Original

Correlation of renal function biomarkers in the first diagnostic approach to canine chronic kidney disease

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ABSTRACT

Objective. To determine the correlation of kidney function biomarkers at the first evaluation in dogs with different risk factors identified for developing chronic kidney disease (CKD). **Materials and methods.** A descriptive, prospective study of cases and controls of 388 animals, divided into five groups: control group (CG), and four groups of potentially kidney-diseased dogs (pCKD) was carried out. Clinical history, physical examination, body condition score (BCS), complete blood count, biochemical profile with symmetrical dimethylarginine (SDMA), urinalysis, urine protein/creatinine ratio (UPC), and systemic blood pressure were analyzed. Non-parametric statistics were used, and data were expressed as medians and percentiles; for BCS, Xi^2 was used. For biomarkers, Spearman's correlation was performed. **Results.** For SDMA and serum creatinine (sCr), a moderate correlation was observed for pCKD (r=0.69, p<0.001). Significant differences were observed in the variables age (p=0.002) and BCS (p<0.001) between the CG and the pCKD. Animals with mild azotemia (sCr 125–250 μmol/L) and/or with an SDMA value of 18–35 μg/dL, with or without proteinuria, had a greater probability of presenting an increase in SDMA when the BCS was below 5/9 (OR=3.55, p=0.005). **Conclusions.** SDMA is a useful complementary biomarker in pre-azotemic stages and advanced stages where there is cachexia and sarcopenia. Biomarkers must be evaluated together to have a complete perspective of renal function in animals with risk factors for developing CKD.

Keywords: Chronic kidney disease; comorbidities; creatinine; canine; renal function biomarker; symmetrical dimethylarginine (SDMA) (*Source: ICYT Animal Biology*).

RESUMEN

Objetivo. Determinar la correlación de los biomarcadores de funcionamiento renal en la primera evaluación en perros con diferentes factores de riesgo identificados para desarrollar enfermedad renal crónica (ERC). **Materiales y métodos.** Se realizó un estudio descriptivo, prospectivo de casos y controles de 388 animales, divididos en cinco grupos: grupo control (GC), y cuatro grupos de perros potencialmente enfermos renales (pERC). Se analizaron historia clínica, examen físico, condición corporal (CC), hemograma, perfil bioquímico con dimetilaginina simétrica (SDMA), urianálisis, ratio

proteinuria/creatininuria (UPC) y medición de presión arterial sistémica. Se utilizó estadística no paramétrica y los datos fueron expresados en medianas y percentiles; para la CC se utilizó Xi^2. Para los biomarcadores se realizó correlación de Spearman. **Resultados.** Para SDMA y la creatinina sérica (CrS) se observó una correlación moderada para pERC (r=0.69, p<0.001). Se observaron diferencias significativas en las variables edad (p=0.002), y CC (p<0.001) entre el GC y los pERC. Los animales azotemia leve (CrS 125-250 μmol/L) y/o con un valor de SDMA de 18-35 μg/dL, con o sin proteinuria tuvieron una mayor probabilidad de presentar un incremento de SDMA cuando se presentó una CC por debajo de 5/9 (OR=3.55, p=0.005). **Conclusiones.** El SDMA es un biomarcador complementario útil en etapas preazotémicas y en estadios avanzados donde existen caquexia y sarcopenia. Los biomarcadores deben evaluarse en conjunto para tener perspectiva completa de la función renal en los animales con factores de riesgo para desarrollar ERC.

Palabras clave: Enfermedad renal crónica; comorbilidades; creatinina; perro; biomarcadores de función renal; dimetilarginina simétrica (SDMA) (*Fuente: ICYT de Biología Animal*).

INTRODUCTION

Chronic kidney disease (CKD) is a common cause of morbidity and mortality in dogs, affecting 1–3% of a population (1); it is a progressive and irreversible pathology. Diagnosis in the early stages of the disease, where there are no clinical signs, allows improving the quality and life expectancy of the patient, and establishing appropriate therapeutic strategies (2).

The diagnostic approach begins with the identification of the risk factors that are currently considered for the presentation of CKD and are divided into: _Susceptibility factors_ such as advanced age and breed; _initiation factors_ that are associated with comorbidities, previous anesthesia, and exposure to nephrotoxic substances (3,4).

Subsequently, renal function biomarkers should be used to demonstrate the presence and severity of kidney disease and identify other risk factors: such as disease _progression factors,_ which are renal proteinuria, arterial hypertension, and hyperphosphatemia; and _end-stage factors_ such as anemia, hypoalbuminemia, cachexia (3,4).

Conventionally, the determination of serum creatinine (sCr) is considered the standard for evaluating the glomerular filtration rate (GFR). However, the current diagnostic trend is to identify the disease early, which makes this a late biomarker since its values increase with a 75% loss of functional nephrons. In addition, diagnostic accuracy is variable since it depends on the muscle mass of the individual (5). The use of other markers that detect CKD in pre-azotemic stages or in advanced stages where muscle mass is decreased is necessary (6),

and in response to these demands, the use of biomarkers such as serum concentration of symmetrical dimethylarginine (SDMA) and the urine protein/creatinine ratio (UPC) in urine, have been annexed to the protocol of the International Renal Interest Society (IRIS) (7).

SDMA is a derivative of the post-translational methylation of arginine residues contained within the nucleus of cells. After its proteolysis, it is released into the circulation and 90% is eliminated through the kidneys, making it an endogenous biomarker of GFR (8). In dogs, it increases when there is a 40% loss of functional nephrons, and without any renal signs (9). Although SDMA has been part of the CKD diagnostic protocol since 2015, its diagnostic utility remains under study (9).

Another form of early detection of CKD is the determination of the UPC, which quantitatively evaluates the loss of protein through the urine. Proteinuria can occur from the early stages, allowing the disease to be detected in pre-azotemic stages when it the only finding; in addition, together with systemic hypertension (HTN), they are considered factors indicating of CKD progression (10).

Some studies have investigated the correlation between SDMA and different biomarkers of kidney function, but none have been carried out in a large hospital population, considering the relationship of the different biomarkers of kidney function in the first evaluation of the patient. The objective was to determine the correlation of renal function biomarkers at the first evaluation in dogs with different identified risk factors for developing CKD.

MATERIALS AND METHODS

Type of study. A descriptive, prospective study of cases and controls was carried out with 388 dogs that attended for consultation at the internal medicine hospital section.

Location. The animals were treated at the Hospital Veterinario para Pequeñas Especies de la Facultad de Medicina Veterinaria y Zootecnia de la Universidad Autónoma del Estado de México (HVPE-UAEMex), with the following coordinates, 19° 17' 29 N and 99° 39' 38 W, during the period from August 2016 to July 2018.

Data collection. Data were obtained from the dogs that attended consultation for the first time; risk factors for developing CKD (such as susceptibility, initiation, and/or progression) were considered, and the clinical diagnostic protocol established by the veterinary community was used (7).

Data from the clinical history and physical examination were considered. For each case, data on sex, age, breed, and body condition score (BCS) were considered, including thin animals (1/9–4/9) and those with an ideal BCS or being overweight (5/9–9/9) for all groups (11). Blood and urine samples were taken following fasting from solids for eight hours. A laboratory profile (Pro Cyte and Catalyst One IDEXX, USA) was performed, with determination of sCr, urea, and phosphorus (P). In addition, serum SDMA was determined by liquid chromatography mass spectroscopy (LC-MS) analysis; the samples were sent to the reference laboratory with the necessary care for their correct transport and conservation (IDEXX Laboratories, Inc. Westbrook, Maine USA). Urine samples were obtained by cystocentesis to perform urinalysis and UPC determination. For urinalysis, urine specific gravity (USG) was determined by refractometry, chemical examination using reactive strips (VetLab UA and UA strips IDEXX), and microscopic examination for urinary sediment analysis by means of an optical microscope. Observing inactive urinary sediment, the UPC was determined by quantifying protein in urine using the pyrogallol red method and molybdate in an acid medium and measuring urinary creatinine using the picrate method in an alkaline medium (BTS-350, Biosystems SA Barcelona, Spain), and the subsequent equation.

For non-invasive blood pressure, we used an oscillometric monitor (Vet 20, SunTech Medical Inc. USA). The animals were placed in right lateral recumbency, and the cuff was placed on the left thoracic limb, on the radial artery. The measurement was performed according to the established protocol, taking as reference a value ≥160 mmHg for systolic blood pressure (SBP) as HTN (1).

The animals were classified according to the following parameters (7):

Control group (CG): animals with some risk factor for susceptibility or initiation to develop CKD, but without alterations in GFR (sCr<125 μmol/L and SDMA<15 μg/dL), or proteinuria (UPC<0.5), with variable USG, according to the state of hydration.

For the remainder of the groups, those patients who presented USG below the critical point (<1.030) and who also presented the following characteristics were considered:

Group 1 (G1): non-azotemic animals (sCr<125 μmol/L) and SDMA 15–17 μg/dL, with or without proteinuria (UPC<0.5).

Group 2 (G2): animals with mild azotemia (sCr 125–250 μmol/L) and/or an SDMA value of 18–35 μg/dL, with or without proteinuria (UPC>0.5). If sCr values were found to be within the ranges mentioned, with SDMA values >35 μg/dL, these patients were classified as G3.

Group 3 (G3): animals with moderate azotemia (sCr 251–440 μmol/L), and/or an SDMA value of 36–54 μg/dL, with or without proteinuria (UPC>0.5). In case of showing sCr values within the mentioned intervals, with SDMA values >54 μg/dL, these patients were classified in G4.

Group 4 (G4): animals with severe azotemia (sCr>440 μmol/L) and/or an SDMA value >54 μg/dL, with or without proteinuria (UPC>0.5).

The cases in which the sCr and SDMA values established for each group did not coincide were classified with the value of the analyte with the highest value, representing the true state of the GFR.

Exclusion criteria: Cases with evidence of dehydration, not considered hemodynamically stable, as well as azotemia and post-renal proteinuria, with a diagnosis of acute kidney injury (AKI), and with chronic diseases previously diagnosed or under medical treatment were excluded from this study.

Statistical analysis. A Kolmogorov–Smirnov normality test was performed and, due to the non-normal distribution, non-parametric tests were used for statistical analysis. The results are reported in medians and percentiles, taking into consideration the variables age, BCS, USG, sCr, urea, SDMA, UPC, P, and SBP for all groups. Xi^2 was performed for the relationship of BCS (1–4/9, 5–9/9) and the increase in SDMA in the study groups, and the Kruskal–Wallis test and the post hoc Dunn's multiple comparisons' test to identify differences between groups. Spearman's correlation coefficient was performed for the variables USG, sCr, urea, SDMA, UPC, P, and SBP for the CG, and for the possible chronic kidney disease group (pCKD), interpreting the values (Table 1) according to Mukaka (12). Statistical significance was considered at a value of $p < 0.05$. The statistical analysis of the data was performed with the Graph Pad Prism program, version 8.1.1 (California, USA, 2018); the scatter diagram was made with IBM SPSS Statistics for Windows, version 20.0 (New York, USA, 2011).

Table 1. Interpretation of the correlation coefficients according to Mukaka, 2012 (12).

Correlation value	Interpretation
0.90 to 1.00 (−0.90 to −1.00)	Very high correlation
0.70 to 0.90 (−0.70 to −0.90)	High correlation
0.50 to 0.70 (−0.50 to −0.70)	Moderate correlation
0.30 to 0.50 (−0.30 to −0.50)	Low correlation
0.00 to 0.30 (0.00 to −0.30)	Very low correlation

Positive correlation values (Negative correlation values).

RESULTS

Population distribution. A total of 545 cases were analyzed, of which 388 met the inclusion criteria; of these, 51.8% (n=201) were females. The median age was nine years, with a range of 1–18 years. Forty-nine different breeds were identified, the most frequent were Poodle (16.5%), Miniature Schnauzer (13.6%), Chihuahua (6.9%), Cocker Spaniel (5.6%), and Golden Retriever (4.1%); mixed breeds´ represented corre13.9% of cases. No significant risk of presenting CKD in any specific breed was observed.

Distribution of the groups. The 388 clinical cases studied were distributed into different groups:

The **CG** was composed of 226 animals, corresponding to 58.2% of the total cases. Of these, 57.1% (129/226) were females; with a median age of eight years, of which 45 animals had a BCS of 1/9–4/9, and 181 a BCS of 5/9–9/9.

G1 was formed by 86 animals, being 22.1% of the total cases. Of these, 51.2% were females (44/86), with a median age of ten years. Twenty-six animals had a BCS of 1/9–4/9, and 60 a BCS of 5/9–9/9. Of these, 25.6% (22/86) presented GFR alterations identified by SDMA values between 15–17 µg/dL, 59.3% (51/86) presented proteinuria, and 15.1% (13/86) presented alterations in both biomarkers simultaneously, 9.3% (8/86) presented HTN.

In **G2**, 48 dogs were included, representing 12.5% of the total cases. Of these, 29.2% were females (14/48), with a median age of ten years. Twenty-seven animals had a BCS of 1/9–4/9, and 21 a BCS of 5/9–9/9. In 33.3% (16/48) the SDMA and sCr coincided according to the established criteria: 16.7% (8/48) showed azotemia with an SDMA value <18 µg/dL; and 50.0% (24/48) presented SDMA values between 18–36 µg/dL without azotemia; 56.3% (27/48) were proteinuric and 10.4% (5/48) had HTN.

In **G3,** 15 cases were included, corresponding to 3.9% of the total population. Of these, 46.6% were females (7/15), with a median age of 11 years. Ten animals had a BCS of 1/9–4/9, and five a BCS of 5/9–9/9. Of these, 20.0% (3/15) presented SDMA values between 36–54 µg/dL and sCr between 251–440 µmol/L, coinciding as established for this group. On the other hand, 20% (3/15) presented SDMA values below the established range. The remaining 60.0% (9/15) presented SDMA values <36 µg/dL and a sCr between 125–250 µmol/L. Proteinuria was observed in 66.6% (10/15) of the animals, and the frequency of HTN was 13.3% (2/15).

G4 was comprised of 13 cases, corresponding to 3.3% of the cases studied. Of these, 53.8% were females (7/13), and had a median age of nine years. Nine animals had a BCS of 1/9–4/9, and four a BCS of 5/9–9/9. In 61.5% (8/13) of the cases, relation was observed between the values of SDMA (>54 µg/dL) and sCr (>440 µmol/L), 15.4% (2/13) presented values of SDMA below the suggested range, but this sCr corresponded to this group; 23.1% (3/13) had a concentration of SDMA >54 µg/dL and sCr ≤440 µmol/L and 77.0% (10/13) of the animals were proteinuric. The HTN was observed in 23.0% (3/13) of these patients.

Odds ratio was performed in the study groups with respect to BCS and the probability of presenting an increase in SDMA. It was observed that the animals in G2 had a 3.55 times greater probability (OR=3.55, p=0.005) of presenting an increase in SDMA when the BCS was below 5/9 (Table 2).

Table 2. ORs calculated for the study groups with respect to the increase in SDMA and BCS.

Group	BCS 1-4/9	BCS 5-9/9	OR	CI 95%	p-value
G1	15	20	0.89	0.419-1.898	0.767
G2	17	7	3.55	1384-9136	**0.005**
G3	6	3	2.56	0.619-10.645	0.180
G4	3	1	3.77	0.384-37.052	0.223

Differences between variables by group. The medians of the variables age, BCS, USG, sCr, urea, SDMA, UPC, and P were statistically different between the study groups (Table 3). For the variable age as a risk factor for susceptibility, a significant statistical difference was observed between the CG with a median of eight years, and G2 with a median of ten years (p=0.002). In the case of BCS, significant differences were observed between the CG and G2, G3, and G4, as well as between G1 and G3.

For the USG variable, a significant statistical difference was observed between the CG and G2, G3, and G4. The variables sCr, urea, and SDMA showed a gradual increase in the medians according to the classification of the groups. The sCr of the CG and G1 were significantly different from those of G2, G3, and G4. In the case of urea, the CG was statistically different from G2, G3, and G4, and G2 was different from the others. For the SDMA variable, there were differences between the CG and the pCKD, in addition, G1 was different from G2, G3, and G4. In the case of UPC, significant differences were observed between the CG and the pCKD. For the P variable, a higher median was observed in G3 and G4 with respect to G2, G1, and CG. For the SBP variable, there were no statistical differences between the groups.

Table 3. Distribution of medians and percentiles for the variables age, weight, and biomarkers in the study groups.

n= 388	CG 226	G1 86	G2 48	G3 15	G4 13	p-value
Age (years)	8 (3.0-14.4) a	10 (2.1-15.8) a, b	10 (2.35-16) b	11 (4.35-15.3) a, b	9 (1.9-14) a, b	**0.002**
BCS (1/9-9/9)	5 (3-9) a	5 (1-9) a, b	4 (3-9) b, c, d	3 (2-6) c, d	3 (1-7) d	**<0.0001**
USG	1.034 (1.007-1.056) a	1.021 (1.005-1.029) a	1.015 (1.010-1.027) b	1.013 (1.007-1.019) b	1.013 (1.010-1.016) b	**<0.0001**
sCr (µmol/L)	80 (35-124) a	71 (44-115.8) a	125 (70.1-248) b	221 (138.95-409) b	723 (376.4-1201) b	**<0.0001**
Urea (mmol/L)	5 (2.1-11.81) a	5.4 (1.8-15.63) a	8.6 (2.61-31.9) b	27.1 (11.09-46.4) c	46.2 (21.78-65.68) c	**<0.0001**
SDMA (µg/dL)	11 (5.6-14) a	14 (7-17) b	21 (12.17-29) c	40 (19.2-69.65) c	76 (41-108.4) c	**<0.0001**
UPC	0.15 (0.03-0.48) a	0.77 (0.03-7.16) b	0.62 (0.03-4.06) b	1.05 (0.19-5.48) b	1.49 (0.08-8.58) b	**<0.0001**
P (mmol/L)	1.22 (0.65-1.88) a	1.35 (0.73-1.91) b	1.36 (0.84-2.41) b	2.13 (1.33-5.2) c	4.74 (0.66-8.28) c	**<0.0001**
SBP (mmHg)	138 (109-166) a	139 (109-174) a	147 (126-179) a	140 (119-224) a	144 (118-179) a	0.165

BCS, body condition score; USG, urine specific gravity; sCr, serum creatinine; SDMA, symmetric dimethylarginine; UPC, protein/creatinine ratio; P, phosphorus; SBP, systolic blood pressure. Median and percentiles in parentheses (0.025-0.975). Different literals indicate significant statistical differences p<0.05; Analysis performed with Kruskal-Wallis.

Correlations. Correlations were performed according to (Table 1) Mukaka (12). In the CG, the results of the statistically significant correlations were determined as very low (Table 4).

The correlations of the pCKD (G1, G2, G3, and G4) were analyzed together (Table 5). Where correlations of the variables are shown, moderate correlation values were observed between the variables sCr, urea, and SDMA.

Low correlations can also be observed for the variables USG in relation to SDMA and P in relation to sCr, urea, and SDMA (Table 5).

Table 4. Correlations of the variables USG, sCr, urea, SDMA, UPC, P, and SBP in the CG.

	USG	sCr (µmol/L)	Urea (mmol/L)	SDMA (µg/dL)	UPC	P (mmol/L)	SBP (mmHg)
USG	1.00	-0.05	0.27*	-0.09	-0.08	-0.01	-0.06
sCr (µmol/L)	-0.05	1.00	0.24*	0.23*	-0.25*	-0.15*	0.05
Urea (mmol/L)	0.27*	0.24*	1.00	0.08	-0.11	0.03	0.06
SDMA (µg/dL)	-0.09	0.23*	0.08	1.00	-0.07	0.06	0.035
UPC	-0.08	-0.25*	-0.11	-0.07	1.00	-0.03	-0.017
P (mmol/L)	-0.01	-0.15*	0.03	0.06	-0.03	1.00	0.18
SBP (mmHg)	-0.07	0.06	0.06	0.04	-0.02	0.02	1.00

USG, urine specific gravity; sCr, serum creatinine; SDMA, symmetric dimethylarginine; UPC, protein/creatinine ratio; P, phosphorus; SBP, systolic blood pressure. *The correlation has a statistical significance $p<0.05$.

Table 5. Correlations of the variables USG, sCr, urea, SDMA, UPC, P, and SBP for pCKD.

	USG	sCr (µmol/L)	Urea (mmol/L)	SDMA (µg/dL)	UPC	P (mmol/L)	SBP (mmHg)
USG	1.00	-0.28*	-0.15	-0.41*	-0.03	-0.25*	-0.24*
sCr (µmol/L)	-0.28*	1.00	0.65*	0.69*	-0.05	0.31*	0.05
Urea (mmol/L)	-0.15	0.65*	1.00	0.58*	0.19*	0.45*	-0.09
SDMA (µg/dL)	-0.41*	0.69*	0.58*	1.00	-0.04	0.43*	0.077
UPC	-0.03	-0.05	0.19*	-0.04	1.00	0.28*	0.17
P (mmol/L)	-0.25*	0.31*	0.45*	0.43*	0.28*	1.00	0.14
SBP (mmHg)	-0.24*	0.055	-0.09	0.07	0.17	0.14	1.00

USG, urine specific gravity; sCr, serum creatinine; SDMA, symmetric dimethylarginine; UPC, protein/creatinine ratio; P, phosphorus; SBP, systolic blood pressure. *The correlation has a statistical significance $p<0.05$.

The relationship between the sCr and SDMA variables in the study groups is shown in the scatter diagram (Figure 1). An increase in both biomarkers was observed in relation to the severity of the disease (CG: r=0.23, p<0.001, pCKD: r=0.69, p<0.001).

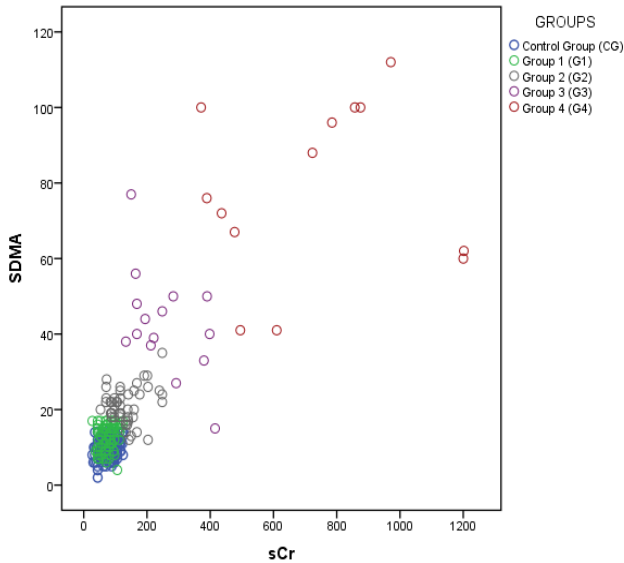**Figure 1.** Scatter plot of sCr (μmol/L) and SDMA (μg/dL) in the study groups.

DISCUSSION

Recognition of risk factors for susceptibility and disease onset is the key to the early diagnostic approach to CKD. In this study, no significant differences were found regarding gender, as described in other works, where gender was not shown to represent a risk factor for developing CKD (13,14). Regarding age, 88.2% of the animals in the pCKD groups were more than six years, with a median age of nine years, similar that reported in other populations (15). These findings are attributed to the fact that renal function is affected by age, being associated with chronic-degenerative diseases that are related to CKD (13, 16).

The cases studied were of different breeds and their frequency of appearance differed from that reported by other authors. O'Neill et al (13) observed a higher frequency in animals of the Yorkshire terrier, Jack Russell terrier, and West Highland White terrier breeds. Pelander et al (14) observed a higher frequency of Bernese Mountain, Miniature Schnauzer, and Boxer breeds. These differences in the frequency of

breeds with CKD are attributed to the preference of the owners depending on the geographical areas where the studies were carried out. In Mexico, the animals of Poodle, Schnauzer, Chihuahua, Cocker Spaniel, and Golden Retriever breeds are among the most popular, and this is reflected in this study. It has been documented that some of these breeds may present a breed predisposition to kidney diseases; future studies would be necessary for this population (7, 13).

Of the total pCKD cases analyzed, 22.1% were classified as G1, considered the pre-azotemic stage of CKD by the ranges of GFR biomarkers used (7), with a decrease in GFR being evidenced in a quarter of these cases due to a slight increase in SDMA. Different authors have reported that SDMA can be used as an early biomarker of CKD since its serum concentration increases before sCr, when there is an approximately 40% reduction in functional nephrons (17, 18). In addition, almost two-thirds of the cases included in this group presented proteinuria as the only finding, making the use of the UPC fundamental in the diagnostic process, which allows the identification of the disease in early non-azotemic stages (10). Proteinuria has been considered a biomarker of kidney disease and a factor indicating of progression of great value. In many cases, increases occur before observing increases in SDMA or sCr (10). On the other hand, only 15.1% of G1 cases showed an increase in SDMA and proteinuria simultaneously, suggesting a more advanced degree of disease in these cases due to GFR alteration (19), but without reaching the azotemic phase.

Using the ranges proposed by IRIS in 2019 (7), a relation was observed between the sCr and SDMA ranges for each group in half of the cases studied. In 23.0% (12/52) of the azotemic animals, the BCS was below 5/9, which limited the use of the sCr concentration as a classification criterion and forced the use of SDMA values for this purpose. In these cases, since they did not have a good muscular condition, and since 95% of the body's creatinine is found in skeletal muscle (5), the finding of lower sCr values was not usually representative of renal function, tending to its overestimation (17). In this study, a higher frequency of BCS from 1/9–4/9 was observed in animals from G2, G3, and G4, similar to that observed by Rudinsky et al (15), where a lower BCS in patients in advanced stages, associated with the presence of cachexia and sarcopenia were shown. This is generated by multifactorial pathophysiological mechanisms that include

an increase in energy requirements, decreased absorption of nutrients, decreased energy intake, and alterations in the patient's metabolism (20). In addition, weight loss in patients with CKD has been related to decreased survival of patients in advanced stages (21). In these cases, SDMA should be used as a GFR biomarker, since it is not affected by muscle mass (17,22).

On the other hand, in 19.2% (10/52) of the cases, SDMA was found to be within the reference range (<18 μg/dL) in the presence of azotemia (sCr>125 µmol/L), attributing this finding to analytical interferences or lack of hemodynamic balance (23), although in this study it was considered that these cases were hemodynamically stable at the time of sampling; based on the information obtained in the consultation of each of the patients. This reaffirms the importance of re-evaluating patients to confirm the diagnosis of CKD (7). In addition, it has been reported that the sole determination of SDMA does not adequately differentiate between CKD and AKI (23), making it essential to carry out a detailed evaluation of the clinical history, clinical and laboratory findings, as well as follow-up of the patient over time, to differentiate between these processes, and achieve a diagnosis and determine the staging of CKD (24).

Regarding the behavior of the variables sCr, urea, SDMA, and P, in the study groups, an increase in their values related to the probable severity of the disease was evidenced.

Concerning SBP, various studies have shown that CKD is one of the main pathologies associated with the finding of HTN (1, 13); in this study, there were no statistical differences between the groups. On the other hand, in the pCKD groups, HTN was more frequent in the groups corresponding to advanced stages of the disease, being the result of different pathophysiological alterations and the interaction between them, such as sodium retention, activation of the renin-angiotensin system aldosterone, increased sympathetic activity, with the consequent increase in cardiac output and peripheral vascular resistance (25). In azotemic patients, the magnitude of HTN has been related to the presence of renal proteinuria, which is more frequent in advanced stages (1).

Concerning the correlations in the biomarkers, the association of SDMA and sCr showed a very low correlation in the CG; similar correlations have been observed in healthy patients. Hall et al (22) observed a correlation of r=0.32 (p=0.04) in a population.

With reference to the correlation of pCKD, in human medicine, data from 2100 patients were analyzed, observing a high correlation between SDMA and sCr of r=0.75 (p<0.001) (8). In veterinary medicine, a high correlation of r=0.84 (p<0.001) was observed in a retrospective study in a colony of Beagle dogs under controlled conditions (18). In another study, a correlation of r=0.74 (p<0.0001) in dogs with CKD (23) was reported. In 2019, McKenna et al (7) evaluated the correlation between iohexol, SDMA, and sCr in 119 non-azotemic dogs, observing a low correlation of r=0.33 (p<0.0001). These correlation values are because SDMA has more than 90% renal excretion, an essential characteristic that allows it to be considered a biomarker of renal function, which is related to the excretion of sCr. When the GFR is altered, there is a serum increase of both biomarkers. In this study, the value of the correlation in pCKD could be due to the non-homogeneous distribution of patients due to the way of approaching the disease due to risk factors, observing a lower frequency of azotemic animals.

The correlation observed between the urea and SDMA variables was considered moderate, the existing association between the serum variations of urea due to hepatic metabolism, and diet, among others, make it an unstable renal biomarker (6).

It was observed that the correlations of USG with the other markers were negative because when the critical point of USG decreased and reached isosthenuria, in patients with impaired functional renal mass, GFR markers tended to increase. This is associated with the inability to concentrate urine and eliminate waste substances (26).

Regarding the other biomarkers evaluated (UPC, P, SBP), the observed correlations were considered low or very low, since these are markers of disease progression, but are not considered GFR markers. Specifically, for P, a low correlation was observed with sCr, urea, and SDMA; since by decreasing GFR, renal P excretion is reduced, causing hyperphosphatemia, common in advanced stages of the disease (2).

Based on the above, the rationale for adding SDMA to the CKD diagnostic protocol is based on two premises: the first is that it favors reaching the diagnosis of the disease in pre-azotemic stages (≥40% decrease in GFR), and the second is that allows corroborating the diagnosis of CKD in patients with loss of muscle mass; where sCr may not adequately determine renal function, especially in cachectic animals. It is important to emphasize that under no circumstances should SDMA be used as the only biomarker in the evaluation of renal function in dogs (24).

In conclusion, in this study, the usefulness of the clinical application of a diagnostic approach based on risk factors was demonstrated to identify CKD in its early stages. The first evaluation carried out comprehensively, considering the evaluation of early renal function biomarkers, allows both determining if the animals require a reassessment of renal function, and providing relevant information on the patient's renal function.

The correlation of the SDMA and sCr biomarkers was significant in the pCKD groups, lower than that reported in other studies because natural kidney disease was analyzed.

It is worth mentioning that UPC was useful as a marker of kidney disease, so it should be included in the diagnostic approach to patients with risk factors. In our experience, the use of the complete protocol allows for knowing, from a comprehensive perspective, the state of renal function in the first diagnostic approach to CKD.

The pCKD animals were older than the controls, increasing the probability of developing CKD due to the decrease in functional nephrons secondary to advanced age. In addition, a decrease in BCS was evidenced in the groups corresponding to advanced stages of CKD.

Conflict of interests

The authors of this study declare that there is no conflict of interest with the publication of this manuscript.

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