Standard Article

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Variability of Symmetric Dimethylarginine in Apparently Healthy Dogs

M.A. Kopke, R.K. Burchell, C.G. Ruaux, S.E. Burton, N. Lopez-Villalobos, and A. Gal

Background: Symmetric dimethylarginine (SDMA) is a screening tool for early kidney dysfunction and monitoring treatment in cases of chronic kidney disease (CKD). There are no current studies describing the suitability of this test for use with published population-based reference intervals.

Hypothesis/Objectives: To determine the components of biological variability, the index of individuality (IOI), the critical difference between sequential measurements (C_D) and the number of measurements required to assess the homeostatic set point (HSP), for both SDMA and serum creatinine (sCr), in apparently healthy dogs.

Animals: Twenty apparently healthy adult dogs owned by clients or staff at a veterinary teaching hospital.

Methods: Prospective, observational study. Blood was collected from each dog on 9 occasions, and SDMA and sCr were measured in duplicate using commercially available assays.

Results: SDMA and sCr had intermediate and low IOI values of 0.87 and 0.28, respectively. The C_D of SDMA and sCr, was 1.34 μg/dL and 0.89 μmol/L, respectively. The sample numbers required for estimation of an individual's HSP (with 90 and 95% CI) for SDMA and sCr were 8 and 45, and 2 and 12 sequential measurements, respectively.

Conclusions and Clinical Importance: Based on our findings, in comparison to sCr, SDMA is better suited for use with population-based reference intervals. False-negative test results could occur when comparing a single test result from an individual to such intervals. Ideally C_D should be used with sequential measurements.

Key words: Canine; Chronic kidney disease; Critical difference; Index of individuality; Serum creatinine; Symmetric dimethylarginine.

Symmetric dimethylarginine (SDMA) is a relatively new indirect biomarker of glomerular filtration rate (GFR), and kidney function.[1,2] It has several advantages over serum creatinine (sCr) in detecting decreased GFR. SDMA detects loss of renal function earlier and is less affected by extra-renal factors such as age, sex, breed, and lean body mass.[1–6] The use of SDMA has thus become more extensive, not only in screening for early kidney dysfunction, but also as a therapeutic monitoring tool in chronic kidney disease (CKD).[1,2] SDMA has been incorporated into the International Renal Interest Society[a] CKD Staging Guidelines on a preliminary basis.

The clinical utility of a reference interval and the performance of a screening test, are dependent on the total biological variability (TBV) of the test. The intraindividual variability, interindividual variability, and analytical variability of the assay comprise the TBV.[7–9] Determination of the TBV of a biomarker establishes the limits of normality and thus the inability to conclude abnormality, if the result falls within the limits of the established reference interval (Fig 1).[10] Tests with high individuality possess low intraindividual and high interindividual variability and may not provide clinically useful information when interpreting a single result from an individual, if that result happens to fall within the reference interval. The index of individuality (IOI) describes the relationship between the intraindividual and interindividual variability for a given diagnostic test, with IOI values classified as low (<0.6) or high (>1.4).[7,9] The individual's homeostatic set point (HSP) is derived from the mean and confidence intervals (CI) of the test variable with repeated measurements. Tests with a low IOI will have the individual's HSP spanning a small fraction of the reference interval and are thus not well-suited for use in population-based reference intervals.[7,8] With such tests, it becomes important to calculate the critical difference (C_D) which is the minimum change necessary between sequential results for an individual to exceed the TBV of the test.[7]

Several studies have investigated the TBV and clinical utility of various tests in veterinary medicine including; hematological variables,[11] platelet function,[12] hemostatic variables,[13]

Abbreviations:

C_D	critical difference
CI	confidence intervals
CKD	chronic kidney disease
CV_A	analytical coefficient of variation
CV_G	interindividual coefficient of variation
CV_I	intraindividual coefficient of variation
CV_T	total biological coefficient of variation
GFR	glomerular filtration rate
HSP	homeostatic set point
IOI	index of individuality
IRIS	international renal interest society
sCr	serum creatinine
SDMA	symmetric dimethylarginine
TBV	total biological variability

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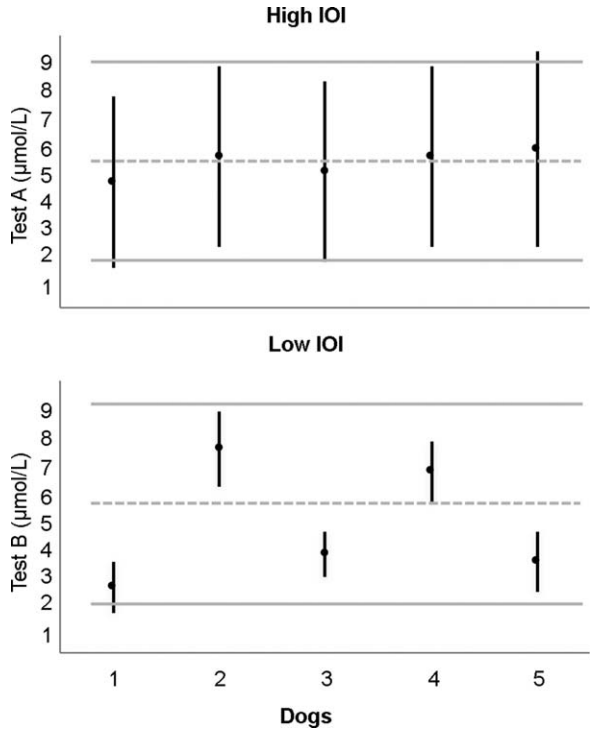

Fig 1. Graphic representation of 2 hypothetical diagnostic tests explaining the concepts of high and low IOI, respectively. Tests with high individuality have a low IOI value and vice versa. Test A demonstrates high IOI because of high intraindividual variability and low interindividual variability. With a high IOI, the values span a large proportion of the population-based reference interval and thus is suitable for a single value to be interpreted using such an interval. Test B demonstrates a low IOI, with low intraindividual variability and high interindividual variability. With a low IOI, the values only span a small proportion of the population-based reference interval and thus such tests are not suitable for interpretation of a single value for an individual, using such an interval. In the case of test B, a normal test result will not necessarily accurately identify deviation from normal for a specific individual. IOI, index of individuality; gray solid lines, upper and lower reference limits of the population-based reference range; vertical line with a dot in the center, each dogs' test mean and confidence interval for the mean.

cardiac troponin I,[14] N-terminal pro-brain natriuretic peptide,[14,15] serum cystatin C,[16,17] C-reactive protein,[18] specific canine pancreatic lipase immunoreactivity,[18] canine serum cortisol concentration post-adrenocorticotropic hormone stimulation,[19] and canine serum thyrotropin concentration.[20]

Based on the current veterinary literature regarding SDMA, we hypothesized that SDMA would have a lower individuality than sCr because of less influence from extrarenal factors. The aims of the study were to (1) determine the IOI of SDMA and sCr, (2) estimate the C_D for both tests, and (3) estimate the number of samples required to determine the HSP for an individual dog, for both biomarkers. We approached the study aims by obtaining serial measurements of SDMA in apparently healthy dogs to determine the components of TBV of SDMA. We also obtained serial measurements of sCr in order to contrast SDMA with creatinine.

Materials and Methods

Animals

Blood was collected from 20 adult dogs weighing over 10 kg that were privately owned by clients or staff members at the Massey University Veterinary Teaching Hospital. The owners of the dogs considered the dogs to be in good health and a veterinarian examined the dogs before the commencement of the study. These dogs were fasted overnight and had free access to water before and after the blood collection. Diets and housing conditions differed depending on owner preference. Table 1 describes the characteristics of the cohort of dogs included in this study.

Study Design

The Massey University Animal Ethics Committee approved the study (protocol #16/102).

The prospective analysis was performed on 20 apparently healthy dogs. To minimize pre-analytical variation, both sample collection and processing were standardized. A total of 9 separate blood samples were collected between 0800 and 1000 hours from each dog after an overnight fast (≥8 hours). Each 2 mL sample was obtained after gentle manual restraint (with the dog in a sitting position) and drawn via jugular venipuncture using a 20 G needle and a 3 mL syringe.[b] The blood samples were collected on Days 1, 3, 5, 12, 19, 26, 40, 54, and 68. After collection, blood was transferred into serum tubes.[c] Samples were allowed to clot at room temperature, were centrifuged[d] at 3,000 g for 15 minutes, and the serum was then decanted and stored in sealed aliquots at −80°C until analysis. At the end of the study period, the samples were submitted to a reference diagnostic laboratory[e] for analysis. SDMA[3],[f] and serum creatinine (sCr)[g] were measured for each sample, in duplicate, with commercially available, previously validated assays. Both assays were performed according to the manufacturers' recommendations. The lower and upper limits of quantification of the 2 assays were 0–100 µg/dL and 4–4420 µmol/L for SDMA[f] and sCr,[g] respectively.

Statistical Analysis

We performed all statistical analyses in R 3.3.3.[21] We used the lm() function in the lme4 package[22] to perform analyses of variances of the

Table 1. Dog demographics.

Variable	Value
Sample size (n)	20
Age[a] (mean ± SD)	76 ± 36.9
Weight[b] (median and range)	26.25 (15–46)
BCS[c] (median and range)	5 (3–7)
Sex	
Male entire	4
Female entire	3
Male neutered	6
Female spayed	7
Breed	
Huntaway (New Zealand Sheepdog)	6
Collie	6
Greyhound	3
Harrier Hound, Jack Russell Terrier, Labrador Retriever, Maremma Sheepdog, Huntaway/ Labrador Retriever crossbreed	1 each

BCS, body condition score; SD, standard deviation.
[a]Age in months.
[b]Body weight in kg.
[c]BCS in a scale of 1–9.

dependent variables "SDMA" and "creatinine" with a linear model that included the random effects of "dog," "sample," and "duplicates."[7] Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. The C_D was calculated by the formula, $C_D = 1.96[2\ (Var_I + Var_A)]^{1/2}$, where Var_G, Var_I, and Var_A are the interindividual, intraindividual and analytical variance components derived from the expectation of the mean squares as outlined by Fraser and Harris.[7] The coefficients of variation (CV) for intraindividual (CV_I), interindividual (CV_G), and the analytical (CV_A) components of the total variance were calculated by dividing the SD of each by the mean. The IOI was calculated as $(CV_I^2 + CV_A^2)^{1/2}/CV_G$. The number of specimens that should be assayed to be X% confident of achieving an estimate of the HSP within D% of an individual dog was calculated from the formula $n = Z_X^2(CV_I^2 + CV_A^2)/D^2$, where Z_X is the percentile of the standard normal distribution and D is the desired percentage closeness to the HSP ($Z_X = 1.645$ for $X = 90\%$ and $D = 10\%$; $Z_X = 1.96$ for $X = 95\%$ and $D = 5\%$).[19] We used the outliers package[23] cochran.test() function to test if any of the dogs was significantly influencing the variance from the other dogs and the BlandAltmanLeh package[24] bland.altman.plot() function to generate the Bland–Altman plot. We used the lmerTest package[22,25] lmer() function to perform a regression analysis for repeated measures, to evaluate the relationship between the fixed effect factor "day of sampling" and the dependent variables "SDMA" and "creatinine"; a random intercept was fitted for each dog. A P value < 0.05 indicated the presence of statistical significance.

Results

The means and 90% confidence intervals (CI) for SDMA and serum creatinine (sCr) of 20 apparently healthy dogs and the group mean with respect to the reference laboratory population-based reference interval are presented in Figures 2 and 3, respectively. The results of the mean, coefficients of variation (CV) for CV_G, CV_I, CV_A, the variances (Var) for Var_G, Var_I, Var_A, the critical difference (C_D), C_D %mean, and IOI for both SDMA and sCr, and the numbers of measurements required to assess the HSP for either test with CI of 90 and 95%, are summarized in Table 2. The CV_G, CV_I and CV_A for SDMA were 19.5, 14, and 9.5%, respectively. In comparison the CV_G, CV_I, and CV_A for sCr were 30.1, 8.32, and 2%, respectively. Furthermore, the 90 and 95% CI for the individual's HSP for SDMA required 8 and 45 sequential measurements, respectively, in comparison to 2 and 12 sequential measurements for sCr. Finally, we calculated IOI values of 0.87 and 0.28 for SDMA and sCr, respectively. The mixed-effect regression analysis indicated that there was a significant effect of the sampling day on SDMA and sCr. Relative to Day 1, SDMA (mean, SE) on Days 12 and 26 was lower by -1.52 ± 0.46 ($P = 0.001$) µg/dL and -1.43 ± 0.46 ($P = 0.002$) µg/dL, respectively. The effect of time on sCr was opposite to that of SDMA. Relative to Day 1, sCr (mean, SE) on Day 40 was greater by 3.95 ± 1.8 ($P = 0.03$) µmol/L. There was a significant ($P < 0.001$) moderate to high correlation ($r = 0.7$) between SDMA and sCr (Fig 4). The Bland–Altman plot (Fig 5) indicated a proportional bias for sCr relative to SDMA; that is, the difference between the 2 measurements becomes greater at higher values for the 2 tests.

Discussion

The purpose of our study was to estimate the IOI for SDMA and sCr to determine their suitability for use with a population-based reference interval. Our study demonstrated intermediate and low IOI values (ie, high individuality) of 0.87 and 0.28 for SDMA and sCr, respectively. According to our results, in comparison to sCr, SDMA is better suited for use in population-based reference intervals.

A subject's HSP for a laboratory test is defined as the mean value from repeated measurements for that individual, bounded by the confidence interval for the mean.[7] In healthy individuals, the HSP should almost always exist

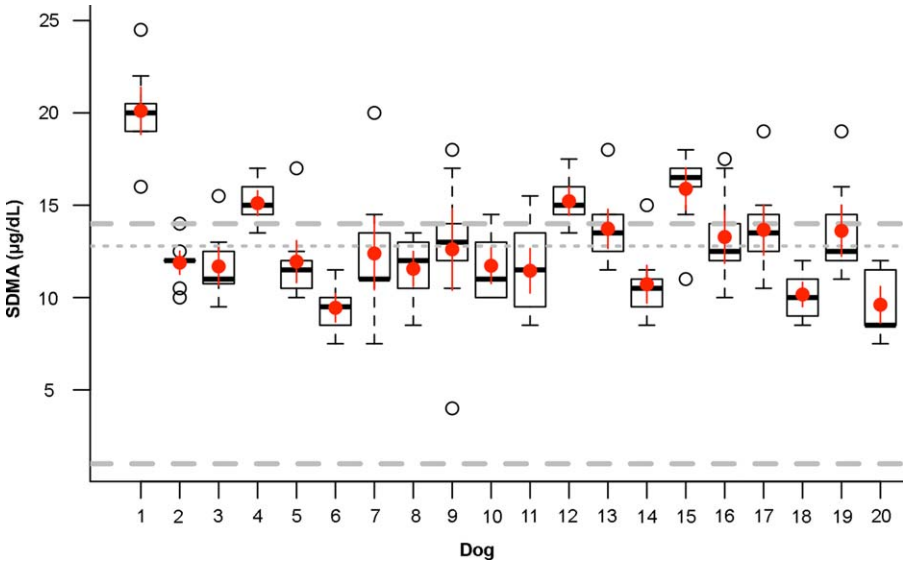

Fig 2. Serum symmetric dimethylarginine (SDMA) concentrations in 20 apparently healthy dogs sampled 9 times at varying intervals. The box and whiskers represent the median and 1.5× interquartile ranges for SDMA from 9 measurements. The red dot with error bars represent the mean and 90% CI (ie, the homeostatic set point of the individual). The gray dashed lines represent the population-based reference intervals 0–14 µg/dL. The gray dotted line represents the group's mean. Gray empty circles represent outlying data.

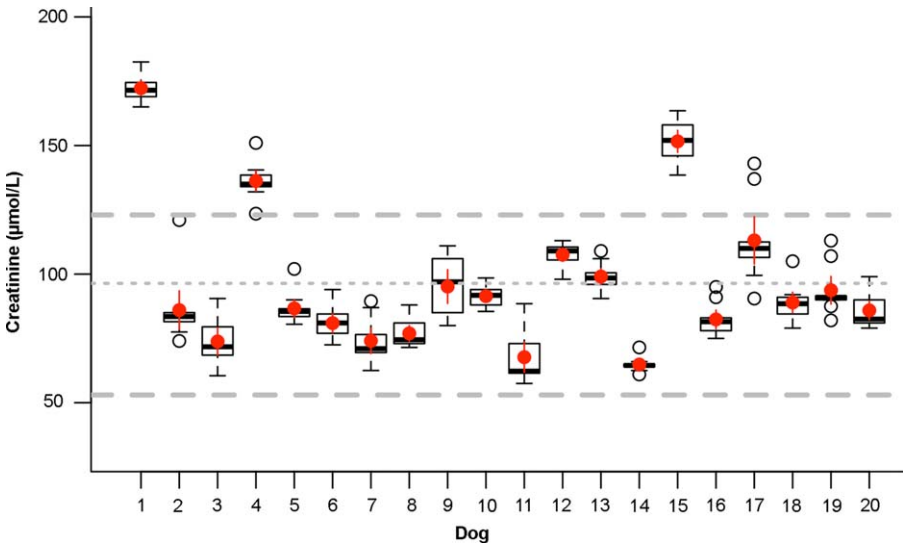

Fig 3. Serum creatinine (sCr) concentrations in 20 apparently healthy dogs sampled 9 times at varying intervals. The box and whiskers represent the median and 1.5× interquartile ranges for sCr from 9 measurements. The red dot with error bars represent the mean and 90% CI (ie, the homeostatic set point of the individual). The gray dashed lines represent the population-based reference intervals 53–123 µmol/L. The gray dotted line represents the group's mean. Gray empty circles represent outlying data.

within the population-based reference interval for the test. The IOI measures the degree of individuality of a laboratory test, with tests possessing high individuality demonstrating a low IOI value. In the case of a laboratory test with a high individuality, the individual's HSP spans a small fraction of the population-based reference interval. Therefore, a test with high individuality has the limitation that if its value falls within the population-based reference interval, but outside of the individual's HSP, it could be falsely interpreted as normal (ie, false negative).

The general recommendation for assays with a low to intermediate IOI is to use the critical difference (C_D) between sequential measurements rather than to rely on population-based reference intervals. In that situation, a difference between 2 measurements that is equal to or smaller than the C_D could be the result of random variation rather than a true biological change in the individuals test variable. In this study we found that the C_D's of SDMA and sCr, were 1.34 µg/dL and 0.89 µmol/L, respectively. Given that the C_D of sCr is 0.93% of the group mean, a slight difference between sequential measurements >0.93% would be considered abnormal. The International Renal Interest Society (IRIS) staging protocol[a] suggests that the assessment of sCr should take place at least twice in the stable patient to overcome the biological variability. According to the results of our study as depicted graphically in Figure 3, none of our dogs were stable because they all had at least 2 measurements that were above the calculated C_D. Hence, the IRIS algorithm for IRIS Stage 1 would be more accurate if the HSP was calculated. According to our results, 12 measurements would be required to find the HSP with 95%

Table 2. The results of the mean, CV_I, CV_G, CV_A, Var_I, Var_G, Var_A, C_D, C_D %mean, and IOI for SDMA and sCr, and the numbers of measurements required to assess the HSP with 90 and 95% CI, for SDMA and sCr.

Parameter	SDMA (µg/dL)	sCr (µmol/L)
Mean	12.7	96.1
Var_G	6.1	836
Var_I	3.2	64
Var_A	1.5	3.7
CV_G	19.5	30.1
CV_I	14	8.3
CV_A	9.5	2
C_D	1.34	0.9
C_D %mean	10.5	0.93
IOI	0.87	0.28
CI 90%	8	2
CI 95%	45	12

Var_G, interindividual variance; Var_I, intraindividual variance; Var_A, analytical variance; CV_G, interindividual coefficient of variation; CV_I, intraindividual coefficient of variation; CV_A, analytical coefficient of variation; C_D, critical difference; IOI, index of individuality; CI, confidence interval.

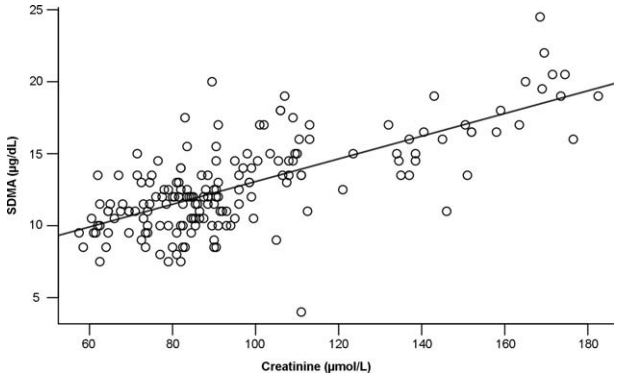

Fig 4. Scatter plot of symmetric dimethylarginine concentrations (µg/dL) against serum creatinine (µmol/L) from 20 apparently healthy dogs, showing a positive correlation between the 2 variables ($R^2 = 0.49$; $P < 0.001$).

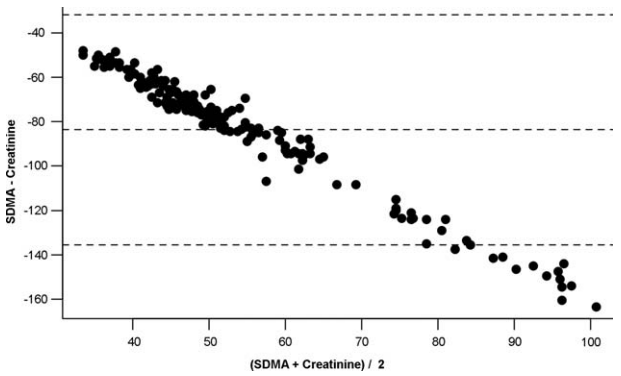

Fig 5. Bland–Altman plot demonstrating a progressive proportional bias between the 2 tests. As the renal function declines there is a proportional increase in serum creatinine relative to symmetric dimethylarginine. The middle dashed line represents the mean difference between SDMA and sCr. The upper and lower dashed lines are 2 SD above and below the mean difference between SDMA and sCr (middle dashed line).

confidence interval. Future studies on dogs with IRIS Stages 2, 3, and 4 will determine if the number of measurements needed to establish the HSP is similar or different from our results. Opinions vary as to whether such studies should be conducted in healthy individuals or those with stable disease. In the human literature, no significant difference has been documented in studies aimed at evaluating the components of biological variability when performed in the healthy versus the stable disease state.[10,26,27]

Our study indicates that, from the perspective of biological and analytical variability, SDMA is superior to sCR as a biomarker for detecting early kidney dysfunction. As shown in Figures 2 and 3, SDMA had less interindividual variability. The decreased interindividual variability and increased C_D of SDMA relative to sCr decreases the probability that a given SDMA test result would be outside the individual's HSP for SDMA. An explanation for the increased interindividual variability with sCr might be the variances in age and lean body mass across our study, shown in Table 2. Age and lean body mass influence sCr concentration but do not influence SDMA concentration.[4]

When assessing the biological variance of several biomarkers of GFR in healthy dogs, an intermediate IOI value of 0.8 for sCr was estimated in a previous study,[16] in contrast to the low IOI value of 0.28 in our study. This same study[16] found marked variation between their findings and yet another study estimated an IOI value of 1.15.[26] A potential reason for such marked difference between both previous studies and ours would be that short-term studies tend to yield higher individuality when compared to long-term studies.[7,16,28] Factors such as storage time, temperature and sample type could also influence the degree of individuality during the storage period.[29]

In this study, the calculated SDMA mean was 12.7 μg/dL (Table 2). The SDMA mean was in the top quarter of the population-based reference interval (Fig 2). This could argue for revision of the current published reference interval. The reference laboratory utilized in this study made use of published population-based reference intervals established for use in healthy dogs older than 1 year of age.[h] With adoption of such intervals there is the risk that in some cases the reference interval may not be appropriate for either the method of measurement or alternatively the population of animals to which it is applied. To avoid such problems, a transference study should be performed to determine whether the population-based reference interval is suitable for the population being evaluated.[9] A transference study is an extended correlation determining the amount of bias between the established population-based reference interval of the first population and the target population. The published reference interval is then adapted to fit the target population, using the amount of bias determined. The advantage of a transference study is that a smaller number of subjects is required for such an analysis compared to the number that is required to determine a new population-based reference interval.[9]

A previous study[1] documented a moderately high correlation ($r = 0.84$) between SDMA and sCr. In agreement with that study, our study demonstrated a mildly lower correlation between SDMA and sCr concentrations ($r = 0.7$), depicted graphically in Figure 4. Despite the moderate to high correlation between sCr and SDMA, the Bland–Altman plot in Figure 5 indicates a proportional bias for sCr relative to SDMA. This proportional bias can be attributed to progressive increase in sCr values or a progressive decrease in SDMA values. The proximal convoluted tubules are responsible for a small component of active secretion of creatinine. The measured sCr is the sum of this small component and the large component of glomerular filtration. In the early stages of loss of renal function there is a sharp exponential drop in GFR. The population-based reference interval for sCr fails to show the early fall in GFR as sCr values fall within the reference interval. In contrast to sCr, SDMA is freely filtered without a component of tubular active excretion. Hence, we suspect that this is causing a proportional bias of sCr relative to SDMA. Alternatively, if SDMA and sCR have non-linear, differing, relationship to GFR, it is not unexpected that differences between the 2 measurements will become greater at higher values for the 2 tests.

We found that time of sampling had a significant effect on both SDMA and sCr albeit in an opposite direction. In other words, SDMA and sCr concentrations were significantly lower and higher, respectively, from Day 1 on various days. A dietary effect and an environmental effect such as weather are unlikely because the effect of time on SDMA and sCr was opposite and dogs were not sampled simultaneously to each other to experience the same weather conditions. Altering the diet was found to affect SDMA after 3 and 6 months.[4] However, the dogs in this study were on different diets and as mentioned above, we sampled them sequentially and not simultaneously. The pattern of days affected seems random, and highlights the significant effect that biological variability may have.

Several limitations were identified with this study, the first being slight breed overrepresentation, with the majority of animals enrolled being Huntaway/Huntaway cross (New Zealand sheepdog) and collies. Although this large percentage of working dogs accurately reflects the dog population in New Zealand, this is not globally representative. In addition to this, apart from 1 Jack Russell terrier, no small breed

dogs were enrolled in the study. That being said, extra-renal factors such as breed and lean body mass have not been shown to affect SDMA concentrations in dogs.[4,6] Another potential limitation of our study was the use of a competitive ELISA assay[f] for SDMA measurement, rather than mass spectrometry. The latter is considered the gold standard for SDMA quantification with better selectivity and sensitivity, however, the above assay has been shown to have a high correlation with mass spectrometry.[3] The use of a published population-based reference interval in this study, without a transference study having been performed to ascertain its suitability for the population being sampled, was another limitation of our study. Assessment of the biological variability of SDMA in apparently healthy animals without information on their urine specific gravity, might be another potential limitation if an inference on azotemic dogs is sought. However, as mentioned previously, no significant difference in biological variability has been documented in similar studies performed in healthy versus stable disease state individuals in the human literature.[10,26,27]

In conclusion, both SDMA and sCr were found to have moderate to high individuality with a intermediate to low IOI that could account for significant false-negative test results in dogs with early kidney dysfunction; more so with sCr than SDMA. Hence, according to our results, we highlight the limitation of both tests as screening tests when comparing a single value for an individual to published population-based reference intervals. This emphasizes the importance of serial monitoring, when trying to diagnose early kidney dysfunction and the need for application of C_D. We found that for SDMA, C_D should be >1.34 μg/dL to ensure that the difference, whether an increase or decrease between sequential measurements, is not because of biological variability. Future studies should involve further investigation into sCr and SDMA in the diseased state, according to IRIS CKD Staging Guidelines, to assess the potential use of the C_D for monitoring changes in renal function.

Footnotes

[a] IRIS Staging of CKD. International Renal Interest Society website. Iris-kidney.com/guidelines/staging.html. 2015, © 2016 International Renal Interest Society (IRIS)

[b] BD PrecisionGlide Needle 20G 1TW and 3 mL Luer-Lok Tip Syringes, Singapore

[c] BD Vacutainer Red Top Tubes, Singapore

[d] Heraeus Multifuge X3, Thermo Fisher Scientific, Waltham, MA

[e] IDEXX Laboratories, Inc, Palmerston North, New Zealand

[f] Patch D, Obare E, Xie H. High throughput immunoassay that correlates to gold standard liquid chromatography mass spectrometry (LC-MS) assay for the chronic kidney disease (CKD) marker symmetric dimethylarginine (SDMA). J Vet Intern Med 2015;29:1216 (abstract)

[g] Beckman Coulter AU CREA Creatinine (Enzymatic), Australia (http://www.accrediweb.fr/Biochimie/Gamme_AU/Notices_Utilisation/Metabolites/CREATININE_ENZYMATIQUE_BLOSR6x204_07_FR.pdf)

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Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-Label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

Institutional Animal Care and Use Committee (IACUC) or Other Approval Declaration: The Massey University Animal Ethics Committee approved the study (protocol #16/102).

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