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Reliability of symmetric dimethylarginine in dogs with myxomatous mitral valve disease as kidney biomarker

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Abstract

The most common cause of heart failure in the canine population is myxomatous mitral valve disease, sometimes complicated by chronic kidney disease. Many studies have been done on the use of symmetric dimethylarginine as biomarker of renal impairment in dogs affected by chronic kidney disease, few studies have examined his reliability as biomarker in dogs affected by heart diseases. Aim of this study was to evaluate symmetric dimethylarginine in dogs affected by mitral valve disease in order to assess his reliability in heart diseases. This was a retrospective case-control study on a clinical population of dogs affected by mitral valve disease (cases) vs healthy dogs (controls). Both groups underwent a complete physical evaluation, echocardiographic examination, complete blood count, biochemical panel, including serum creatinine and urea and urine analysis with protein-to-creatinine ratio. Serum was frozen and sent to IDEXX laboratories for symmetric dimethylarginine determination. General linear model was applied to data. A total number of 24 cases and 7 controls were included. Symmetric dimethylarginine value was in the reference value in the 75% (n=18) of cases, and in the 43% (n=3) of controls. Once set symmetric dimethylarginine as dependent variable, no statistical significant differences were found for each variable considered (breed, age, sex, weight, class of cardiomyopathy, presence/absence of valvular disease, presence/absence of congestive heart failure, pharmacological therapy, creatinine and urea concentration). Blood concentration of SDMA resulted not influenced by the variables mentioned above, so it could be considered a reliable marker of early renal impairment in dogs affected by mitral valve disease.

Keywords: Biomarker, Cardiovascular-renal disorder, Dog, Myxomatous mitral valve disease, Symmetric dimethylarginine.

Introduction

The most common acquired heart disease affecting dogs and leading to congestive heart failure (CHF) is myxomatous mitral valve disease (MMVD), also known as endocardiosis, that can reach a prevalence of 100% in small breed dogs in their old age (Mattin *et al.*, 2015).

The presence of both heart and kidney failure can be defined as cardiorenal syndrome (CRS) (Ronco, 2010; Ronco and Maisel, 2010). In 2015, a Consensus Group was formed to develop definitions and recommendations for CRS approach in veterinary medicine (Pouchelon *et al.*, 2015). The consensus renamed CRS as “Cardiovascular-Renal Disorder” (CRvD) and defined it as a “disease, toxin or drug-induced structural and/or functional damage to the kidney and/or cardiovascular system, leading to disruption of the normal interactions between these systems, to the ongoing detriment of one or both” (Pouchelon *et al.*, 2015).

Martinelli *et al.* (2016), reported a 25% prevalence of chronic kidney disease (CKD) associated with

hyperazotemia in dogs with MMVD. Moreover, the presence of CKD associated to MMVD in dogs decreases survival time (Martinelli *et al.*, 2016).

An early diagnosis of renal impairment in patients affected by cardiovascular disease is therefore very important. Glomerular filtration rate (GFR) is currently considered the gold standard for quantitative determination of kidney filtration, although not routinely performed on dogs and cats. In fact, the procedure requires the intra-venous injection of a marker of filtration, followed by multiple blood samples at determined time, making the process expensive and time consuming (Relford *et al.*, 2016; Yerramilli *et al.*, 2016).

Currently, the cornerstone in the diagnosis of CKD in veterinary practice is the measurement of serum Creatinine (sCr), that is the most common marker of GFR. Indeed, the use of sCr has some limitations, since its concentration tend to rise when almost the 75% of the renal activity is lost and its levels are affected by muscle mass, making the evaluation of renal function more difficult in cachectic and geriatric animals.

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Many studies have been carried out to assess the efficacy of novel biomarker in the early detection of kidney failure. Due to its characteristics, symmetric dimethylarginine (SDMA) has raised, in the last few years, as the most promising novel biomarker in the early detection of kidney failure. SDMA is one of the methylated forms derived from the arginine metabolism, with asymmetric dimethylarginine (ADMA) and monomethylarginine (MMA) (Kakimoto and Akazawa, 1970). While ADMA is largely cleared by enzymatic hydrolysis, SDMA is primarily eliminated by renal excretion (McDermott, 1976), suggesting its role as endogenous marker of GFR (Kielstein *et al.*, 2006; Schwedhelm and Böger, 2011). SDMA increases earlier than creatinine and can identify a GFR decline as early as a 30% (Polzin, 2016). A large meta-analysis from Kielstein *et al.* (2006) showed that SDMA strongly correlated with inulin clearance and sCr. A study from Relford *et al.* (2016) reported that age and lean body mass cannot influence SDMA concentration in small animals.

Major non-renal systemic disease, including hepatic and endocrine diseases have not been consistently identified as reasons of elevation in serum SDMA concentrations, neither in humans nor in animals, and the effects of both heart and kidney failure on SDMA elimination are still undefined (Schwedhelm and Böger, 2011; Veldink *et al.*, 2013; Bum-Sul *et al.*, 2017).

The aim of this study was to determine the reliability of serum SDMA as a marker of early renal impairment in dogs affected by MMVD in different American College of Veterinary Internal Medicine (ACVIM) classes, and with sCr within the normal range of values.

Materials and Methods

This was a retrospective case-control study on a clinical population of dogs affected by MMVD (cases) vs healthy dogs (controls). The animals enrolled belonged to the population of dogs referred to the Cardiology Service of our institution between February 2015 and July 2016.

Inclusion criteria were: diagnosis of MMVD, absence of any other concurrent pathology and no hematological alteration compatible with CKD (sCr < 1,4 mg/dl). Dogs with other heart or systemic diseases were excluded. Control group was composed by healthy dogs that were evaluated at the veterinary teaching hospital for periodic screening and then enrolled. For all dogs, informed consent for every procedure signed by the owner was obtained, as routinely done in our clinical practice.

Both groups underwent a complete physical evaluation, chest x-ray, echocardiographic examination, complete blood count (CBC) and serum biochemical panel. A complete echocardiographic examination was performed in both groups using standardized thoracic

imaging planes, according to the recommendations of the American Society of Echocardiography (Thomas *et al.*, 1993). Dogs without any clinical, radiographic and echocardiographic sign of cardiac disease were enrolled as controls. Echocardiography was performed using an Esaote MyLab50 Gold cardiovascular, with phased array multifrequency probes (7.5-10 Mhz and 2.5-3 Mhz).

The investigators performing the echocardiographic exams were all focused on specialty cardiology practice (AS, CL, PGB). Dogs diagnosed with MMVD were subdivided in 4 classes, from B1 to D, in line with the ACVIM classification. As recommended by the ACVIM guidelines, stage B included dogs affected by MMVD without signs of CHF (namely, in stage B1, dogs without evidence of cardiac enlargement; in B2, dogs with signs of cardiac remodeling as left atrium (LA) and/or left ventricle (LV) enlargement); stage C included dogs with clinical signs of CHF due to MMVD, and stage D included subjects presenting both clinical signs of CHF and refractoriness to standard medical treatments (Atkins *et al.*, 2009; Boswood *et al.*, 2016).

All dogs underwent indirect systemic blood pressure evaluation by Doppler flow meter as reported in ACVIM guidelines (Brown *et al.*, 2007). Based on the International Renal Interest Society (IRIS) guidelines, dogs were classified in 5 classes, from 0 to 4. No azotemic patients (normal sCr) were enrolled, and all the dogs were classified as IRIS class 0 (dogs at risk for the development of CKD). An IRIS substage of CKD based on blood pressure and on the presence of proteinuria was performed, when possible, as reported in the IRIS guidelines (IRIS, 2016).

Blood samples were collected from the cephalic vein during routine clinical evaluation, and CBC and selected biochemical parameters were performed. For CBC, EDTA was used as anticoagulant, while, for biochemical evaluation, blood was placed in tubes without anticoagulant and centrifuged at 3250 x g for 5 minutes. CBC was performed using an automated laser hematology analyzer (Sysmex XT-2000iV, Sysmex corporation, Kobe, Japan), while biochemistry was carried out with an automated spectrophotometer (Roche Cobas Mira Classic Biochemistry Analyzer).

The biochemical panel performed was composed by: glucose, urea, sCr, total proteins, sodium, potassium, chloride, alanine transaminase, alkaline phosphatase. An aliquot of serum (at least 300 µl) was frozen and sent to IDEXX laboratories for SDMA determination with the EMIT (Enzyme Multiplied Immunoassay Technology). SDMA in dog was considered normal when < 14 µg/dL (Nabity *et al.*, 2015). Urine were collected via spontaneous micturition or cystocentesis and complete urinalysis with sediment and Urine Protein to Creatinine Ratio (UPC) was performed.

Normality of data distribution was tested with Shapiro - Wilk test. For baseline descriptive statistics, normally distributed continuous variables were presented as mean and standard deviation, non- normally distributed variables as median and interquartile range (IQR). Data were analyzed with IBM SPSS Statistics 23 [Release 23.0.0.0] using a multivariate generalized linear model (GLM) with backward elimination, applied on ACVIM class (compared with breed, sex, age, weight, SDMA values, Urea values and sCr values) and on SDMA (compared with breed, sex, age, weight, MVD presence or absence, ACVIM class, presence of clinical symptomatology, pharmacological therapy, Urea values and sCr values).

To apply the model, a transformation of ACVIM classes into numbers categories was made, with ACVIM class B1 corresponding to 1, ACVIM B2 to 2, ACVIM C to 3 and ACVIM D to 4. We evaluated if severity of MMVD, CHF symptomatology or pharmacological treatment can influence a single measurement of SDMA levels in dogs affected by the MMVD. $P < 0.05$ was set to indicate statistical significance.

Results

An overall number of 367 privately owned dogs were admitted to the Cardiology Service of DIMEVET of the University of Milan between February 2015 and July 2016; among these, 148 (40%) were diagnosed with MMVD. The medical archives were reviewed in order to include only patients with complete medical and laboratory information. Thirty-one dogs were enrolled, 24 in the case group and 7 in the control group.

The median age of dogs enrolled in case group was 11.5 years (± 2.83 SD), and the median body weight was 13.8 kg (± 9.91 SD). Most of the dogs included were neutered females ($n=11$; 46%), followed by intact males ($n=8$; 33%), intact females ($n=3$; 12%) and neutered males ($n=2$; 9%). The most represented breeds were Mongrels ($n=8$; 33%), Cavalier King Charles ($n=2$; 8%), Chihuahua ($n=2$; 8%) and Maltese dogs ($n=2$; 8%). Breeds with less than two dogs were grouped and listed as others (51%). In this group were also listed Dachshund and Jack Russel terrier.

The median age of the control group was 8.2 years (± 1.92 SD), and the median body weight was 21 kg (± 9.17 SD). Most of the controls included were neutered females ($n=5$; 71%), followed by intact males ($n=2$; 29%). The most represented breed were mongrels ($n=3$; 43%). Thirteen dogs over 24 were classified as ACVIM B1 (54%), 8 as ACVIM B2 (33%) and 3 as ACVIM C (13%). Symptomatic and non-symptomatic dogs for CHF were respectively 13% ($n=3$) and 87% ($n=21$). The 3 dogs classified as ACVIM class C were all under standard triple therapy, composed by furosemide, an ACE inhibitor, and pimobendan.

Renal function was tested measuring serum urea, sCr

and SDMA. sCr was normal in all dogs, as it was an inclusion criteria. Serum urea (NV= 20 – 60 mg/dL) was over the reference interval in 8 of the cases (33%). None of the controls presented alterations in urea values.

The 25% ($n=2$) of dogs classified as ACVIM B1 presented urea values above the reference interval, so the 24% ($n=3$) of ACVIM B2 and the 100% of ACVIM C. Only medical records of dogs presenting urinalysis were retrospectively included. Urine specific gravity (USG) was normal (NV=1020 - 1060) in 87% of cases (21/24) and in 100% of the controls for which urinalysis was available. For the case group, 2/3 dogs presented USG lower than reference interval (66%), while one presented USG over the reference interval (34%). Both of the dogs with low USG were classified as ACVIM C and were assuming furosemide. No proteinuria was detected in any of the dog considered (UPC < 0.5) (IRIS 2016). SDMA value was normal (< 14 $\mu\text{g/dL}$) in the 75% ($n=18$) of cases, and over the reference interval in the 25% ($n=6$). The 31% of the ACVIM B1 dogs (4/13) and the 25% of the ACVIM B2 (2/8) presented SDMA values over the reference interval. None of the dogs classified as ACVIM C had SDMA above the references. SDMA was above the reference intervals in 4/7 of controls (57%).

According to the new IRIS guidelines, the 25% of MMVD dogs should be shifted from IRIS class 0 to IRIS class 1, while the 57% of dogs classified as controls switched from IRIS class 0 to IRIS class 1.

Once set SDMA as dependent variable in the GLM, the results showed no statistical significant difference for each variable considered (breed, age, sex, weight, ACVIM class, healthy/affected, presence of CHF symptomatology, pharmacological therapy, sCr, urea) (Table 1, Table 2).

Once set ACVIM class as dependent variable in the GLM, the results only showed a statistical significant difference in the variable age for higher ACVIM classes (C) ($P = 0.014$; OR = 1.3) (Table 3, Table 4).

Discussion

The population of dogs affected by MMVD enrolled in this study presented the typical characteristics reported in literature. In fact, the dogs were mainly of medium age and body weight, mongrels or pure small breed dogs (Borgarelli and Buchanan, 2012). Most of the dogs were asymptomatic for CHF (87%). Age resulted correlated with ACVIM classes, as reported in literature (Borgarelli and Buchanan, 2012). Interestingly, multivariate analysis showed that for each year of life of the dog, the risk of belonging to a higher ACVIM class increases of 1.3 times.

According to literature, the prevalence of azotemia in dogs with heart diseases ranges between 7.4 and 25%. (Atkins *et al.*, 2002; Nicolle *et al.*, 2007; Guglielmini *et al.*, 2013; Martinelli *et al.*, 2016).

Table 1. Linear generalized univariate model analysis with binomial negative response; dependent variable: SDMA.

Variable	Factor	OR	IC 95%		p-value
			INF	SUP	
Sex	F	1.661	0.153	18.075	0.677
	FC	1.072	0.166	6.931	0.942
	M	1.397	0.212	9.200	0.728
	MC (reference)	1			
Breed	Mongrel	1.036	0.357	3.012	0.948
	Pure (reference)	1			
Age (years)	Continuous Variable	1.032	0.855	1.246	0.740
Weight (Kg)	Continuous Variable	0.994	0.937	1.055	0.848
sCr (mg/dL)	Continuous Variable	4.865	0.070	339.044	0.465
Serum urea (mg/dL)	Continuous Variable	0.997	0.963	1.033	0.862
ACVIM	Continuous Variable	0.804	0.321	2.013	0.641
CHF symptoms	No	1.241	0.052	29.905	0.894
	Yes (reference)	1			
Pharmacological treatment (CHF)	ACE-inhibitor	1.361	0.231	8.013	0.733
	ACE- inhibitor and diuretic	1.317	0.052	33.426	0.868
	ACE- inhibitor and inotropic agent	2.219	0.025	198.372	0.728
	ACE- inhibitor, inotropic agent and diuretic	1.681	0.101	27.989	0.717
	No treatment (reference)	1			

Table 2. Linear generalized multivariate model analysis with binomial negative response [after backward elimination]; dependent variable: SDMA

Variable	Factor	OR	IC 95%		p-value
			INF	SUP	
sCr (mg/dL)	Continuous Variable	2.143	0.242	19.001	0.494

Table 3. Linear generalized univariate model analysis with ordinal logistic response; dependent variable: ACVIM.

Variable	Factor	OR	IC 95%		p-value
			INF	SUP	
Sex	F	10.015	0.175	573.395	0.265
	FC	0.458	0.024	8.880	0.606
	M	15.623	0.754	323.923	0.076
	MC (reference)	1			
Breed	Mongrel	1.270	0.214	7.542	0.793
	Pure (reference)	1			
Age (years)	Continuous Variable	1.300	0.924	1.829	0.132
Weight (Kg)	Continuous Variable	0.929	0.843	1.023	0.135
sCr (mg/dL)	Continuous Variable	15.479	0.502	477.462	0.117
Serum urea (mg/dL)	Continuous Variable	1.036	0.975	1.101	0.253

Table 4. Linear generalized multivariate model analysis with ordinal logistic response; dependent variable: ACVIM

Variable	Factor	OR	IC 95%		p-value
			INF	SUP	
Age (years)	Continuous Variable	1.323	1.058	1.656	0.014

In our population, all dogs presented normal sCr values. No statistically significant differences in urea values have been found among different ACVIM classes, although a positive increasing trend is evident and the totality of dog in ACVIM class C presented urea values above the reference interval. The progression of MMVD and the development of CHF reduce renal perfusion and can result in urea increase (Aronson *et al.*, 2004; Kazory, 2010). Also, the activation of the renin angiotensin aldosterone system (RAAS), which increases the absorption of sodium and water, can contribute (Aronson *et al.*, 2004; Kazory 2010). The increase may also be due to the administration of diuretic drugs, prescribed for ACVIM class C patients (Atkins *et al.*, 2009; Pouchelon *et al.*, 2015).

The 75% of patients at any stage of MMVD presented normal values of SDMA. SDMA has been compared with all the variables considered in our study, and none of them gave statistically significant result. SDMA appears as a promising early biomarker of kidney function in patients affected by any stage of MMVD, since the presence of this cardiovascular disease doesn't result in influences on the parameter. According to literature, our study showed that SDMA is not influenced by lean body mass and weight (Hall *et al.*, 2015), differently from creatinine; therefore, those data cannot be considered as confounding factors. Moreover, SDMA is not influenced by age: this is an important aspect since the canine population affected by MMVD is usually in the old age. Pedersen *et al.* (2006) reported also that SDMA levels were not altered by the presence of asymptomatic mitral regurgitation, but the study did not consider any dog presenting CHF and was conducted only on Cavalier King Charles Spaniels dogs (Pedersen *et al.*, 2006).

More recently, Bum-Sul *et al.* (2017) reported that SDMA levels in dogs with MMVD, mostly symptomatic, were not correlated to age or body weight, but closely correlated to the severity of HF and some echocardiographic markers, like LA/Ao ratio (Bum-Sul *et al.*, 2017). Based on our population, since SDMA is considered a biomarker of GFR (Nabity *et al.*, 2015), our data seems to suggest that the 25% of our population could present a reduction of GFR despite the normal sCr values. Even the comparison between SDMA and creatinine didn't result statistically significant, differently from what reported in literature (Nabity *et al.*, 2015), but this data is probably attributable to the inclusion criteria used in this study. In fact, all the patients presented normal creatinine values.

It can be noted that, in the control group, there was a greater percentage of patients with SDMA values higher than normal compared to the group of cases (25% vs 57%): this result is of difficult interpretation since, in veterinary medicine, few studies have been

made about influences other than CKD on SDMA values and due to the lack of information about abdominal ultrasound. However, all control dogs in our population presented normal urinary specific gravity and no proteinuria, so kidney damage seems less likely. Many different causes have been investigated to justify high levels of SDMA and ADMA concentration in human patients, like in diabetes (Krzyzanowska *et al.*, 2007) or sepsis (Koch *et al.*, 2013), but all the principal diseases that can alter SDMA values have been excluded from our population. We cannot exclude the presence of pre-analytical errors in SDMA determination. Moreover, a recent study reported the presence of a small percentage of animals with elevated SDMA but normal sCr in which kidney function must be assessed (Polzin, 2016).

Although no agreement is present on the therapeutic options in dog presenting alteration of SDMA without other signs of renal impairment, the evaluation of this parameter could be important from a clinical point of view, and allows us to suggest for these patients a more targeted diagnostic procedure with a full assessment of kidney function, including second evaluation of SDMA within one month, complete biochemical renal profile examination (creatinine and urea), urinalysis with UPC ratio and ultrasound renal evaluation, as recommended by IRIS guidelines (IRIS, 2016). Moreover, according to Hall *et al.* (2017) the administration of a renal diet to dogs currently classified as IRIS-Stage 1 CKD can result in reduction of blood concentration of kidney biomarkers, in particular SDMA and sCr, besides improving patient's quality of life (Hall *et al.*, 2017). A previous longitudinal study on geriatric dogs fed with commercial renal protective food showed that dogs consuming test food showed significant reduction in the concentrations of serum SDMA, sCr, and BUN. Moreover, serum sodium concentrations were seen to be statistically significant lower in dogs assuming this kind of diets, that can be of help in dogs with heart disease in which restriction of sodium is indicated. Finally, a kidney protection food containing more biologically available protein sources with respect to other commercially available dry foods could be of help in preventing cardiac cachexia, loss of muscular masses and mobility in patients affected by MMVD at various gravity levels (Hall *et al.*, 2016). This is a retrospective study and, as such, has some inherent limitations. Firstly, the abdominal ultrasound was not systematically assessed in our population of dogs. Secondly, the small number of animals included in higher ACVIM class C and the single determination of the parameter could affect the statistical significance and must be taken into account in future studies. In fact, a single determination of SDMA, not followed by a second reevaluation within one month or abdominal imaging, cannot be considered diagnostic of CRvD.

In conclusion, our study showed that SDMA is free from correlation with breed, age, sex, weight, presence/absence of MMVD, presence of CHF symptoms and pharmacological therapy as well. SDMA can be actually considered a reliable parameter for evaluation of renal function in dogs affected by MMVD, especially in those patients with a non-advanced stage of disease (ACVIM class B2), for which an early diagnosis of the onset of kidney failure is fundamental in order to plan a diuretic therapy. SDMA repeated measurements over time, as recommended by IRIS guidelines, are necessary (IRIS, 2016), because one determination does not allow us to exclude definitely a later onset of the renal impairment and then to be considered diagnostic in order to highlight a possible onset of CRS.

Conflict of interest

The authors declare that there is no conflict of interest.

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