The new age of renal biomarkers – does SDMA solve all of our problems?

Hannah Sargent BVetMed (Hons), MRCVS

Jonathan Elliott MA, Vet MB, PhD, Cert SAC, Dip ECVPT, MRCVS

Rosanne E. Jepson BVSc MVetMed PhD DipACVIM DipECVIM MRCVS

Royal Veterinary College, Hawkshead Lane, North Mymms, Herts, AL9 7TA

hsargent@rvc.ac.uk; jelliott@rvc.ac.uk; rjepson@rvc.ac.uk

Corresponding author contact number: 07966807751

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Abstract

Within clinical small animal practice, diagnosis of both chronic kidney disease (CKD) and acute kidney injury (AKI) is common. To assess renal function, measurement of glomerular filtration rate (GFR) is considered the gold standard. Currently, routine tests of kidney function include surrogate markers of GFR such as serum creatinine, and urea, each with their own limitations, whilst urine protein to creatinine ratio gives an indication of glomerular and tubular handling of protein, and urine specific gravity information about urine concentrating ability by the kidney. These parameters are used together with historical and physical examination data to give a diagnosis of kidney disease following which creatinine, proteinuria and blood pressure are used to stage CKD and, together with urine output, grade AKI according to the International Renal Interest Society (IRIS). However, there has been much concern that creatinine is insensitive when used to indicate early decline in renal function and this has highlighted the need for additional methods of diagnosing and monitoring these patients, with the potential to allow earlier therapeutic intervention. Symmetric dimethylaginine (SDMA) is a novel biomarker, which has been shown to perform as a surrogate marker of GFR in small animals. This article will review current research on SDMA and the ways in which it may be utilised in small animal practice; current research supports the use of SDMA as a screening test for detection of early CKD according to IRIS guidelines, but further research is required in to the usefulness of SDMA as a tool for monitoring disease and the effect of non-renal influences.

Introduction

Renal disease encompasses both chronic kidney disease (CKD) and acute kidney injury (AKI) and affects both cats and dogs, with CKD in particular being a common diagnosis in general practice (Bartlett et al. 2010; O'Neil et al. 2013; O'Neil et al. 2015). AKI is characterised by a rapid decrease in glomerular filtration rate (GFR), for which there are a wide array of potential aetiologies both community and hospital acquired, though in many instances an underlying aetiology is not identified (Khan & Khan 2015). In contrast, CKD is defined as the presence of functional or structural abnormalities affecting one or both kidneys for a period of at least 3 months. Again, although certain specific conditions can be identified e.g. polycystic kidney disease, frequently the major histopathological finding is non-specific tubulointerstitial nephritis particularly in cats. (Chakrabarti et al. 2012; Reynolds & Lefebvre 2013).

In veterinary medicine, it is routine to use a combination of plasma or serum creatinine (Scr) and urea concentrations with urine specific gravity and protein content to assess renal function. Limitations of these parameters mean that the early diagnosis of CKD remains challenging. Nevertheless, this is of significant clinical importance as early intervention may slow the rate of progression of disease, potentially improving quality of life and prolonging survival (Grauer 2005; Tenhündfeld et al. 2009). In addition, early diagnosis possibly gives the best chance of identifying and treating the underlying cause. This has led to the advent of a new age of research with the aim of identifying biomarkers of renal function that can be utilised to diagnose early disease.

Current methods of assessing kidney function in clinical practice

The rate of glomerular filtration (GFR) is defined as the volume of ultra-filtrate formed in the nephrons of both kidneys per unit of time and is directly correlated to the functional renal mass (DiBartola 2012; Kerl & Cook 2005).Therefore, measuring the clearance of a marker of filtration is considered the best method for assessing renal functional mass (Kerl & Cook 2005). The exogenous filtration marker inulin is considered the gold standard marker for determination of GFR via urinary clearance (Haller et al. 1998). However, determination

of urinary clearance is challenging clinically compared to obtaining blood samples and therefore plasma clearance of a marker, typically iohexol, remains the most useful filtration test for clinical practice (Finch 2014). Within clinical practice, serum or plasma creatinine and urea concentration combined with urine specific gravity (USG) are the most frequently used markers of renal function, being easily quantified from a single blood or urine sample. Having identified azotaemia on a clinical biochemistry, pre and post-renal azotaemia should always be excluded before attributing azotaemia to intrinsic kidney disease. It is widely reported that in dogs with pre-renal azotaemia, USG should exceed 1.030 whilst in cats it should exceed 1.035 (Wamsley & Alleman 2017). However, this is complicated by the fact that cats may also maintain urine concentrating ability despite significant renal mass reduction (Adams et al. 1993; Mathur et al. 2002). Anecdotally, cats with early CKD may still maintain some degree of urine concentrating ability (USG 1.030-1.045) even in the face of mild azotaemia.

Scr concentration is an indirect estimate of glomerular filtration. Creatinine, a by-product of endogenous muscle metabolism (Linnetz & Graves 2010), is filtered at the glomerulus and negligible renal secretion or reabsorption occurs in healthy animals (Finco et al. 1991). Plasma or serum concentrations have been shown to correlate inversely with GFR (Finch 2014). However, the use of Scr concentration as a surrogate marker of GFR has limitations; two of the most clinically relevant being non-renal influences and poor sensitivity to early disease.

Non-renal influences on serum creatinine

Circulating creatinine is a breakdown product of creatine phosphate in muscle and therefore Scr concentration will depend on muscle mass and may be significantly decreased in animals with low muscle mass such as geriatric or immature individuals or in (Braun et al. 2003). Scr is also influenced by age and breed in dogs and, to a lesser degree cats (e.g. Birman) (Gunn-Moore et al. 2002), however breed specific reference intervals are not routinely used (Reynolds et al. 2010; Misbach et al. 2014; Rørtveit et al. 2015). Data suggests that plasma creatinine concentrations increase gradually over the first year of life in

dogs but then remain stable(Braun et al. 2003) whilst in kittens creatinine concentrations are relatively high at birth but are similar to or lower than adults by 8 weeks of age (Levy et al 2006). Furthermore, studies have shown that Scr can be affected by diet with increases in Scr following meat ingestion (Watson et al. 1981). A recent study of healthy geriatric cats aged between 8 and 19 years, revealed cats below 12 years had higher total lean body mass determined by dual-energy X-ray absorptiometry, Scr and GFR compared with cats over 15 years old (Hall et al. 2014b). This is of particular clinical relevance with the demographic of cats being monitored for CKD generally being geriatric. To allow for these limitations, it is recommended that Scr is always measured on a fasted blood sample and interpreted considering the breed, muscle mass and age of the animal. In general, non-renal disease conditions are believed to have minmal influence on creatinine concentration aside from their potential impact on muscle condition. The exception is thyroid disorders, where the alteration in endogenous production of creatinine may have an impact on plasma creatinine concentration (Panciera & Lefebvre 2009).

The interpretation of Scr may also be influenced by analytical variability (Hokamp & Nabity 2016). Within human medicine, the clinical implications of inconsistencies between instruments and laboratories, have been recognised and the standardisation of Scr results between laboratories is a priority, with consensus based International Organisation for Standardisation (ISO) standards being made available (Peake & Whiting 2006). However, similar standardisation has not been implemented in veterinary medicine. A recent study of reference laboratories in Northern Europe revealed laboratories to have good precision amongst instruments but revealed large variation between different laboratories (Ulleberg et al. 2011) highlighting the importance of consistency in the analyser and laboratory used when monitoring trends in creatinine.

Sensitivity of creatinine as a maker of glomerular filtration rate

Perhaps the most widely reported limitation of using a marker of GFR to assess renal function and the greatest challenge in early diagnosis of CKD is the curvilinear relationship between the marker and GFR, specifically the steep curvilinear relationship between Scr and GFR (Figure 1). Consequently, a significant reduction in GFR from normal is required before a corresponding increase in Scr is seen (Finch 2014) whilst in advanced disease, a smallhange in GFR will have a large impact on Scr making Scr a useful marker of progression in the later stages of CKD.

Increases in creatinine can be identified in early disease but the changes are small, often still within reference interval and potentially below the precision of instruments measuring creatinine. Nevertheless, such subtle changes and trending of creatinine within reference interval can be used to determine change in GFR with time, providing the patient's muscle mass, volume and hydration status remains constant (Hokamp & Nabity 2016; Nabity et al. 2015). Creatinine is widely used in this context particularly with reference to acute kidney injury in human (RIFLE classification (Risk of renal dysfunction, Injury to the kidney, Failure of renal failure, Loss of renal function and End-stage renal disease) and Acute Kidney Injury Network (AKIN)) (Lopes & Jorge 2013) and veterinary medicine (IRIS 2013).

Use of creatinine for IRIS grading of acute kidney injury and staging of chronic kidney disease

Within veterinary medicine, IRIS recommends the use of fasting blood creatinine concentration to grade AKI and stage CKD in cats and dogs (IRIS 2019). The presentation of animals with AKI is frequently reported to be different to that in people, with animals often suffering a community acquired insult (e.g. toxin) rather than hospitalised acquired AKI. Such patients typically present with clinical signs referable to uraemia, and clinical biochemistry confirms azotaemia. In this situation, creatinine would be anticipated to perform well as a marker of GFR as creatinine values will fall on the steep region of the exponential relationship. However, IRIS grade I AKI includes non-azotaemic animals with a progressive increase of

greater than 26.5umol/L (0.3mg/dl from baseline creatinine concentration within a 48 hour period (IRIS 2013). This can aid early identification of AKI, for example in those patients who require monitoring after nephrotoxin ingestion and means that our recognition and therefore the prevalence of hospital acquired AKI is likely to increase.

In both dogs and cats, a specific diagnosis of CKD is made on the basis of historical information, physical examination findings together with laboratory diagnostics and diagnostic imaging. The diagnosis of CKD must be made before IRIS staging can be applied. Based on creatinine the IRIS system defines stage 1 CKD as a non-azotaemic animal (Scr < 125 umol/L, 1.4mg/dl in dogs; Scr <140 umol/L, 1.6 mg/dl in cats) with some other renal abnormality present: persistent inadequate urine concentrating ability with no identifiable non-renal cause, abnormal renal palpation or imaging, persistent proteinuria of renal origin, abnormal renal biopsy results or an increase in blood creatinine concentration in serially collected samples (IRIS 2019). For patients with more advanced stable CKD, it is more straight forward to identify CKD based solely on persistently increased creatinine concentration. However, the identification of IRIS stage 1 and stage 2 animals (Scr 125-180 umol/L, 1.4-2 mg/dl in dogs; Scr 140-250 umol/L, 1.6-2.8 mg/dl in cats) where creatinine may be within laboratory reference interval, can be challenging and requires clinicians understand the importance of documenting trends in creatinine concentration within reference interval, in addition to evaluating for other clinical evidence of CKD. An early biomarker that eliminated the need to monitor trends and which had a high sensitivity to detect an early change in renal function for both acute and chronic disease, would have considerable clinical utility (Hokamp & Nabity 2016).

History of symmetric dimethylarginine

In the 1970s scientists investigated previously unclassified amino acids in human urine and identified a group of compounds which they classified as N-methylated derivatives of lysine and arginine. This group included N^G, N^G- dimethylarginine (asymmetrical dimethylarginine; ADMA) and N^G, N'^G- dimethylarginine (symmetrical dimethylarginine; SDMA) (Kakimoto & Akazawa 1970). Subsequently, it has become clear that SDMA and ADMA are produced in all cells through methylation of arginine residues, a post translational modification in which a

methyl group is added to protein residues to alter their detection or activity , subsequently SDMA and ADMA are released into the circulation during proteolysis (Bedford et al. 2009; Kielstein et al. 2006). In humans, ADMA is primarily metabolised in the tissue by the enzyme dimethylarginine dimethylaminohydrolase, with renal clearance being estimated at 20% (Schwedhelm & Böger 2011). In contrast, although a small amount of hepatic clearance has been reported in people (Siroen et al. 2005), renal clearance of SDMA is estimated at 90% in people (Schwedhelm & Böger 2011), highlighting the suitability of SDMA as an endogenous kidney biomarker. Twenty years after SDMA and ADMA were first discovered, Vallance and
colleagues (1992) identified their accumulation in human patients with renal failure and published a landmark paper focusing on the pathogenicity of ADMA via inhibition of nitric oxide (NO) synthase, hypothesising that ADMA may contribute to the complications of hypertension, immune dysfunction and cardiovascular disease, associated with human CKD. Consequently, until recently, research has focused on ADMA which has emerged as a cardiovascular risk factor (Zeller et al. 2008; Ronden et al. 2012). In contrast, for many years little attention was paid to SDMA.. However, in recent years, with the growing interest in novel biomarkers of renal function, research has focused on how SDMA may be utilised in veterinary clinical practice.

Quantification of SDMA until recently has been the preserve of research studies due to the requirement for measurement by liquid-chromatography-mass spectrometry (LC-MS). However, since 2015, SDMA quantification has been available to practitioners worldwide through IDEXX[a], a commercial veterinary diagnostic laboratory. A notable advantage compared to creatinine therefore is the potential for reduced analytical variability given that a single company is offering this test. However, it should be noted that reducing analytical variability relies on a number of factors including variability of the immunoassay methodology, the environmental conditions of the laboratory and the instruments used within labs and appropriate internal quality control should be performed. Reference intervals were initially generated using serum samples from a population of 120 clinically healthy, adult dogs aged between 1 and 15 years and of varying breeds and sex, with SDMA

measured using LC-MS and results analysed with a nonparametric model, using a 2-sided 95% confidence interval (Relford et al. 2016). The same methodology was used for 86 clinically healthy adult cats, aged between 6 and 15 years and of varying breeds (Relford et al. 2016). Neither study included GFR measurement or necropsy evaluation. Subsequently IDEXX[a] have developed a high-throughput competitive immunoassay and usedthe LC-MS derived reference interval for clinically healthy animals in both species at ≤14ug/dl (Relford et al. 2016). This patented technology uses anti-SDMA monoclonal antibody and glucose-6-phosphate dehydrogenase (G6PDH) conjugate to quantify SDMA in serum or plasma with an immunoassay. The anti-SDMA antibody is reported to be specific to SDMA showing no significant cross reactivity with closely related molecules arginine, monomethyl arginine and ADMA(Patch et al. 2015). The analyte competes with a fixed amount of labelled analyte, SDMA-G6PDH conjugate, for a limited amount of anti-SDMA antibody. The amount of antibody bound to labelled analyte is a function of the total concentration of both labelled and unlabelled analyte and quantification is achieved through measuring label (G6PDH) activity. The accuracy of the assay was confirmed in human, feline and canine biological samples by comparison with LC-MS analysis which is the gold standard for SDMA measurement (Patch et al. 2015; Prusevich et al. 2015). In the UK, SDMA is currently quantified by immunoassay, although at USA IDEXX an LC-MS methodology is available for clinical research purposes. To date, studies that have been published have used both methodologies for SDMA quantification. Good agreement between methods (cats r=0.97, dogs r=0.97, humans r=0.99) has been reported in abstract form (Patch et al. 2015) and a recent comparison of paired samples from healthy geriatric cats attending two UK first opinion practices (n=19) also showed good agreement between methods (r=0.97, personal communication). In 2018, IDEXX released the option to perform SDMA quantification by immunoassay on their in-house Catalyst® analyser (IDEXX 2018). This in-house assay has been assessed in terms of performance in comparison to LC-MS, precision, analytical specificity and the impact of interfering substances and performs well. Further information about the validation of this assay can be found in the IDEXX white paper (IDEXX 2018). This development transitions SDMA measurement to be a bed-side test for the practitioner, therefore complementing creatinine as an in-house test of renal function.

Other assays are commercially available including the DLD SDMA ELISA which was designed to detect SDMA in human serum and which has been shown to be less accurate and precise than the IDEXX SDMA immunoassay in macroscopically normal feline and canine serum, when compared to the reference method of LC-MS (Ernst et al. 2018). No other comparisons of other commercially available assays have been published and therefore, based on the current literature, the IDEXX SDMA test is currently most suitable for use in a clinical setting.

Non-renal influences on symmetric dimethylarginine (Specificity of SDMA)

As previously discussed, non-renal influences are a significant limitation on serum creatinine, notably, the influence of muscle mass. A recently published study reports that there will be some expected biological and individual day to day variability for SDMA, as for other analytes such as creatinine and that a critical difference of 5.98 ug/dl between serially obtained samples is required in dogs to be a genuine difference in SDMA using the high-throughput immunoassay methodology (Kopke et al. 2018; Kopke et al. 2019) This study also demonstrated a decreased interindividual variability of serum SDMA when compared to creatinine which the authors attribute to the influence of lean body mass and age on creatinine. However, it should be noted that intraindividual and analytical variation were both higher for SDMA than creatinine. Overall, SDMA is considered a very stable analyte (4 days at room temperature, 14 days at 4°C or long-term when frozen) and can be measured on both serum (preferred) and plasma samples with no interference in quantification reported with lipaemia or icterus although severe haemolysis may cause a decrease in the measured result (Nabilty et al 2015). It is always recommended that patients are fasted prior to biochemical evaluation, however there is some evidence that SDMA is not influenced by recent protein ingestion (Hall et al 2014b).

There is evidence that, unlike creatinine, SDMA is not influenced by muscle mass, showing no correlation with lean body mass in healthy adult dogs or geriatric cats (Hall et al. 2014a; Braff et al. 2014; Hall et al. 2015). Similar to Scr, age may be an influencing factor on SDMA although not after the onset of maturity. A single study in dogs from age 2 months to 1 year reported that SDMA did not correlate with body condition, age or weight (Nabity et al. 2015).

However, young healthy dogs in this study did have plasma SDMA concentrations up to 16 µg/dL.There is limited information in the published literature currently relating to the effect of breed in dogs and cats on SDMA values. One study has suggested that SDMA may be a more accurate reflection of renal function in Birman cats than creatinine concentration although direct GFR and renal imaging were not performed (Paltinieri et al. 2018). A recent study comparing serum SDMA concentrations in clinically healthy greyhounds to that in other breeds, concluded that greyhounds havesignificantly higher mean serum SDMA concentrations compared to dogs of other breeds (13.1 µg/dL and 10.2 µg/dL respectively) (Liffmann et al 2018). A reference interval for serum SDMA concentration in greyhounds was established as 6.3-19.9 µg/dL. No effect of sex on SDMA has been reported. Overall, the data to date supports that for those patients with sarcopenia, SDMA is likely to provide a more accurate representation of GFR than creatinine and based on this concept, SDMA has been encorporated into the IRIS staging system. It demonstrates reduced variability across breeds of dogs again most likely related to alteration in body size and muscle mass and the inherent limitations of creatinine in this regard. However, some caution should be applied when interpreting the SDMA results in juvenile animals in practice when SDMA concentrations slightly higher than the normal reference interval may not indicate renal disease.

It is particularly important to understand the possible non-renal influence of concurrent disease and drug administration on circulating SDMA. Studies have reported that time of day, white coat effect and short-term administration of the angiotensin-converting-enzyme inhibitors enalapril and quinapril (0.5mg/kg per os q24hrs for 7 days), have no influence on circulating SDMA (Moesgaard et al. 2005; Moesgaard et al. 2007, Pedersen et al. 2006). One study in dogs has shown no correlation of serum SDMA concentration with the presence/absence of myxomatous mitral valve disease or with the symptoms of ,or pharmacological therapy for, congestive heart failure (Savarese et al 2018). Furthermore, no correlation has been found between the cardiac biomarker N-terminal pro-brain natriuretic peptide or between the liver enzymes alkaline phosphatase, alanine transaminase or aspartate aminotransferase and SDMA concentrations in

dogs, providing preliminary although not complete evidence that cardiac disease including hypertrophic cardiomyopathy in cats (Langhorn et al. 2017) and hepatic disease have limited effect on SDMA (Relford et al. 2016). However, there is conflicting information about the potential effect of neoplastic disease on SDMA values, with studies presented only in abstract form exploring this issue.One study reviewing dogs and cats with neoplastic disease that had undergone post-mortem examination identified that, when SDMA was elevated above the upper reference limit (> 14ugdl), there was histopathological evidence of CKD whilst creatinine was within reference interval in all but one of these patients (Yeramilli et al. 2017). Another, which evaluated SDMA concentrations in dogs with lymphoma, some dogs had elevated SDMA concentrations whilst remaining non-azotaemic, and showed normalisation of their SDMA concentrations when they achieved clinical remission after chemotherapy (Abrams-Ogg et al. 2017). Concern was raised that, given that SDMA originates from all nucleated cells, large tumour burden could result in elevated SDMA concentration rather than reduced renal function. Further work is necessary to establish any potential relationship between SDMA concentrations and neoplasia.

Other conditions which have undergone preliminary investigation include nephrolithiasis and diabetes mellitus. There is evidence that SDMA concentrations may be increased in cats with nephrolithiasis although it is likely that this reflects early alteration in renal function rather than a non-renal influence on SDMA (Hall et al. 2017). However, significantly lower SDMA concentrations were observed in cats with diabetes mellitus (n= 17) compared to controls (n=20) (Langhorn et al. 2018). This has also been reported in humans where lower serum SDMA concentrations have been reported in humans with poorly controlled type 2 diabetes compared to control (Can et al. 2011; Dimitroulas et al. 2015) and warrants further investigation.

A further area of current interest is the effect of thyroid status on SDMA concentrations. As previously discussed, aside from the effects of hyperthyroidism on GFR, creatinine has limitations for both hypo- and hyperthyroid patients given the alteration in muscle mass and potential alteration in production of creatinine with these conditions. A marker that accurately reflected GFR would be of particular utility in hyperthyroid cats where CKD is also commonly diagnosed. Studies demonstrate that it can take up to 3-6 months for creatinine to

accurately reflect GFR after treatment of hyperthyroidism, at least partly due to muscle wasting and therefore SDMA offers an attractive alternative (Boag et al. 2007). A very small study, presented only in abstract form, raised concern that SDMA did not differentiate hyperthyroid cats with and without azotaemic CKD, but the study was substantially underpowered (Williams 2017). Peterson and colleagues (2018) reported SDMA concentrations in the lower half of the reference interval (≤10 µg/dl) in 71% of a population of untreated hyperthyroid cats (n=262) anhypothesised this was due to the increased GFR associated with a hyperthyroid state. Of interest, in those cats that went on to develop azotaemic CKD following ^{131}I treatment (n=42), 33% had an elevated pre-treatment SDMA concentration (>14 µg/dl), showing poor sensitivity for predicting azotaemia (33.3%) but good specificity (97.7%). In the same study sCr at a cut off just below the upper limit of the reference interval (>1.9 mg/dl 167 µmol/L RI >2.1mg/dl 186 µmol/L), performed poorly with a test sensitivity of 11.9%, specificity was 84.4%. Results of this study indicate that a high SDMA raises concern for CKD in cats diagnosed with hyperthyroidism but that it is in no way a perfect test for prediction of the development of azotaemia after treatment of hyperthyroidism. Buresova and colleagues (2019) also examined the effect of ^{131}I treatment on the renal parameters of a group of 47 client-owned cats. Of particular interest in this study was a subgroup of 10 cats in which GFR was estimated via exogenous creatinine clearance at 4-48hrs prior to ^{131}I treatment (T0) and 1 month after treatment (T1). Correlation between GFR and serum SDMA concentration was low and not significant at T0 and T1(τ_b= -0.35, P=.17 at TO and τ_b = -0.22, P=.41 at T1), whereas correlation between GFR and sCr was moderate and significant at both T0 and T1 (τ_b= -0.52, P= < .05 at TO and τ_b = -0.53, P= < .05 at T1).. All 47 cats in the study remained non-azotaemic at T0 and T1, though a significant increase in median Scr concentrations between before and after treatment with no significant difference in median SDMA concentrations was observed. The authors conclude that neither Scr or SDMA perform well as renal biomarkers in hyperthyroid cats and that a mildly increased serum SDMA in a hyperthyroid cat should be interpreted with care considering concentrations may return to normal following treatment, as in 4 of the 6 cats with elevated SDMA at T0. In a recent study examining changes in thyroid and renal function following bilateral thyroidectomy, SDMA was relatively higher in hyperthyroid cats compared to euthyroid or hypothyroid cats in a group of 68 client-owned cats (Covey et

al. 2019). Of interest, this study reported a high number of discordant results. Thirty percent of samples had a creatinine within reference range (<177 µmol/L, <2.0 mg/dl) with an elevated SDMA (> 14µg/dL). Four of these samples were in hyperthyroid cats prior to treatment; the remaining 26 samples were cats following bilateral thyroidectomy. Five percent (8 cats) of the samples had a normal SDMA (≤ 14µg/dL) with a creatinine above reference range following bilateral thyroidectomy (in a euthyroid or hypothyroid state). Five of these cats had a concurrent measured USG, all of which were below 1.035, indicating a renal azotaemia. Data on the SDMA and creatinine plasma concentrations before and after thyroidectomy for individual cats was not available. The authors conclude that it is likely thyroid status influences the concentrations of both creatinine and SDMA, relative to GFR but also to one another. We have many years of clinical experience interpreting creatinine concentrations in patients with altered thyroid function with studies exploring its limitations. These studies highlight that, further research and experience is required in order to expand our understanding of the effect of thyroid status on SDMA measurement in dogs and cats and with treatment for thyroid disorders.

Within human medicine SDMA has been demonstrated to be upregulated in critically ill patients, especially in conditions of sepsis and to be independently associated with organ failure and death (Koch et al 2013). A preliminary study of 7 healthy control and 22 critically ill dogs with heterogeneous disease, excluding acute kidney injury, found no difference in median SDMA concentrations between critically ill and control dogs (Koster et al. 2018). However, this study did not specifically include septic dogs and is significantly underpowered and further larger studies examining this relationship are warranted.

To summarise these studies provide preliminary evidence that some concurrent diseases may influence SDMA concentrations in cats and dogs and highlights the need for further research. Interestingly, within the literature, there are reports of animals who are considered to have normal renal function with elevated SDMA (Langhorn et al. 2018) and anecdotally this has been observed in clinical practice (authors personal communication). This highlights the importance of exploring the effects of concurrent disease and also

documenting persistence of elevation in SDMA when using this parameter to make a diagnosis of early non-azotaemic CKD.

Sensitivity of SDMA as a marker of GFR

In an early meta-analysis of 18 studies providing a total of 2136 human patients, SDMA showed a strong negative correlation with GFR measured by inulin clearance (r=.85, P< .0001) (Kielstein et al. 2006). Similarly, in both dogs and cats serum SDMA has been shown to be an endogenous surrogate marker of GFR. In a study of 69 geriatric cats, serum SDMA concentrations were significantly increased in those cats with azotaemic CKD and were positively correlated with plasma creatinine (r = .741) (Jepson et al. 2008). In adolescent dogs with x-linked hereditary nephropathy (XLHN), both serum SDMA and Scr correlated strongly and inversely with GFR measured by exogenous plasma iohexol clearance (r= - .95, - .98 respectively) (Nabity et al. 2015) indeed suggesting that there was little to differentiate creatinine and SDMA as markers of GFR in this small and very specific population of dogs. Similarly, in a retrospective analysis of 10 client owned cats selected to give a range of GFRs, the reciprocal of serum SDMA and plasma creatinine showed a linear relationship with GFR measured by exogenous plasma iohexol clearance (R^2= .82, .81 respectively P< .001) whether cats were azotaemic or not (Braff et al. 2014). Based on these studies, the relationships between SDMA and Scr and GFR are very similar. Both markers demonstrate an exponential relationship with GFR and as such will be subject to the same limitations in terms of detection of early decline in GFR. It is mainly on the basis of clinical longitudinal studies that the ability of SDMA to detect early decline in renal function has become apparent. To date studies that explore the potential benefits of trending SDMA as discussed for creatinine have not been performed but may hold potential merit.

A recent abstract examined serum SDMA concentration in a group of dehydrated dogs rehydrated over 24 hours with standard fluid therapy. Strong correlation was observed between serum SDMA and creatinine concentrations in these dogs and SDMA was seen to normalise to within reference interval following rehydration (Choi et al. 2017). These results indicate, as would be expected of a marker of GFR, that SDMA can be influenced by pre-renal factors and volume status and highlights the importance of performing concurrent urine

specific gravity to assess urine concentrating ability whenever evaluating a marker of GFR such as SDMA or creatinine.

Sensitivity of symmetric dimethylarginine for the diagnosis of chronic kidney disease

In a recent study which measured GFR, Scr and serum SDMA in 21 geriatric cats with CKD and 21 healthy geriatric cats, the upper reference interval for SDMA (14ug/dl) corresponded to a GFR of 1.47ml/min/kg, a 24% decrease from the median GFR of healthy controls (Hall et al. 2014a). In a similar study of 19 dogs with CKD and 20 healthy controls, the upper reference limit for SDMA corresponded to a GFR decrease of 49% from median of healthy controls (Hall et al. 2016c). One possible explanation for this discrepancy between species could be the clinically healthy populations used to establish reference intervals. Without GFR measurements from these reference populations, it is not possible to know if animals were included that had reduced renal function, and if so, if the number of animals with reduced GFR differed between the dog and cat populations.

There are a number of studies evaluating the sensitivity of serum SDMA as a marker for detection of early CKD and comparing it to Scr. Importantly, studies suggest that SDMA detects a decrease in GFR prior to Scr, when based on the established reference limits. In a study of 8 male adolescent XHLN dogs with rapidly progressing CKD, serum SDMA increased above reference interval an average of 5 weeks prior to an increase in Scr, based on their respective reference limits (Nabity et al. 2015)In a study of Beagle colony dogs with naturally occurring CKD, serum SDMA was elevated an average of 9.8 months prior to elevation of Scr (Hall et al. 2016c). Similarly, in a study of 21 geriatric laboratory cats with naturally occurring CKD, SDMA was elevated an average of 17 months prior to elevation of creatinine above the upper reference interval of 186 umol/L (2.1 mg/dl) (Hall et al. 2014a).

In the feline study, using a decrease of greater than 30% GFR from the median of healthy controls as a significant decrease in GFR, SDMA had 100% sensitivity, 91 % specificity, 100% negative predictive value and 86% positive predictive value (Hall et al. 2014a.). The perfect sensitivity and negative predictive value indicates that cats with SDMA within

reference interval (≤14 ug/dl) had a normal GFR, but that an elevation in SDMA above reference interval (>14 ug/dl) may detect a decrease in GFR of less than 30% in 14% of cases. In comparison, the upper reference limit for Scr used in this study was 186 umol/L (2.1 mg/dl), this had a 100% specificity and positive predictive value, but sensitivity was 17% and negative predictive value was 70%. The perfect specificity and positive predictive value indicates that cats with elevation of Scr above reference interval (> 186umol/L, 2.1mg/dl) all had a decrease in GFR of greater than 30%. However, poor sensitivity means that many cats with creatinine within reference interval (≤ 186 umol/L, 2.1 mg/dl) may also have a decrease in GFR of greater than 30% and remain undetected by this upper reference limit. Of interest, the clinically healthy cats in this study had Scr of less than 141 µmol/L (1.6 mg/dl) with a mean of 106 µmol/L (1.2 mg/dl). Specificity and sensitivity of a clinical test is based on reference limits, which are based on the central 95% of a healthy reference population used by a specific laboratory. Using a lower cut off below the laboratory reference interval for Scr, which reflected the Scr of the clinically healthy cats in the study, would have improved the sensitivity of creatinine, although would not necessarily have meant that Scr outperformed SDMA in this situation. Similarly, a recent prospective study of 62 non-azotemic dogs with a clinical suspicion of CKD, observed that elevation of SDMA above reference interval (>14 µg/dl) was more sensitive than elevation of creatinine above reference interval (>125 umol/L, 1.4 mg/dl) at detecting a 40% decrease in GFR from a breed specific reference interval (72%, 25.6% respectively). However, SDMA generated false positive results in 32% of cases compared to 100% specificity of creatinine (Pelligand et al. 2017). Subsequently, a recent study of 97 client owned clinically stable dogs with or without CKD, assessed the overall performance of creatinine, SDMA and cystatin C as markers of decreased GFR measured by scintigraphy (mGFR) and found that, although the diagnostic performance of cystatin C was inferior to both creatinine and SDMA, overall performance of creatinine and SDMA as markers of decreased mGFR were the same (Pelander et al. 2019). Usingpredefined cut-offs of 14 µg/dL for SDMA and 115 µmol/L for creatinine, a cut-off currently used clinically within the study hospital, resulted in almost exactly the same sensitivity and specificity (90% specificity 90% sensitivity and 87% specificity and 90% sensitivity respectively) for detection of an

abnormal mGFR (<30.8 mL/min/L). The authors concluded that SDMA had no overall diagnostic benefit over creatinine and would be more suitably used as an adjunct to creatinine for diagnosis of reduced GFR.

One study has assessed the diagnostic utility of SDMA for detecting CKD in the specific context of canine leishmaniosis (Torrent et al. 2018). Proteinuric nephropathy, as a result of glomerular damage from high levels of circulating immune complexes, can initially manifest as proteinuria, progressing to excretory dysfunction with decreased GFR and hypertension resulting in progression of CKD. The severity of renal lesions in canine leishmaniosis is currently classified by UPC and creatinine concentrations. Torrent and colleagues (2018) aimed to determine whether SDMA was increased in dogs with leishmaniosis. Fifty-three dogs with Leishmaniosis, 39 of whom had a 6 month follow up, were compared to 41 healthy controls. Increased UPC, SDMA and creatinine concentrations above their respective reference intervals were found in 47.1%, 15.1% and 9.4% of dogs with leishmaniosis on the day of diagnosis and the median of all 3 parameters were found to be significantly higher in those dogs that went on to develop CKD compared to those who did not. However, no advantage for the use of SDMA in predicating CKD compared to the use of UPC or creatinine concentrations was demonstrated.

These studies together highlight that SDMA has an exponential relationship with GFR similar to that of creatinine. On a clinical basis, in some cases, it does appear to detect changes in renal function earlier than could be detected by creatinine outside the reference interval and therefore can have utility as an early marker of CKD.

Symmetric dimethylarginine and IRIS staging

IRIS guidelines were first modified to include serum SDMA concentration in 2015 and updated in line with current research in 2019. The recommendations include how to interpret SDMA in all stages of CKD. In patients with Stage 2 and 3 CKD based on blood creatinine concentrations, it is suggested that SDMA concentrations over the threshold of 25 µg/dl in cats or 35 µg/dl in dogs and 38 µg/dl in cats or 54 µg/dl in dogs respectively indicates an underestimation of the degree of renal dysfunction and

guidelines advise that treatment recommendations for the subsequent IRIS stage should be used (IRIS 2019). This is based on the concept that SDMA is not influenced by muscle mass and therefore will provide a more accurate reflection of GFR in patients with reduced muscle condition. Use of SDMA in this manner provides helpful information, particularly where clinicians feel less comfortable extrapolating the implications of sarcopenia on reported Scr concentrations.

Where SDMA may be of particular value is in the identification of those animals in early non-azotaemic CKD. IRIS guidelines suggest that a persistent mild increase of SDMA above reference interval (i.e. >14 and < 18 µg/dl on at least two occasions) with a creatinine of less than 125 umol/L (1.4 mg/dl) in dogs and 140 umol/L (1.6 mg/dl) in cats, may indicate reduced renal function (IRIS 2019) and the patient staged as IRIS Stage 1. Those with a more severe increase in SDMA (persistently > 18 µg/dL) can be considered IRIS Stage 2. It should be noted that guidelines specify a 'persistent' increase in SDMA, stressing the requirement for multiple samples prior to diagnosing a patient as having CKD and undertaking IRIS staging.

It should be noted that although serum SDMA can aid in early diagnosis of CKD, both serum SDMA and serum creatinine are biomarkers of GFR only and do not inform on the metabolic status of an animal. Neither creatinine or SDMA alone are able to inform about the presence of chronic kidney disease- mineral and bone disorder (CKD-MBD) which results in derangements in parathyroid hormone (PTH), fibroblast growth factor -23 (FGF-23), 25-dihydroxyvitamin D, serum calcium and phosphate (Moe et al. 2006). A recent study aiming to identify a safe level of dietary phosphate in adult cats demonstrated significant increases from baseline in FGF23 after 2 weeks on a high phosphate test diet. However, no significant increase in creatinine or SDMA concentrations was seen until 13 to 21 weeks on diet, at which time 3 cats had developed CKD, having both elevated creatinine and SDMA concentrations above reference interval (Alexander et al. 2019). Further research is therefore required to establish the derangements in phosphate homeostasis that can be identified with stageCKD on the basis of normal creatinine but a persistent elevation in SMDA where clinical intervention i.e. dietary modification may be recommended.

Symmetric dimethylarginine, dietary intervention and progression of chronic kidney disease

SDMA is a useful screening tool for early detection of decreased kidney function however, its use as a monitoring tool for progression of CKD is not as well established. To date there are no studies that have specifically evaluated whether SDMA is a superior marker for monitoring change in renal function or progression of disease in CKD patients although it could be hypothesised that this may be the case, particularly with continued weight and muscle loss with advancing disease and the known effect that this will have on Scr. There have however, been several studies that have begun to evaluate the impact of dietary intervention in either geriatric or IRIS stage 1 dogs and cats and the nutritional effect on SDMA and Scr in this context.

Feeding a phosphate and protein restricted diet is the primary intervention in canine and feline CKD shown to provide survival advantage and slow progression of disease (Elliott et al. 2000; Ross et al. 2006). Historically, serial creatinine measurements are routinely used to monitor progression (Sparkes et al. 2016). In dietary intervention studies in clinically healthy geriatric dogs and cats, some of which were suspected to have IRIS stage 1 CKD, feeding test diets containing functional lipids, antioxidants and high quality protein have demonstrated that this resulted in significant decreases in variable combinations of renal function markers including SDMA and creatinine when compared to owners choice diets (Hall et al. 2016a; Hall et al. 2016b). In these studies, the authors speculate that improvement in renal function secondary to the effect of the test diet, may explain the stability of, or decrease in, circulating SDMA concentrations. However, no direct GFR assessments were performed in these studies to confirm this, nor were evaluations performedo explore the significance of changes in Scr in the face of stable serum SDMA. The true protein concentrations of test and owner diets are difficult to determine from these publications.

In human medicine, it is generally accepted that the rate of SDMA production is relatively constant with little biological variation in the healthy individual (Blackwell et al. 2007). However, there is evidence in humans to support that there may be an association between whole body protein synthesis and catabolism of protein and SDMA

concentrations (Marliss et al. 2006) such that protein turnover could play an important role in regulating plasma levels of methylarginines and particularly in patients with end stage renal disease (Cupisti et al. 2009). This is of interest when assessing the significance of changes in serial SDMA measurements from an individual where nutritional status or disease process may have altered protein synthesis or catabolism, and when comparing SDMA between groups with differing nutritional status. It should be remembered that Scr will be substantially influenced by muscle synthesis and catabolism and therefore is not an ideal marker in this regard. Therefore, it can be speculated that, even if SDMA is to some extent influenced by protein turnover, it may still prove superior to Scr. However, a better understanding of non-renal influences on circulating SDMA including dietary intervention (i.e. protein and phosphate restricted renal diets) and changes in serum SDMA concentration over time in CKD is required to determine whether serial SDMA measurement is a superior monitoring tool for canine and feline CKD than serial assessment of sCr.

Symmetric dimethylarginine and acute kidney injury

To date, the majority of research into SDMA in veterinary medicine has focused on SDMA in the context of CKD. However, more recently the use of SDMA in diagnosing AKI has been explored. In a recent study, comparing serum SDMA and Scr of healthy dogs with dogs in IRIS CKD stage 2 and AKI grade II (Scr 141-220 umol/L, 1.7-2.5 mg/dl) or above, median SDMA concentration were 8.5 µg/dl, 35 µg/dl and 39.5 µg/dl respectively (Dahlem et al. 2017). Serum SDMA concentrations were significantly higher in dogs with AKI ($P < .0001$) or CKD ($P < .0001$) in comparison with healthy dogs although there was no significant difference between AKI and CKD dogs. Scr concentration was significantly higher in dogs with AKI (583 umol/L, 6.6 mg/dl) compared to dogs with CKD (318 umol/L, 3.6 mg/dL, $P = .0002$) and in both groups when compared to healthy controls (80 umol/L, 0.9 mg/dl). This study indicates that, as expected, SDMA is a marker of GFR and is therefore increased in patients with AKI. It emphasises that the differentiation of azotaemic AKI from CKD must be made on the basis of other parameters, such as historical information, rather than on the severity of decline in renal function. Interestingly, creatinine appeared to be able to differentiate the AKI and CKD groups, however, without direct GFR measurement it is

impossible to say whether the variability in Scr reflects true differences in renal function or could be accounted for by differences in, for example, muscle mass. This study does not allow a definitive conclusion to be made in terms of superiority of either marker for the detection of AKI or CKD. In particular, the lack of patients with hospital acquired AKI meant that evaluation of SDMA as a marker of grade I AKI was not possible and warrants exploration. Given that hospital acquired AKI patients may have more normal body condition (dependent on their underlying disease process) then it could be speculated that there may be less difference between Scr and SDMA for the detection of early AKI. Further studies, are warranted to explore this concept and will become more feasible with the introduction of bed-side SDMA testing. #xa0;

Conclusion

SDMA is a useful endogenous marker of GFR in small animal practice. The apparent lack of influence from muscle mass makes SDMA a useful tool for assessing renal function in patients with low muscle mass where Scr has limitations. However, further studies are needed to explore the influence of other non-renal factors. Current research supports the use of SDMA as a screening test for detection of early CKD according to IRIS guidelines typically performed in conjunction with Scr and urinalysis including urine specific gravity. However further research is needed to establish whether SDMA is a more sensitive tool for monitoring and predicting disease outcome, fully understanding how concurrent disease may influence SDMA concentrations, and how best to manage patients where SDMA does allow an early diagnosis of either CKD or AKI to be made.

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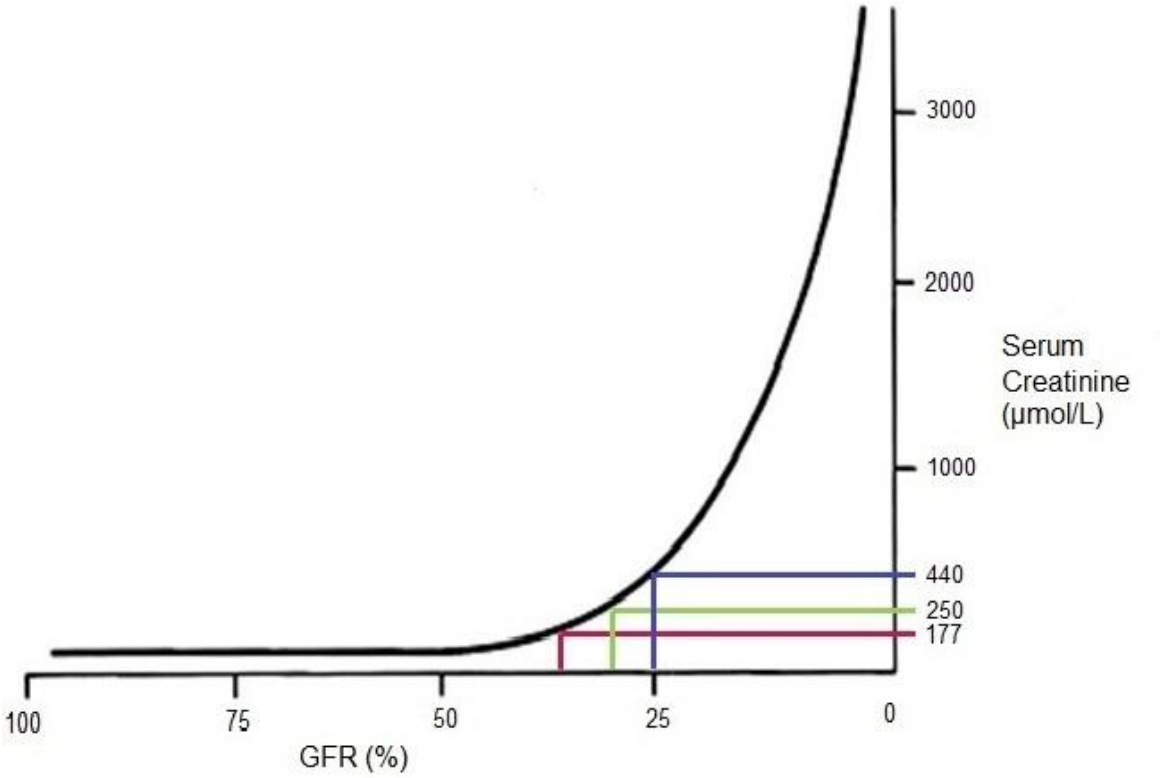

Figure 1 Curvilinear relationship between GFR and serum creatinine