily (≥90%) by renal clearance.[21,22] Unlike serum creatinine, SDMA is unaffected by lean body mass.[16] SDMA has an exponential relationship with GFR but may be a more sensitive marker of declining GFR than creatinine.[19] A caveat is that data pertaining to the effects of concurrent disease on SDMA remain somewhat limited.

A study by Nabity *et al.* comparing SDMA with serum creatinine concentration and GFR for early detection of decreasing kidney function in dogs with X-linked hereditary nephropathy demonstrated that SDMA increased as the disease progressed, correlating with increases in serum creatinine and decreasing GFR.[19] SDMA detected, on average, a <20% decrease in GFR, which was earlier than serum creatinine using any comparison method.[19] In addition, Hall *et al.* demonstrated that serum SDMA increased before serum creatinine by a mean of 9.8 months (range 2.2-27 months) in dogs with chronic kidney disease (CKD).[20] Although the two aforementioned studies described the relationship between GFR, SDMA and serum creatinine, the study populations in both cases were relatively small populations of research colony dogs. Data relating to the relationship between GFR, SDMA and serum creatinine in a general population of client-owned dogs in a clinical setting where kidney disease is suspected based on their clinical presentation are lacking.

The aim of this study was to describe the relationship between GFR, SDMA and serum creatinine in a population of client-owned dogs presenting to both referral and first-opinion

practice, and to compare the clinical utility of SDMA to the gold standard of GFR estimation via serum iohexol clearance for the detection of pre-azotemic CKD.

Materials and Methods:

Data Acquisition and Analysis

The medical records of dogs who had samples submitted for GFR estimation via iohexol clearance to the Royal Veterinary College from 9/3/12-4/11/17 were assessed. The project was reviewed and approved by the Royal Veterinary College (RVC) clinical research and ethical review board which allowed for access to joint iohexol clearance test submission forms held by deltaDOT Ltd. and the RVC, with subsequent submission of serum samples for SDMA and serum creatinine measurement via batch analysis to Idexx Laboratories Ltd. Additionally, contact with the veterinarians for access to the clinical records of the patients under investigation and for completion of a short questionnaire regarding patient outcomes was approved. This contact was performed prior to final implementation of the General Data Protection Regulation (EU) 2016/679.

Iohexol clearance protocol

A standard protocol was used for performing the iohexol clearance tests via a limited sampling technique, as previously described by McKenna *et al.*[23] GFR was estimated from the iohexol clearance data by application of a compartmental model and correction formula previously described by Bexfield *et al.,*[13] normalised to body weight in kilograms. For data analysis, dogs were divided into the four weight quartiles previously described by Bexfield *et al.*;[13] 1.8-12.4kg, 13.2-25.5kg, 25.7-31.6kg and 32.0-70.3kg. In the event that a dog's body

weight did not fall within the range of one of these body weight categories, the dog was included in the body weight category to which its body weight was closest. GFR estimation results were interpreted in light of the categorisation criteria previously defined by McKenna *et al.*:[23] GFR group 1: GFR increased or <20% decreased from the mean GFR of the body weight category; kidney disease considered excluded or very unlikely as a cause of presenting clinical signs, GFR group 2: ≥20%-30% decrease in GFR from the mean GFR of the body weight category; kidney disease considered possible but unconfirmed as an aetiology for presenting clinical signs, GFR group 3: ≥30%-40% decrease in GFR from the mean GFR of the body weight category; kidney disease considered likely as an aetiology for presenting clinical signs, GFR group 4: ≥40% decrease in GFR from the mean GFR of the body weight category; kidney disease considered almost certain as an aetiology for presenting signs.

Using the same serum that was submitted for GFR estimation via iohexol clearance testing, SDMA and serum creatinine concentrations were measured. After storage in a -80°C freezer for a median of 166 days (range 9-1360 days), all samples were submitted to Idexx Laboratories Ltd., to either Idexx Wetherby (UK) or Idexx Ludwigsburg (Germany). Identical machines and methodology were used to measure SDMA and serum creatinine in both labs. The reference intervals for SDMA and serum creatinine used in this study were set at 0-14µg/dL and 0.23-1.5mg/dl respectively on 10/29/2017.

The veterinarian(s) who submitted each sample set for iohexol clearance to be measured were contacted via email and asked to complete a short questionnaire regarding patient outcomes. Data collected included patient status (i.e. alive/dead), date of euthanasia/death,

reason for euthanasia/death if known and whether or not a diagnosis was reached for the clinical signs/ routine laboratory findings that prompted GFR estimation. If no response to the questionnaire was received or the answers were insufficient to provide outcome information, veterinarians were contacted directly by telephone to request the full clinical history and laboratory reports for the patients in question from the time of iohexol clearance sample submission to the time of follow-up.

Statistical Analysis

Data are presented as median (range) unless otherwise stated. GFR estimation results were expressed as % deviation from the mean GFR of that patient's body weight category as defined by Bexfield *et al.*[13] and interpreted in light of the categorisation criteria defined by McKenna *et al*.[23] Statistical analyses were performed using statistical software Prism 8.[24] To investigate the associations between SDMA and GFR, serum creatinine concentration and GFR, and SDMA and serum creatinine concentration, SDMA and serum creatinine results were plotted against GFR data, and against each other. Best-fit equations were derived from the resulting data plots in order to measure the associations between variables. Log and power transformations were applied as required to obtain linear relationships between the variables. Data from the 116 dogs for which GFR results and creatinine +/- SDMA results were available were included in the calculation of these associations.

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of different cut-off points for SDMA (>10µg/dl, >12µg/dl, >14µg/dl, >16µg/dl,

>18µg/dl and >20µg/dl) and different cut-off points for serum creatinine (≥1.0mg/dl, ≥1.1mg/dl, ≥1.2mg/dl, ≥1.3mg/dl, ≥1.4mg/dl) for detecting decreases in GFR (GFR decreases of ≥20%, ≥30% and ≥40% below the mean GFR of the patient's bodyweight category respectively) were calculated, using a 95% confidence interval. For calculation of sensitivity, specificity, PPV and NPV only those dogs for which GFR, SDMA and serum creatinine results were simultaneously available were included (n=105).

Receiver operating characteristic (ROC) curves were developed to assess the trade-off between the sensitivity and (1-specificity) across a series of cut-off points for SDMA (>10µg/dl, >12µg/dl, >14µg/dl, >16µg/dl, >18µg/dl and >20µg/dl) and serum creatinine (≥1.0mg/dl, ≥1.1mg/dl, ≥1.2mg/dl, ≥1.3mg/dl, ≥1.4mg/dl and >1.5mg/dl) to detect decreases in GFR of ≥20%, ≥30% and ≥40% below the mean GFR of a patient's body weight category.

Results:

GFR, serum creatinine and SDMA results

A total of 132 dogs had samples submitted for GFR estimation between 9/3/12 and 4/11/17. The characteristics of this study population have previously been described.[23] Serum creatinine data were available for 117/132 (88.6%) dogs. In 7/117 (6.0%) dogs for which serum creatinine was available, serum creatinine was above the upper limits of the reference interval (>1.5mg/dl). The 7 azotemic dogs were excluded from the data set and from further analysis, leaving a total of 125 dogs in the data set. After exclusion of the 7 azotemic dogs,

serum creatinine data was available for 110/125 (88%) dogs. SDMA was available for 111/125 (88.8%) dogs with concurrent SDMA and creatinine available for 105 (84%) dogs.

In the 116 dogs for which either serum creatinine or SDMA was available, median GFR was 2.21ml/kg/min across all body weight categories with a range of 1.16-4.04ml/kg/min**.** Percentage deviation from the mean of the body weight category ranged from -55% to +68.0% with a median deviation of -10.7% from the mean. In 39/116 (33.6%) dogs the percentage deviation from the mean of the body weight category was ≥20% decreased.

Median serum creatinine for all 110 non-azotemic dogs for which it was available was 1.1mg/dl (range 0.38-1.5g/dl) whilst median SDMA for all 111 dogs for which it was available was 15μg/dl (range 6-27μg/dl).

In 57/111 (51%) dogs, SDMA was above the upper limits of the reference interval (>14μg/dl). The median SDMA for these 57 dogs was 17μg/dl (range 15-27μg/dl). Median change in GFR from the mean GFR for the patient's body weight category in the 57 dogs with increased SMDA was -16.73% (range -55% to +40.8%). In the 57 cases with increased SDMA, GFR was increased or <20% decreased below the mean GFR for the patient's bodyweight category in 32/57 (56%) of cases. Further description of SDMA and serum creatinine results by GFR category is provided in Table 1:

Table 1: GFR (defined as % deviation from the mean GFR of the patient's body weight category) results for patients with normal (≤14μg/dl) or increased (>14μg/dl) SDMA and serum creatinine concentrations above and below the cut-off for stage 1 chronic kidney disease (≥1.4mg/dl), as per the International Renal Interest Society (IRIS) guidelines.[25]

GFR results are expressed as per the categories defined by McKenna et al.:[23] GFR group 1: GFR increased or <20% decreased from the mean GFR of the body weight category; kidney disease considered excluded/ or very unlikely, GFR group 2: ≥20%-30% decrease in GFR; kidney disease considered possible but unconfirmed, GFR group 3: ≥30%-40% decrease in GFR; kidney disease considered likely, GFR group 4: ≥40% decrease in GFR; kidney disease considered almost certain.

Variable	GFR ↑ or ↓<20%	GFR ↓ ≥20- 30%	GFR ↓ ≥30- 40%	GFR ↓ ≥40
SDMA ≤14μg/dl n=54	43 (80%)	3 (5.5%)	5 (9%)	3 (5.5%)
SDMA >14μg/dl n=57	32 (56%)	8 (14%)	5 (9%)	12 (21%)
Creatinine <1.4mg/dl n=99	72 (73%)	11 (11%)	8 (8%)	8 (8%)

Creatinine ≥1.4mg/dl n=11	2 (18%)	0 (0%)	2 (18%)	7 (66%)

The relationship between SDMA and serum creatinine concentration with GFR

Serum creatinine and SDMA were moderately associated with GFR (R^2=0.46 and 0.27 respectively, P<0.0001) and with each other (R^2=0.23, P<0.0001). The relationship between serum creatinine and SDMA was linear (Figure 1) while the relationships between SDMA and GFR (Figure 2) and serum creatinine and GFR (Figure 3) were non-linear. The relationships between the reciprocal of serum creatinine and GFR (Figure 4), and the reciprocal of SDMA and GFR (Figure 5) were linear again with moderate but significant association (R^2 = 0.37 and R^2 = 0.25 respectively, P<0.0001).

Utility of SDMA for detecting decreases in GFR

The sensitivity and specificity of different cut-off points for SDMA (>10μg/dl, >12μg/dl, >14μg/dl, >16μg/dl, >18μg/dl and >20μg/dl) and different cut-off points for serum creatinine (≥1.0mg/dl, ≥1.1mg/dl, ≥1.2mg/dl, ≥1.3mg/dl and ≥1.4mg/dl) for detecting decreases in GFR (GFR decreases of ≥20%, ≥30% and ≥40% below the mean GFR of the patient's bodyweight category respectively) are outlined in Table 2. The positive and negative predictive values of these cut-off points for SDMA and serum creatinine for detecting decreases in GFR are outlined in Table 3.

254 Table 2: Ability of different cut-off points of SDMA and serum creatinine to detect different

255 categories of % GFR decrease below the mean GFR of the patient's body weight category.

Variable	Sample Size	Sensitivity % GFR ↓ ≥20% (95% CI)	Specificity % GFR ↓ ≥20% (95% CI)	Sensitivity % GFR ↓ ≥30% (95% CI)	Specificity % GFR ↓ ≥30% (95% CI)	Sensitivity % GFR ↓ ≥40% (95% CI)	Specificity % GFR ↓ ≥40% (95% CI)
SDMA >10µg/dl	105	97.0 (84-100)	23.6 (14-35)	100 (85-100)	21.7 (13-32)	100 (77-100)	19.8 (12-29)
SDMA >12µg/dl	105	84.9 (68-95)	43.1 (31-55)	90.9 (71-99)	41.0 (30-52)	92.9 (66-100)	38.5 (28-49)
SDMA >14µg/dl	105	72.7 (54-87)	56.9 (45-69)	72.7 (50-89)	53.0 (42-64)	85.7 (57-98)	52.8 (42-63)
SDMA >16µg/dl	105	57.6 (39-75)	79.2 (68-88)	63.6 (41-83)	75.9 (65-85)	78.6 (49-95)	74.7 (65-83)
SDMA >18µg/dl	105	45.5 (28-64)	94.4 (86-98)	54.6 (32-76)	91.6 (83-97)	71.4 (42-92)	90.1 (82-95)
SDMA >20µg/dl	105	27.3 (13-46)	95.8 (88-98)	40.9 (21-64)	96.4 (90-99)	50.0 (23-80)	94.5 (88-98)
Creatinine ≥1.0mg/dl	105	81.8 (65-93)	52.8 (41-65)	81.8 (60-95)	48.2 (37-59)	78.6 (49-95)	45.1 (35-56)
Creatinine ≥1.1mg/dl	105	78.8 (61-91)	68.1 (56-79)	81.8 (60-95)	62.7 (51-73)	78.6 (49-95)	58.2 (47-69)
Creatinine ≥1.2mg/dl	105	66.7 (48-82)	83.3 (73-91)	77.3 (55-92)	79.5 (69-88)	78.6 (49-95)	74.7 (65-83)
Creatinine ≥1.3mg/dl	105	57.6 (39-75)	90.3 (81-96)	72.7 (50-89)	88.0 (79-94)	71.4 (42-92)	82.4 (73-90)

Creatinine ≥1.4mg/dl	105	30.3 (16-49)	97.2 (90-100)	45.5 (24-68)	97.6 (92-100)	57.1 (29-82)	95.6 (89-99)

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257 Table 3: Positive predictive values (PPV) and negative predictive values (PPV) of different
258 cut-off points for SDMA and serum creatinine concentration to detect different categories
259 of % GFR decrease below the mean GFR of the patient's body weight category.

Variable	**Sample Size**	**PPV % GFR ↓ ≥20% (95% CI)**	**NPV % GFR ↓≥20% (95% CI)**	**PPV % GFR ↓ ≥30% (95% CI)**	**NPV % GFR ↓ ≥30% (95% CI)**	**PPV % GFR ↓ ≥40% (95% CI)**	**NPV % GFR ↓ ≥40% (95% CI)**
SDMA >10µg/dl	105	36.8 (34-40)	94.4 (70-99)	25.3 (23-27)	100	16.1 (15-18)	100
SDMA >12µg/dl	105	40.6 (35-47)	86.1 (73-94)	29.0 (25-34)	94.4 (82-98)	18.8 (16-22)	97 (84-100)
SDMA >14µg/dl	105	43.6 (36-52)	82.0 (72-89)	29.1 (23-37)	88.0 (78-94)	21.8 (17-27)	96.0 (87-99)
SDMA >16µg/dl	105	55.9 (43-68)	80.3 (73-86)	41.2 (30-53)	88.7 (82-93)	32.4 (23-43)	95.8 (89-98)
SDMA >18µg/dl	105	79.0 (57-91)	79.1 (73-84)	63.2 (43-79)	88.4 (83-92)	52.6 (35-69)	95.4 (90-98)
SDMA >20µg/dl	105	75.0 (46-91)	74.2 (70-78)	75.0 (47-91)	86.0 (81-90)	53.3 (33-73)	92.6 (88-96)
Creatinine ≥1.0mg/dl	105	44.3 (37-52)	86.4 (75-93)	29.5 (24-36)	90.9 (80-96)	18.0 (14-23)	93.2 (83-97)
Creatinine ≥1.1mg/dl	105	53.1 (44-62)	87.5 (78-93)	36.7 (29-45)	92.9 (84-97)	22.5 (17-29)	94.6 (86-98)
Creatinine ≥1.2mg/dl	105	64.7 (51-76)	84.5 (77-90)	50.0 (38-62)	93.0 (86-97)	32.4 (23-43)	95.8 (89-98)
Creatinine ≥1.3mg/dl	105	73.1 (56-85)	82.3 (76-87)	61.5 (46-75)	92.4 (86-96)	38.5 (26-52)	94.9 (89-98)
Creatinine ≥1.4mg/dl	105	83.3 (54-96)	75.3 (71-79)	83.3 (54-95)	87.1 (82-91)	66.7 (41-85)	93.6 (89-96)

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A receiver operating characteristic (ROC) curve assessing the trade-off between sensitivity and (1-specificity) across each cut-off point for SDMA (>10µg/dl, >12µg/dl, >14µg/dl, >16µg/dl, >18µg/dl and >20µg/dl) to detect decreases in GFR below the mean GFR of a patient's body weight category of ≥20%, ≥30% and ≥40% is presented in Figure 6. The area under the curve for SDMA assessing for a decrease in GFR ≥20% was 0.76, 0.79 for assessing for a GFR decrease ≥30% and 0.86 for assessing for a GFR decrease ≥40%. Using Youden's J statistic, the optimal SDMA concentration for assessing for a GFR decrease of ≥20% was 16µg/dl (sensitivity 72% (95% CI 55-86), specificity 70% (95% CI 58-80)). The optimal SDMA concentration for assessing for a GFR decrease of ≥30% was 19µg/dl (sensitivity 57% (95% CI 34-77), specificity 90% (95% CI 81-95)) and optimal SDMA concentration for assessing for a GFR decrease of ≥40% was 18µg/dl (sensitivity 80% (95% CI 52-96), specificity 90% (95% CI 73-89)).

A ROC curve assessing the trade-off between sensitivity and (1-specificity) across different cut-off points for serum creatinine (≥1.0mg/dl, ≥1.1mg/dl, ≥1.2mg/dl, ≥1.3mg/dl and ≥1.4mg/dl) to detect decreases in GFR below the mean GFR of a patient's body weight category of ≥20%, ≥30% and ≥40% is presented in Figure 7. The area under the curve for serum creatinine assessing for a decrease in GFR ≥20% was 0.82, 0.83 for assessing for a GFR decrease ≥30% and 0.80 for assessing for a GFR decrease ≥40%. Using Youden's J statistic, the optimal serum creatinine concentration cut-off for assessing for a GFR decrease of ≥20% was ≥1.25mg/dL (sensitivity 67% (95% CI 48-82), specificity 89% (95% CI 79-95)). The optimal serum creatinine concentration for assessing for a GFR decrease of ≥30% was ≥1.31mg/dL (sensitivity 73% (95% CI 50-89), specificity 90% (95% CI 82-96)) and optimal

serum creatinine concentration for assessing for a GFR decrease of ≥40% was ≥1.37mg/dL (sensitivity 64% (95% CI 35-87), specificity 93% (95% CI 86-98)).

Out of the 57 dogs who had increased SDMA measured, in 32 dogs (56%) their GFR result was not considered consistent with renal disease (GFR increased or <20% decreased from the mean GFR of their bodyweight category). Out of these 32 dogs, reviewing the available follow up data, a final diagnosis was available for 23 (71.8%), with a median time to follow up of 344 days (range 2-951). A range of different final diagnoses for the patients' presenting clinical signs were obtained: psychogenic polydipsia (n=8), idiopathic dermatopathy (n=5), clinically normal (n=3), central diabetes insipidus (n=2), colonic adenocarcinoma (n=1), urinary tract infection (n=1), urinary incontinence (n=1), pyelonephritis (n=1), and renal mass (n=1). Follow-up data on dogs who had a decrease in GFR ≥20% below mean is further discussed in a separate publication by McKenna *et al*.[23]

Discussion

As anticipated the relationships between GFR and both serum creatinine and SDMA were non-linear. The regression associations between SDMA and GFR, and between serum creatinine and GFR in this study were lower than previously reported by Nabity *et al.*[19] A possible explanation for this is that the dogs in this study represented a heterogenous population of client-owned dogs, which were ultimately diagnosed with a variety of medical conditions that may have influenced serum creatinine and/or SDMA concentrations.

Increased SDMA above the reference interval of 14µg/dl was sensitive (85.7%) but relatively non-specific (52.8%) for detection of a ≥40% GFR decrease below the mean GFR of the patient's body weight category; a category that is considered as clear indication for a renal etiology of disease. Using ROC curve analysis and Youden's J statistic, the optimal SDMA concentration for assessing for a GFR decrease of ≥40% was 18µg/dl (sensitivity 80%, specificity 90%). Given the relatively low specificity of SDMA >14µg/dl for detecting of a GFR decrease of ≥40% , the authors suggest that applying a cut-off of >18µg/dl may be more appropriate than the previously-described reference interval when used as a screening test for decreased renal function in dogs with a clinical presentation suggestive of non-azotemic renal disease as a cause for polyuria and polydipsia or other urinary tract signs. When using SDMA to screen for non-azotemic renal disease, if using the traditional reference interval of >14µg/dL, only about half (52.8%) of cases will ultimately have significantly decreased GFR (defined as a GFR decrease of ≥40% below the mean GFR for the patient's bodweight category). The specificity is greatly improved (90%), while maintaining acceptable sensitivity (80%), if the SDMA cut-off is changed to >18µg/dL even if serum creatinine is within the reference interval.

Fifty six percent of patients who had SDMA measured above reference interval (> 14µg/dL) did not have a GFR result consistent with decreased renal function (defined as an increased GFR or <20% decreased below the mean GFR of the patient's bodyweight category) raising concern for false positive results in this scenario had SDMA alone been used (as a single data point) to interpret renal function in dogs being screened for non-azotemic renal disease. Evaluating the final diagnosis from follow-up clinical data for dogs with increased

SDMA but normal GFR estimation results, several fell into the category of idiopathic dermatopathy. Many of these cases had GFR estimation performed to screen for AKI due to the presence of skin lesions, reflecting the recent emergence of cutaneous renal glomerular vasculopathy in the United Kingdom.[26] Three dogs with increased SDMA and an increase/≤20% decrease from the mean GFR of their bodyweight category were considered clinically normal by the submitting veterinarians at the time of follow-up; unfortunately this assessment was based on spontaneous resolution of the dogs' clinical signs (polyuria-polydipsia in all cases) rather than on longitudinal monitoring of their kidney function. Therefore, the authors cannot exclude the possibility that these patients may have had undetected kidney disease at the time of follow-up, and that progression of kidney disease would have been documented either through serial assessment of serum creatinine concentration or repeat GFR estimation. The ultimate classification of these dogs as normal can therefore be questioned. Serial assessment of SDMA and GFR estimation in these dogs would have been of interest to determine the long-term outcome of carefully monitored renal function in these dogs with initially elevated SDMA concentrations. However, this was unfortunately not possible within the scope of this study.

A common rationale for measuring SMDA or performing GFR estimation in dogs is to determine whether a renal etiology exists for presenting clinical signs, typically polyuria and polydipsia, in non-azotemic patients. All dogs included in the statistical analysis for this study were non-azotemic at the time of GFR estimation. Using ROC curve analysis and Youden's J statistic, the optimal serum creatinine cut-off for assessing for a GFR decrease of ≥40% was ≥1.37mg/dL (sensitivity 64%, specificity 93%). This cut-off for serum creatinine

had lower sensitivity (64% vs. 80%) but similar specificity (93% vs. 90%) to a using cut-off of >18mg/dl for SMDA for detection of a GFR decrease of ≥40%. Using such a cut-off for serum creatinine therefore offers no significant advantage over using a cutoff of >18mg/dl for SDMA for detection of decreased renal function, but the presence of a high-normal serum creatinine concentration in conjunction with an SDMA result of >18mg/dl may further support clinical suspicion for the presence of decreased renal function.

The authors acknowledge that using breed-specific reference ranges for serum creatinine or serial monitoring of serum creatinine concentrations may have increased the sensitivity of creatinine for detection of decreased renal function without the requirement for GFR estimation in some of the dogs in the present study. However, breed-specific reference intervals and serial creatinine concentrations from the same laboratory were not available, so it was not possible to compare the sensitivity of SMDA to the use of breed-specific creatinine reference intervals or to serial creatinine measurements. Such a comparison could be a focus for a future study.

The authors recognise the limitations of this study. Firstly, the study was retrospective in nature. The reference interval for serum creatinine at Idexx Laboratories (at both the German and UK locations) was changed to 0.5-1.5mg/dl (44-133µmol/l) during the course of the study (on 29th October 2017). The reference intervals prior to this date had been 0.23-1.63mg/dl (20-144.5µmol/l) in the UK and <1.4mg/dl (<124umol/l) in Germany. 93 samples were sent to the Idexx's UK laboratory prior to the reference interval change while the

remaining 17 samples were sent to Idexx's German laboratory after the reference interval change. The change in reference intervals did not reflect a change in the equipment or methodology used for measurement of serum creatinine concentration. The most up-to-date reference interval of 0.5-1.5mg/dl (44-133µmol/l) was used for the purpose of interpretation of serum creatinine values in this study. The reference intervals for SDMA did not change during the course of the study.

The authors also acknowledge the limitations of using GFR estimation as the gold standard for assessment of renal function. GFR varies with the size of dog (somewhat accounted for by body weight)[12] and with breed,[27] which may lead to misinterpretation of a patient's renal function if the patient size and breed is not taken into account. It is also possible that comparing a patient's GFR to the mean GFR of the patients' bodyweight category as defined by Bexfield *et al*.[13] may lead to misinterpretation of a patient's renal function; the mean GFR of the patient's bodyweight category may not reflect what is a normal GFR for an individual patient, which could lead to over-estimation or under-estimation of any reduction in GFR.

In conclusion, SDMA can be a sensitive and specific marker for detecting decreased renal function depending on the cut-off used. Using a cutoff of >18µg/dl rather than the traditional cutoff of >14µg/dl increases the specificity of SDMA for detecting decreased renal function.

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Figure 1: relationship between serum SDMA and serum creatinine

Figure 2: relationship between GFR and serum SDMA

Figure 3: relationship between GFR and serum creatinine

Figure 4: relationship between GFR and reciprocal of serum creatinine

Figure 5: relationship between GFR and reciprocal of serum SDMA

Figure 6: ROC curve for SDMA detecting a GFR decrease of ≥20%, ≥30% and ≥40% below the mean GFR of a patient's body weight category.

Figure 7: ROC curve for serum creatinine detecting a GFR decrease of ≥20%, ≥30% and ≥40% below the mean GFR of a patient's body weight category.