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Evaluation of kidney injury through early markers in canine pyometra

Avaliação de lesão renal através de marcadores precoce em piometra canina

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ABSTRACT

Fourteen female dogs diagnosed with pyometra were studied at three separate times: at diagnosis (T0) and 24 h (T1) and 10-15 days (T2) after ovariohysterectomy (OH). The means of the markers, symmetric dimethylarginine (SDMA) (17.71 to 26.54 µg/dL) and the urinary gamma-glutamyl transferase to creatinine ratio (uGGT/uCr) (1.06 to 2.62 U/mg), varied, showing an increase with time. Further, the elevation of gamma-glutamyl transferase (uGGT) (56.61 to 128.12 U/L) and the urinary protein to creatinine ratio (RPC) (0.26 to 1.24) was evident at T0 and T1. A reduction in the means of RPC, uGGT, and uGGT/uCr was observed 10-15 days after OH. Despite the elevation of these markers, the concentration of creatinine (1.11 to 1.40 mg/dL), urea (40.07 to 67.16 mg/dL), and urinary specific gravity (1.027 to 1.028) only presented slight variation. In canine pyometra, complications secondary to acute renal injury may be present that may be mild and transient in most treated animals. As elevation in SDMA and RPC preceded changes in creatinine levels for the evaluation of glomerular filtration, tubular markers could assist in the early identification of renal damage in canine pyometra.

Keywords: Symmetric dimethylarginine. Urinary gamma-glutamyl transferase. Acute kidney injury. Reproductive disorder.

RESUMO

Catorze cadelas com diagnóstico de piometra foram estudadas em três tempos distintos, sendo no momento do diagnóstico (T0), 24 horas (T1) e 10 a 15 dias (T2) após a ovário-histerectomia (OH). O objetivo foi avaliar o uso de diferentes biomarcadores renais em cadelas com piometra e estimar suas precocidades diante do agravo. As médias em dimetilarginina simétrica (SDMA) (17,71 a 26,54µg/dL) e relação gama-glutamil transferase e creatinina urinária (uGGT/uCr) (1,06 a 2,62U/mg) variara, apresentando aumento em todos os momentos. Já a elevação do gama-glutamil transferase (uGGT) (56,61 a 128,12 U/L) e da razão proteína e creatinina urinárias (RPC) (0,26 a 1,24) foram evidenciadas nos dois primeiros tempos. Uma redução na média do RPC, uGGT e uGGT/uCr foi observada 10-15 dias após a implantação do tratamento (OH). Apesar da elevação desses marcadores, a concentração de creatinina (1,11 a 1,40mg/dL), ureia (40,07 a 67,16mg/dL) e densidade urinária (1,027 a 1,028) sofreram poucas variações. Em piometra canina, as complicações renais agudas secundárias podem estar presentes, ainda que leve e transitória nos animais tratados. Os marcadores tubulares foram considerados precoces na injúria renal aguda. Além disso, a SDMA e o RPC antecederam as alterações de creatinina em todos os tempos analisados.

Palavras-chave: Dimetilarginina simétrica. Gama-glutamil transferase urinária. Injúria renal aguda. Afecção reprodutiva.

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Introduction

Pyometra is the most diagnosed reproductive disorder in female dogs, characterized by a purulent exudate due to bacterial proliferation in the endometrium (Hagman, 2018). Consequently, various clinical and pathological manifestations are observed locally and systemically, with renal involvement being a significant complication caused by the deposition of immune complexes in the glomeruli. This suggests that glomerulonephritis results from the release of endotoxins (Hagman, 2014).

A previous study reported contrasting results in an evaluated female dog in which the damage was similar to age-related glomerulosclerosis, as no histopathological evidence related to glomerulonephritis was found (Maddens et al., 2011). However, focal renal changes in glomerular and tubular compartments are evident in pyometra, and its physiopathology has not been completely elucidated (Heiene et al., 2007; Maddens et al., 2010).

Kidney disease evolves silently with no clinical signs or evidence on routine laboratory tests (serum creatinine [sCr]) until approximately 75% of functional nephrons are affected (Dibartola, 2000). As an aggravating factor, changes in the muscle mass of dogs can interfere with the sensibility of sCr and compromise renal evaluation. For these reasons, studies have been conducted using different biomarkers, mainly low-molecular-weight, intratubular, or inflammatory proteins (Cobrin et al., 2013; De Loor et al., 2013).

Early biomarkers are known to assist in differentiating acute and chronic renal impairments and determining kidney damage (Cobrin et al., 2013). In dogs, symmetrical dimethylarginine (SDMA) can be consistently detected with a reduction of 30% in kidney filtration. Therefore, it is better at diagnosing acute kidney injury (AKI) than sCr (Hall et al., 2016; Nabity et al., 2007). Proteinuria is considered an essential indicator of alterations in the glomerular and tubular regions and can be determined by assessing the urinary protein and creatinine ratio (RPC) (Hokamp & Nabity, 2016). Urinary gamma-glutamyl transferase (uGGT) indicates kidney damage and is usually associated with tubular epithelial injury or necrosis (Paltrinieri et al., 2018; Perondi et al., 2019). Thus, an early and consistent biomarker in tubulopathy (Gori et al., 2019) and the ratio of uGGT and urinary creatinine (uCr) are viable parameters for estimating daily enzymatic activity (Lippi et al., 2018).

The urinary enzyme concentrations are elevated in dogs with pyometra, reflecting extensive proximal tubular lesions (Heiene et al., 2001; Maddens et al., 2011). However, further studies are needed to determine the specific urinary biomarkers for localizing kidney localizing dogs with pyometra (Heiene et al., 2001).

This study aimed to compare the serum and urinary biomarkers to indirectly measure the glomerular filtration rate (GFR) and detect the presence of tubular damage that can help in an early assessment of renal impairment in canine pyometra and identify biomarkers for the diagnosis of renal injury.

Material and Methods

The Ethics Committee approved this study on the Use of Animals at the Federal University of Alagoas (UFAL) under protocol 078/18.

Fourteen female dogs (*Canis lupus familiar*) between 3 and 10 years of age with clinical, laboratory, and ultrasonographic diagnoses of pyometra, with no increase in sCr (≤ 1.5 mg/dL and submitted to the surgical procedure of ovariohysterectomy (OH) were selected for the study. Dogs with alterations in compatible complementary exams and diagnosed with blood parasites, based on direct findings of the parasites or analysis in a Snap 4DX IDEXX® test, were omitted. Animals with a clinical history of pre-existing kidney disease from a detailed anamnesis and physical examination were also excluded from the study. The surgical procedure (OH) and complementary therapeutic protocol were implemented up to 24 h after diagnosis.

The dogs were evaluated at three separate times: at diagnosis (T0) and 24 h (T1) and 10-15 days (T2) after OH. Urine and blood samples were collected after 8-hour fasting to evaluate counts and serum biochemicals.

Blood samples were collected through the external jugular vein (8 mL) and placed in tubes with anticoagulant (ethylenediaminetetraacetic acid) (2 mL) for the evaluation of blood cell counts using BC-2800 Vet Mindray and

HumaCount 30 TS and leukocyte differentiation count and erythrocyte morphology through direct evaluation under an optical microscope.

Serum biochemical measurements were performed for blood urea, sCr, alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total serum protein (TSP) using HumaLyzer Primus semiautomatic equipment and Bioclin reagents. Serum SDMA was measured by a high-yield immunoassay technique using the IDEXX Catalyst SDMA One Test equipment (IDEXX®).

The urine samples were collected (5 to 10 mL) exclusively through cystocentesis guided by ultrasound at T0. However, cystocentesis, urethral catheterization, and spontaneous miction were performed at T1 and T2. After collection, the samples were analyzed to measure the levels of proteins (red pyrogallol technique), uCr (Jaffe modified methods), and uGGT (Szazs/Persijn method). A correction of uGGT was performed using urine specific gravity [(uSG)=1.025], and the RPC and uGGT/uCr were calculated. Urine analysis was performed using an automated reading method (Uri-colour check [Wama®]), and urine sediment evaluation was performed using optical microscopy. After centrifugation, the urine supernatant was used to measure the following biochemical parameters: protein, uCr, and uGGT (Bio Plus Bio 2000).

Infectious agents of pyometra were identified by bacterial culture through samples obtained by direct puncture (1 mL) of the uterus after uterine removal, stored in a tube of Stuart medium, and seeded in 48 h in MacConkey medium and 5% sheep blood agar (defibrillated). They were incubated in an oven under aerobic conditions at 37 °C for 48 h and later evaluated by the Gram method and biochemical methods to identify microorganisms.

Descriptive statistical methods of the quantitative data were analyzed using commercial software. The analysis of variance between times was performed using the Shapiro-Wilk normality test and Kruskal-Wallis non-parametric test. Pearson's linear correlations between the serum and urinary biomarkers at the different time points (T0, T1, and T2) were evaluated along with the 95% confidence intervals ($p<0.05$).

Results

Fourteen female dogs were included in this study, averaging 7.1 years (11 months to 12 years). The included dogs were of various breeds without a history of pseudocyesis or breast cancer. The clinical signs usually reported were apathy (85.7%, 12/14), dysorexia (78.6%, 11/14), prostration (71.4%, 10/14), vomiting (57.1%, 8/14), vaginal discharge (57.1%, 8/14), and abdominal enlargement (42.9%, 6/14) fever (42.9%, 6/14), and polyuria/polydipsia (28.6%, 4/14) as evidenced in the anamnesis and the physical exam.

In addition to the sonographic findings of pyometra, such as uterine volume increase with filled anechoic to hypoechoic contents. Unilateral (n=1) and bilateral (n=2) polycystic ovaries were observed, and two cases had mild cystitis without other intra-abdominal morphological changes. Bacterial cultures of uterine content were predominantly gram-negative, mainly *E. coli* (35.7%, 5/14), followed by *Pseudomonas aeruginosa* and Gram-positive *Streptococcus* spp. (7.1%, 1/14). There was no bacterial growth in 28.6% (4/14) of the samples, and 21.4% (3/14) were discarded after 48 h.

All data were completed at T0 and T1. However, five dogs with spontaneous pyometra did not return for the last collection (T2), and one dog died approximately three weeks after being diagnosed with pyometra.

At T0, 57.1% (8/14) of the dogs had mild anemia, 42.9% (6/14) had normochromic normocytic anemia, and 14.3% (2/14) had hypochromic normocytic anemia. Neutrophilic leucocytosis with left shift was observed in 85.7% (12/14) of the dogs, with the regenerative and degenerative types of a left shift in 66.7% (8/12) and 33.3% (4/12) of the dogs, respectively. Deviation to the right shift was observed in 3% (2/14), lymphopenia in 14.3% (2/14), and monocytosis in 71.4% (10/14) of the dogs, without changes in platelets and other cellular components.

In serum biochemical analyses, the average values of ALT remained within the reference range for the species at T0 and T1. However, the values differed significantly from those at T2 ($p=0.0037$ and $p=0.0025$, respectively). About ALP, the activities were within the reference range at T0 but increased at the other time points, with a significant difference between T0 and T2 ($p=0.0178$; Table 1). Progressive hyperproteinaemia was observed in only one dog, up to 13.17 g/dL at T2. Besides, serum protein levels remained within the reference range.

Despite the gradual increase in the sCr concentration over time, it remained within the reference range, with no significant change observed between the time points. A slight increase in the mean serum urea levels was observed at T2. However, this difference was insignificant (Table 1).

The average concentration of SDMA was always higher than the reference range, but the difference was not significant ($p>0.05$) between the time points (Table 1). An increased concentration was observed in 57.1% (8/14) of the dogs at T0 and T1 and 55.6% (5/9) at T2. A concomitant increase

in sCr levels was observed in 28.6% (4/14) of the dogs at T1 and 11.1% (1/9) at T2.

In the urinalysis, the average uSG was within the reference range, with no significant difference between the time points (Table 2). However, isosthenuria and moderately concentrated urine (1.013-1.029) were observed in 14.3% (2/14) and 35.7% (5/14), 14.3% (2/14), and 50% (7/14), 11.1% (1/9) and 44.4% (4/9) of the dogs at T0, T1, and T2, respectively.

Granular and epithelial casts were observed in 42.9% (6/14) of the dogs, with 28.6% (4/14) at T0, 35.7% (5/14) at T1, and 22.2% (2/9) at T2. Three of these dogs presented a persistent urine cast (one dog up to T1; the other two in all time points).

Proteinuria was identified in 57.1% (8/14) of the dogs at T0 and 85.7% (12/14) at T1, with no difference between the two groups. However, at T2, there was a significant reduction in proteinuria (p=0.0098), and only 22.2% (2/9) of the dogs had mild proteinuria.

In the initial study period, a moderate correlation of SDMA was found with uGGT (r=0.751, p=0.001) and uGGT/uCr (r=0.769, p=0.001). However, this correlation gradually decreased with time. Moreover, SDMA showed no correlation with urea and sCr at T0,

Table 1 – Serum biochemical parameters expressed as mean, standard deviation (SD), minimum and maximum values and compared between times (T0, T1, and T2) of canine pyometra

PARAMETERS (Reference Values)	PERIOD	MEAN/SD	MINIMUM	MAXIMUM
sCr (0.5-1.5 mg/dL) (Cowgill, 2016)	T0	1.11 ± 0.29	0.60	1.50
	T1	1.40 ± 0.92	0.23	3.47
	T2	1.39 ± 1.45	0.22	5.17
Urea (21.4-60 mg/dL) (Stockham & Scott, 2011)	T0	40.07 ± 37.64	8.00	147.90
	T1	59.07 ± 65.12	17.00	275.00
	T2	67.16 ± 48.69	15.00	183.80
SDMA (<14 µg/dL) (International Renal Interest Society, 2019)	T0	17.71 ± 11 .13	6.00	44.00
	T1	26.54 ± 24.30	8.00	84.50
	T2	21.89 ± 21.46	10.00	78.00
ALT (21-86 U/L) (Stockham & Scott, 2011)	T0	33.51[a] ± 16.53	18.10	73.00
	T1	33.79[a] ± 13.20	14.55	56.70
	T2	166.73[b] ± 142 .93	32.00	353.86
ALP (20-156 U/L) (Stockham & Scott, 2011)	T0	100.26[a] ± 62.24	36.10	246.00
	T1	179.03 ± 178 .62	25.60	711.00
	T2	286.47[b] ± 179 .25	54.50	524.00
TSP (5.4-7.8 g/dL) (De Schepper et al., 1987)	T0	5.78 ± 2.33	1.00	9.73
	T1	4.87 ± 2.79	1.20	9.52
	T2	6.81 ± 3.48	2.20	13.17

Serum creatinine (sCr), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total serum protein (TSP), and symmetric dimethylarginine (SDMA). T0: At the time of diagnosis (pre-surgical); T1: 24 h after the surgical procedure; T2: 10 to 15 days after the operation. Different letters between the lines highlight the significant difference.

Table 2 – Urinary biochemical parameters expressed as mean, standard deviation (SD), minimum and maximum values and compared between times (T0, T1, and T2) of canine pyometra

PARAMETERS (Reference Values)	PERIOD	MEAN/SD	MINIMUM	MAXIMUM
uSG (1,015-1,045) (Stockham & Scott, 2011)	T0	1028.71± 12.47	1012.00	1052.00
	T1	1027.57 ± 15.03	1012.00	1050.00
	T2	1027.33 ± 12.96	1010.00	1050.00
uGGT (13-92 U/L) (De Schepper et al., 1989)	T0	128.12 ± 130.79	17.50	477.20
	T1	125.33 ± 110.80	25.00	372.73
	T2	56.61 ± 38.34	13.55	118.39
RPC (<0, 5) (Cowgill, 2016)	T0	1.03 ± 1.06	0.04	3.20
	T1	1.24[a] ± 1.08	0.20	3.20
	T2	0.26[b] ± 0.21	0.06	0.60
uGGT / uCr (<0.42 U/mg) (De Schepper et al., 1989)	T0	1.86 ± 1.82	0.21	6.18
	T1	2.62 ± 3.34	0.32	13.12
	T2	1.06 ± 0.77	0.11	2.04

Urinary specific gravity (uSG), urinary γ-glutamyl transferase (uGGT) corrected by uSG (1.025), protein and urinary creatinine ratio (RPC), urinary gamma-glutamyl transferase and urinary creatinine (uGGT/uCr). T0: At the time of diagnosis (pre-surgical); T1: 24 h after the surgical procedure; T2: 10 to 15 days after the operation. Different letters between the lines highlight the significant difference.

a moderate correlation at T1, and a strong correlation at T2 [sCr (r=0.919, p=0.0004) and urea (r=0.872, p=0.003)].

Discussion

Pyometra is a common disease in intact female dogs with an average age of 7 years (Maddens et al., 2011). In this study, the mean age of dogs was 7.1 years. Among the clinical signs, apathy was frequent (85.5%, 12/14) in dogs, as previously described (87%). This is caused by the endotoxin released by invading microorganisms (Hagman, 2018). The bacteria most commonly isolated in pyometra cases are gram-negative, predominating *E. coli* (Hagman, 2014).

Cystitis could be present concomitantly with pyometra and caused by the same etiological agent (Trautwein et al., 2017). Although a urine culture was not performed, a urinary tract infection was identified in 14.3% (2/14) of the female dogs through suggestive ultrasound images and the discrete presence of leukocytes and bacteria in the urine sediment (Smee et al., 2016). Fortunately, these findings had minimal influence on diagnosing renal proteinuria in our study dogs. Several leukocytes and erythrocytes are needed to confirm the diagnosis of intrinsic proteinuria (Harley & Langston, 2012). In addition, none of the dogs presented any history or clinical signs of complications in the urinary tract before pyometra.

The absence of bacterial growth, verified in 21.3% of the uterine secretion cultures, was reported in approximately 20-27% of the female dogs in a previous study (Trautwein et al., 2017). The etiology of sterile pyometra is unknown. However, it is suspected that the uterine defense mechanism could eliminate microorganisms (Yoon et al., 2017). In addition, an inadequate sampling method can impair the growth of bacterial colonies.

Regarding liver enzymes, significantly higher concentrations of ALT and ALP were observed at T2 than at T0 and T1, similar to a previous study (Figueiredo et al., 2017). The change in the levels suggests liver damage from pyometra, which can be justified by hepatocellular lesions due to toxemia (sepsis) caused by the anti-inflammatory response to arterial hypotension and intensified by cellular hypoxia from dehydration and anemia. In contrast, liver disorders are less frequent than renal insult and may be related to mild hepatocyte damage from intrahepatic causes (cholestasis) (Plavec et al., 2006). Persistent and progressive hyperproteinemia in a female dog with pyometra may indicate chronic antigenic stimulation with excessive production of immunoglobulins (Anjos et al., 2021; Srinivas et al., 2018).

The lack of correlation between SDMA, sCr, and SDMA, urea at T0 was expected at the beginning of a renal injury. SDMA is a more sensitive test and increases with approximately 25-40% of renal functional damage before creatinine and urea concentration (Yerramilli et al., 2016). The evolution of insult was found to increase the sensitivity of the urea and sCr, as verified by their correlation with SDMA, which was moderate at T1 and strong at T2, according to the identification of a decline in the GFR (Hall et al., 2016; Hokamp & Nabity, 2016; McKenna et al., 2020). These features highlight the possibility of using SDMA for the early detection of kidney injury.

According to the IRIS guidelines classification, an acute insult could be identified with a progressive increase of 0.3 mg/dL in sCR levels in non-azotemic animals within 48 h. This was observed in almost half of the cases (42.9%) within 24 h, suggesting the presence of AKI. Laboratory changes in urinary and serum biomarkers (SDMA) indicate clinical variation but do not constitute a direct parameter for the staging of AKI (Cowgill, 2016).

A decrease in uSG is expected in dogs with pyometra due to bacterial endotoxins, mainly *E. coli,* which promote insipid nephrogenic diabetes by competing with renal cell receptors (Oliveira, 2015). This condition can be aggravated due to reduced urinary concentration capacity, resulting from tubular damage caused by inadequate local resorption and the development of polyuria and compensatory polydipsia (Dibartola, 2000). However, the uSG of dogs did not show a significant decline, and the results differed from studies that observed an average of 1.016 uSG in canine pyometra (Heiene et al., 2007; Maddens et al., 2011). However, these studies included dogs at different disease stages, including azotemic cases. Such cases were not included in the current study, suggesting that the reduction in uSG observed in pyometra occurs when the diagnosis is made in advanced disease stages.

One dog developed progressive azotemia with a marked reduction in uSG between T1 and T2 (T0=1.040, T1, and T2=1.016), whereas a slight increase in uGGT and uGGT/uCr was observed in the absence of proteinuria. SDMA showed progressive growth, and granular casts were evident, proving to be a valuable marker of functional and glomerular lesions. This dog probably evolved to a more advanced AKI stage, where the excretion of enzymes and proteins may be reduced by progressive tubular damage and microalbuminuria, respectively. In this way, RPC can indicate values within the reference range for the species (Grauer, 2016), and tubulopathy can be reinforced by the inability to concentrate urine between time points

(Gori et al., 2019). Besides, the increase in ALT (T0=19.9, T1=20.37, and T2=283.43 U/L) and ALP (T0=63, T1=218, and T2=481 U/L) was successive in the dog, which may indicate worsening of sepsis through inadequate local perfusion and associated lesions in hepatocytes due to the presence of endotoxemia (Plavec et al., 2006).

The evolution of pyometra associated with delayed clinical intervention could aggravate the renal injury, despite the absence of evidence on ultrasound and high values and progressive elevation (from T0) in the levels of serum biomarkers demonstrating alterations. Renal and hepatic complications, aggravated by sepsis, culminated in the dog's death 25 days after being diagnosed with pyometra (T0). This shows the importance of regularly monitoring dogs with pyometra to assess acute insult or chronic damage and urinary markers to diagnose elevated concentrations up to 60 days after OH (Figueiredo et al., 2017).

Pyometra in female dogs is associated with a decreased GFR, which causes a loss of renal perfusion. Renal proteinuria may have different origins, and renal histopathological studies have found a variable extension of the tubulointerstitial lesion associated with pyometra (De Loor et al., 2013; Heiene et al., 2007; Maddens et al., 2010). The damage of tubular origin is significant due to its disposition in the cortical region with 90% blood irrigation and, consequently, greater vulnerability to toxins and cellular ischemia. Thus, tubular markers are more efficient in acute kidney damage (De Loor et al., 2013).

The persistence of proteinuria was identified up to T1, and there was a significant reduction at T2 in the postoperative phase, which was usually observed after pyometra treatment. However, in cases of persistent severe proteinuria, this may indicate kidney disease (Hagman, 2014). After pyometra surgery, monitoring of proteinuric dogs is suggested to optimize their care (Maddens et al., 2011). Animals with three sequential measurements of RPC ≥0.5 at two-week intervals without recognition of non-renal proteinuria could have a chronic glomerular or tubulointerstitial injury (Lees et al., 2005).

The statistical comparison of SDMA did not identify any difference between the time points. However, at least 21.4% (3/14) of the dogs showed increased uGGT and uGGT/sCr levels at T0. However, the increase in SDMA preceded the rise in sCr and urea levels at T0. It was concurrent with the elevation of RPC, uGGT, and uGGT/sCr levels, considered adequate urinary biomarkers of renal damage before azotemia development (Lippi et al., 2018; Sant'Anna et al., 2019).

The uGGT/uCr assessment was like the SDMA assessment. It remained elevated throughout the study, supporting a possible kidney injury and indicating a potential marker of tubular damage (Perondi et al., 2019), as the elevations preceded the increase in sCr level, decrease in GFR or uSG level, and appearance of cylinders in the urine analysis (Pressler, 2015).

A marked reduction in the average uGGT concentration and uGGT/uCr ratio observed between T1 and T2 was by the gradual drop reported 12 days after the surgical procedure (De Loor et al., 2013) by considering the influence of anesthesia and surgical procedures on hypotension followed by ischemia and renal hypoxia (Sear, 2005). Therefore, tubular cell damage is attenuated after the implementation of treatment, which suggests an interaction between urinary markers and the local reduction of insults and acute changes associated with pyometra (Heiene et al., 2007).

Elevated uGGT/uCr are associated with the severity of proximal tubule injury in female dogs with pyometra (Heiene et al., 2001). The decrease in proteinuria and levels of uGGT and uGGT/uCr was concomitant with the reduction in urinary casts identified in the positive dogs at T0 and T1. This contributes to the findings of renal insults since the presence of a granular cast which is common in tubular damage, and its increase highlights the magnitude of AKI (Figueiredo et al., 2017).

Although the study is about a reproductive disease commonly in female dogs and naturally acquired, some limitations were found in the work. The difficulty in obtaining the number of animals included, and the standardization in age groups is because of the high percentage of older adults with associated comorbidities. Other limitations were the animal's attendance at clinical follow-ups, with the impossibility of reassessing and measuring parameters in five animals at the indicated time (T2). However, the stability of the animals was reported. However, it does not rule out the possibility of the perpetuation of ARI in these individuals, which can lead to chronic kidney damage in the long term. Further studies should be conducted to analyze the urine culture in a female dog with pyometra at the time of diagnosis. This was not portrayed in the present study due to insufficient urine during abdominal ultrasound (T0). After all, analyses of urinary biomarkers were prioritized as one of the purposes of this study.

Conclusion

In conclusion, biomarkers of renal tubules predominated in identifying lesions suggest discrete tubular damage and are transient in female dogs with pyometra. In the

functional evaluation, uSG did not perform well as an early marker. However, SDMA was helpful for the initial detection of GFR reduction and could be used to evaluate pyometra in renal damage. Besides, uGGT and uGGT/sCr can also be assessed for up to 15 days after OH to monitor the persistence of tubular insults after pyometra treatment. Further studies should be conducted to define the role of SDMA as an early renal biomarker in the various stages of AKI associated with canine pyometra.

Conflict of Interest

There is no conflict of interest.

Ethics Statement

The Ethics Committee approved this study on the Use of Animals at the Federal University of Alagoas (UFAL) under protocol 078/18.

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