

Original research article

UDK 636.7.09:616.993.161:577.21

<https://doi.org/10.46784/e-avm.v16i2.327>

## EVALUATION OF PHYSIOLOGICAL BIOMARKERS AS POSSIBLE PREDICTIVE FACTORS AND PROGNOSIS MARKERS OF KIDNEY INJURY IN DOGS NATURALLY INFECTED WITH *LEISHMANIA INFANTUM*

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### Abstract

Impaired renal function is one of the main characteristics of dogs infected with *Leishmania infantum*. Early diagnosis of kidney injury is essential for improving patient's prognosis. This study aims to evaluate physiological biomarkers as predictors of kidney injury and prognostic markers. Medical records of fifty-nine dogs of different breed, age, and sex, naturally infected with *L. infantum*, were analyzed. Red blood cells, leucocytes, platelets count, hematocrit, total plasma proteins, plasma globulin, plasma albumin, serum creatinine, serum urea, serum phosphorus, serum symmetrical dimethyl arginine, urine analysis, urinary density, urinary protein creatinine ratio, urinary creatinine, urinary protein, and systemic blood pressure were examined in trial 0. Six months after trial 0, twenty-four dogs returned for clinical and laboratory examination. The second medical record analysis was identified as trial 1. The twenty-four dogs were examined using the same tests performed in trial 0. The physiological biomarker such as platelets and leukocyte count, hematocrit, serum phosphorus, urinary density, and systemic blood pressure, showed a significant correlation as prognostic and predictive factors of kidney injury in dogs. The platelet count was used as the physiological biomarker to show the value as a predictive factor and prognostic marker related to biomarkers of kidney injury in dogs naturally infected with *L. infantum*.

**Key words:** acute kidney injury, CanL, survival

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## EVALUACIJA FIZIOLOŠKIH BIOMARKERA KAO MOGUĆIH PREDIKTIVNIH FAKTORA I PROGNOŠTIČKIH MARKERA OŠTEĆENJA BUBREGA KOD PASA PRIRODNO INFICIRANIH SA LEISHMANIA INFANTUM

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### Kratak sadržaj

Oštećena bubrežna funkcija je jedna od glavnih karakteristika pasa zaraženih sa *Leishmania infantum*. Rana dijagnostika oštećenja bubrega je od suštinskog značaja za bolju prognozu stanja pacijenta. Ova studija ima za cilj da proceni fiziološke biomarkere kao prediktore oštećenja bubrega i prognostičke markere. Izvršena je analiza medicinske dokumentacije za pedeset i devet pasa različitih rasa, starosti i pola, prirodno zaraženih sa *L. infantum*. Crvena krvna zrnca, leukociti, broj trombocita, hematokrit, ukupni proteini plazme, globulini u plazmi, albumin u plazmi, serumski kreatinin, serumska urea, serumski fosfor, serumski simetrični dimetil arginin, analiza urina, gustina urina, odnos urinarnog proteina i kreatinina, kreatinin u urinu, urinarni protein, i sistemski krvni pritisak ispitivani su kao parametri nultog ispitivanja. Šest meseci nakon nultog ispitivanja, dvadeset i četiri psa su se vratila na klinički i laboratorijski pregled. Podaci od drugog pregleda su označeni kao ispitivanje 1. Dvadeset četiri psa su pregledana korišćenjem istih testova izvršenih u ogledu „0“ (nultog ispitivanja). Fiziološki biomarkeri kao što su broj trombocita i leukocita, hematokrit, fosfor u serumu, gustina urina i sistemski krvni pritisak, pokazali su značajnu korelaciju kao prognostički i prediktivni faktori oštećenja bubrega kod pasa. Broj trombocita je korišćen kao fiziološki biomarker da pokaže vrednost kao prediktivni faktor i prognostički marker koji se odnosi na biomarkere oštećenja bubrega kod pasa prirodno inficiranih *L. infantum*.

**Ključne reči:** akutno oštećenje bubrega, lajšmanioza pasa, preživljavanje

## INTRODUCTION

Visceral leishmaniasis (VL) is an infectious, systemic, and zoonotic disease caused by the protozoan *Leishmania infantum* and is transmitted by infected female sandflies, *Lutzomyia longipalpis*, and its main vector is in Brazil (Dantas-Torres and Brandão-Filho, 2006). It is estimated that there are millions of infected dogs in South America, especially in Brazil, where there are high rates of infection (Marcondes and Day, 2019)

Infected dogs can develop canine leishmaniosis (CanL), which can affect several organs and therefore exhibit several clinical characteristics, ranging from apparently healthy to severe illness and death, depending on the immune response triggered by the patient (Soares et al., 2005; Freitas et al., 2012)

Renal function impairment is quite frequent among the clinical changes observed in infected animals (Braga et al., 2015). The formation and deposition of immune complexes secondary to CanL can cause glomerulonephritis and tubulointerstitial lesions (De Oliveira Frazilio et al., 2018). Some authors believe that tubulointerstitial lesions occur secondary to glomerulopathy (Pardo-Marín et al., 2017; De Oliveira Frazilio et al., 2018). Renal proteinuria, which is a reflection of increased glomerular capillary permeability, is associated with the production of immune complexes and may be evidence of kidney injury (D'Amico and Bazzi, 2003; Soares et al., 2005; Freitas et al., 2012; Brown et al., 2013; IRIS 2023). Treatments for CanL in Brazil are based on miltefosine for leishmanicidal action, immunomodulatory medications such as domperidone, and immunotherapy, which consists of three double doses of the Leishtec<sup>®</sup> vaccine with intervals of twenty-one days between applications. Subsequently, a six-monthly booster should be administered with the application of a double dose. Immunotherapy was a good and efficient protocol for reducing clinical manifestations and controlling CanL relapses compared to other treatments (Santos et al., 2007; Ribeiro et al., 2013; Araujo and Gondim, 2020).

This study aims to evaluate physiological biomarkers as predictive and prognostic markers of the evolution of infection, renal injury, and renal failure in dogs naturally infected with *L. infantum*, assisting in the determination of treatment procedures, managing possible alterations identified, and in the prognosis of patients.

## MATERIAL AND METHODS

For the retrospective data collection, no ethical approval was required as no identifying information was included, and no dogs were actively recruited.

Medical records of fifty-nine dogs of different breeds and ages naturally infected with *L. infantum* were examined. Out of the fifty-nine dogs studied, twenty-six were female dogs, while thirty-three were males. The dogs were classified and divided into stages as suggested by Solano-Gallego et al. (2011). The clinical staging of canine visceral leishmaniasis is classified in four stages based on serological status, clinical symptoms and laboratory findings. Dogs in Stage I - Negative to low positive antibody levels; Dogs with mild clinical symptoms such as peripheral lymphadenomegaly, or papular dermatitis. Usually, no clinicopathological abnormalities were observed. Normal renal profile was the following: creatinine <1.4 mg/dl; non-proteinuric: UPC < 0.5. Stage II - Low to high positive antibody levels; Dogs, which apart from the signs listed in stage I, may exhibit the following symptoms: diffuse or symmetrical cutaneous lesions such as exfoliative dermatitis / onychogryphosis, ulcerations (planum nasale, footpads, bony prominences, mucocutaneous junctions), anorexia, weight loss, fever, and epistaxis; Clinicopathological abnormalities such as mild non-regenerative anemia, hyperglobulinemia, hypoalbuminemia, serum hyperviscosity syndrome. Substages: a) Normal renal profile: creatinine < 1.4 mg/dl; non-proteinuric: UPC < 0.5 b) Creatinine <1.4 mg/dl; UPC = 0.5-1,0. Stage III - Medium to high positive antibody levels; Dogs, which apart from the symptoms listed in stages I and II, may show the signs originating from immune-complex lesions: vasculitis, arthritis, uveitis, and glomerulonephritis. Clinicopathological abnormalities listed in IRIS stage II of chronic kidney disease (CKD), creatinine 1.4-2,8 mg/dl, or stage I with UPC > 1. Stage IV - Medium to high positive antibody levels; Dogs with clinical symptoms listed in stage III. Pulmonary thromboembolism, or nephrotic syndrome (marked proteinuria UPC > 5) and final stage renal disease; Clinicopathological abnormalities listed in IRIS stage II CKD, stage III (creatinine 2,9-5mg/dl) and stage IV (creatinine >5 mg/dl).

The dogs from stage II and those undergoing treatment with immunotherapy (do Nascimento et al, 2018; Gouveia et al, 2021) as suggested by Brasileish (2019) were included. Medical records should also present parasitological examinations for *Leishmania sp.* identification by direct or molecular identification or titration above 1:160 in the indirect Fluorescent Antibody Test (IFAT) or sample value four times higher than the cut-off point in the Enzyme-Linked Immunosorbent Assay (ELISA) (Brasileish, 2019; Solano-Gallego et al., 2011; Laia et al., 2011) at trial 0 (T0). Eighteen (30.5%) dogs were classified as stage IIa (Normal renal profile: creatinine < 1.4 mg/dl; non-proteinuric: UPC < 0.5), fifteen (25.4%) as stage IIb (Creatinine <1.4 mg/dl; UPC = 0.5-1), twenty-three (39%) in stage III, and three (5.1%) dogs were classified as stage IV (Solano-Gallego, et al., 2011).

Also, medical records should present complete blood count tests: red blood cell count (RBC), hematocrit (HC), leucocytes (LE) and platelets (PL), total plasma proteins (PROT), plasma globulin (GL), plasma albumin (AL), serum creatinine (CR), serum urea (UR), serum phosphorus (PH), serum symmetrical dimethyl arginine (SDMA), urine analysis, urinary density (UR. D), urinary protein creatinine ratio (UPC), urinary creatinine (CR. U) and urinary protein (UR PTN), all performed in the exact consultation, in the same laboratory. Systemic blood pressure (SBP) was measured using vascular doppler or through the oscillometer method with the animal positioned in lateral decubitus, in line with criteria for cuff size and minimum stress. Three to seven measurements were performed, and the first measurement was discarded to obtain an average of the other measurements, considering the patient's SBP value.

Animals that showed signs of dehydration, episodes of vomiting, diarrhea, inappetence, or lower urinary tract disease were excluded from the study. This first moment is identified as T0. Six months after T0, twenty-four dogs were returned for clinical and laboratory evaluation. The second medical record analysis was identified as trial 1 (T1). These twenty-four dogs were clinically healthy and evaluated through the same exams performed in the same laboratory in T0. Thirty-five dogs were not returned for evaluation or returned for more than six months and were therefore excluded from the study.

### ***Statistical Analysis***

Principal component analysis (multivariate analysis) was performed using numerical variables. This analysis evaluated all numerical variables, considering their correlation, and was performed using Spearman's correlation matrix. Moderate, strong, and robust correlations were reported, with confidence intervals above 95%. Statistical analyses were performed using the R software version ("The R Project for Statistical Computing," 2019).

## **RESULTS**

### ***T0***

The distribution of the dogs evaluated in this study is shown in Figure 1. The arrows facing the same direction indicate biomarkers that are positively correlated with each other. The points in the two quadrants on the right showed higher values for CR, UR, SDMA, PH, UPC, and UR. PTN. In these

quadrants there were higher concentration of dogs classified in stages III and IV, whereas those in the two left quadrants, mostly dogs classified in stages IIa and IIb, had higher values mainly for CR, U, HC, AL, and UR. D. The points in the two upper quadrants had higher PL counts. On the other hand, the points in the two lower quadrants had higher values, mainly for PROT and GL. There was a relative separation in the group IIa, with animals concentrated on the left side of the graph, and group IV, with animals focused on the right side. The chart shows the dispersion and evolution of dogs from T0 to T1. The dogs classified as stage IIa or IIb were in the left quadrant. The dogs in stages III and IV were in the right quadrant, with high levels of CR, UR, SDMA, PH, and UPC, demonstrating that patients classified in the final stages have a greater impairment of the glomerular filtration rate.

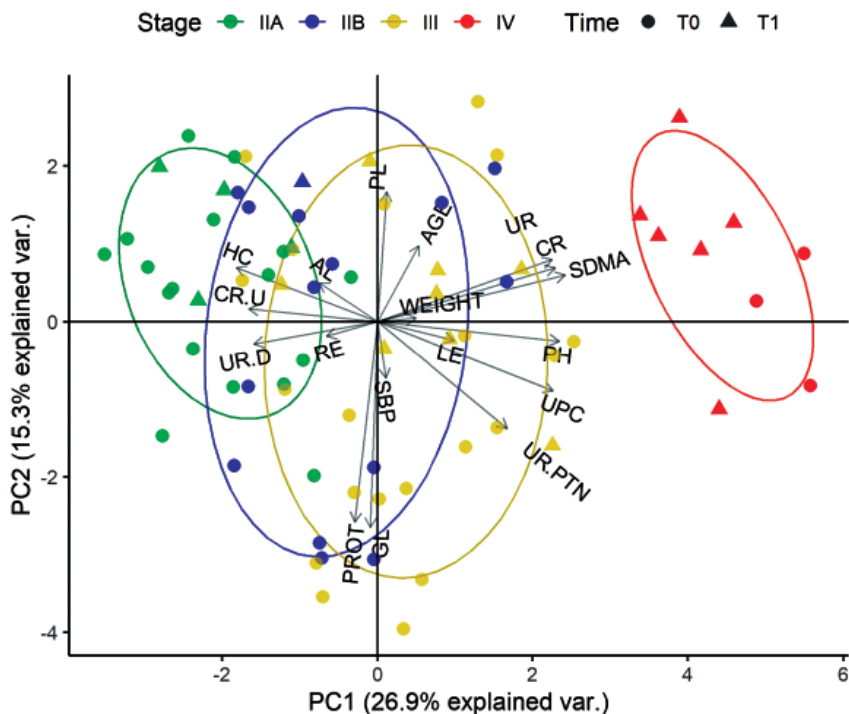


Figure 1: Dispersion of dogs considering the staging and the values obtained in the analytes studied at T0 and T1. The points in the two quadrants on the right showed higher values for CR, UR, SDMA, PH, UPC, and UR. PTN, whereas those in the two left quadrants showed higher values mainly for CR, UR, HC, AL, and UR. D. The points in the two upper quadrants had higher PL counts, while the points in the two lower quadrants had higher values, mainly for PROT and GL.

Anemia was found in twenty-one (35%) dogs out of fifty-nine analyzed in the study. Two (11%) of the dogs were classified as stage IIa, five (33%) as stage IIb, 11 (47%) in stage III, and three (100%) in stage IV present anemia.

Thrombocytopenia was present in seventeen (28%) dogs out of fifty-nine evaluated in the study. Three (16%) of the dogs classified as stage IIa, four (26%) in stage IIb, seven (30%) in stage III, and three (100%) in stage IV had thrombocytopenia.

Out of the thirty-three dogs at T0, classified by Solano-Gallego et al. (2011) IIa and IIb stages evaluated in this study, in thirteen (39%), UPC was the first biomarker to assess kidney injury to have the values higher than reference values. SDMA was the first evaluated biomarker with values higher than reference values in two dogs (6%). SDMA and UPC levels increased simultaneously in two dogs (6 %) classified by Solano-Gallego et al. (2011) IIa and IIb stages.

SDMA had the values above the reference ( $> 18 \mu\text{g/dL}$  IRIS, 2023) in one (5%) dog classified as stage IIa and in four (26%) dogs classified as stage IIb. In stage III, there was an increase in SDMA in eight (34%) dogs, and in stage IV in three (100%) dogs.

Analyzing correlations at T0, they were found to be moderate with values varying between 0.4 and 0.69, and strong between 0.70 and 0.89. SDMA had a moderate positive correlation with UR and CREAT and a moderate negative correlation with HC. UR levels had a strong positive correlation with serum CR levels and a moderate positive correlation with PH levels. PH showed a reasonable positive correlation with UPC and a moderate negative correlation with RBC and HC. PROT levels showed a moderate negative correlation with the PL count. AL levels had a moderate positive correlation with HC and RBC, which was negatively correlated with UPC and UR. PTN levels. UR. D showed a moderate correlation with CR.U.

### ***Comparisons between T0 X T1***

The correlation of biomarkers evaluated at T0 and T1 is shown in Table 1. When correlations were evaluated at T0 and T1, PL (T0) had a negative correlation with UPC and UR. PTN levels at T1. There was no correlation between PL and UPC in T0. The UR. D (T0) and UPC (T1) showed a strong negative correlation. LE (T0) had a moderate correlation with UR (T1). PH (T0) had a moderate correlation with UR. PTN.

In the analysis of the correlation between the variables in T0 and the variables of dogs with CR lower than 1.4 mg/L in T1 is shown in Table 2. UR D (T0) had a negative correlation with the UPC (T1) and the GL (T1). SDMA

(T0) was correlated with CR. U level (T1). PL (T0) correlated with UR and PH levels (T1). UR. PTN (T0) levels are associated with PROT levels (T1). PH and SBP (T0) were correlated with LE (T1). It was not possible to perform the analysis for animals with serum CR equal to or greater than 1.4 mg/L, as due to an insufficient number of dogs, it is not possible to get a basis for interpretable analysis.

Table 1: Correlations between the biomarkers studied at T0 and T1 that were statistically significant.

BIOMARKER	TRIAL 0	BIOMARKER	TRIAL 1	CORRELATION	P
CR.U	T0	UR.D	T1	0.73	0.003
GL	T0	LE	T1	0.48	0.019
CR.U	T0	UR. PTN	T1	-0.71	0.023
PL	T0	UPC	T1	-0.69	0.023
PL	T0	UR. PTN	T1	-0.69	0.026
PROT	T0	GL	T1	0.45	0.027
UR. D	T0	UPC	T1	-0.69	0.027
SDMA	T0	CR. U	T1	0.68	0.029
CR. U	T0	UPC	T1	-0.68	0.03
LE	T0	UR	T1	-0.44	0.031
PROT	T0	LE	T1	0.43	0.031
PL	T0	LE	T1	-0.43	0.036
PH	T0	UR. PTN	T1	0.66	0.036
HC	T0	PTOT	T1	-0.42	0.043
UR. PTN	T0	UPC	T1	0.65	0.043
PL	T0	PH	T1	-0.41	0.048
SBP	T0	LE	T1	0.41	0.049

Hematocrit (HC), leucocytes (LE) and platelets (PL), total plasma proteins (PROT), serum globulin (GL), serum urea (UR), serum phosphorus (PH), serum symmetrical dimethyl arginine (SDMA), urine analysis, urinary density (UR. D), urinary protein creatinine ratio (UPC), urinary creatinine (CR. U), urinary protein (UR PTN) and systemic blood pressure (SBP).



Table 2: Correlation of the variables in T0 together with the variables of dogs that present creatinine lower than 1.4 mg/L at T1 that presented statistically significant.

BIOMARKER	TRIAL 0	BIOMARKER	TRIAL 1	CORRELATION	P
UR. D	T0	UPC	T1	-0.92	0.001
SDMA	T0	CR. U	T1	0.92	0.001
PL	T0	UR	T1	-0.59	0.013
UR. D	T0	GL	T1	0.57	0.016
SBP	T0	LE	T1	0.56	0.018
PL	T0	PH	T1	-0.55	0.022
PH	T0	LE	T1	0.53	0.03
UR. PTN	T0	PROT	T1	0.5	0.042

Hematocrit (HC), leucocytes (LE) and platelets (PL), total plasma proteins (PROT), serum globulin (GL), serum urea (UR), serum phosphorus (PH), serum symmetrical dimethyl arginine (SDMA), urine analysis, urinary density (UR. D), urinary protein creatinine ratio (UPC), urinary creatinine (CR. U) and urinary protein (UR PTN) and Systemic blood pressure (SBP).

## DISCUSSION

The importance of evaluating coinfections in dogs infected with *L. infantum* is well known. Infections with *Babesia canis*, *Anaplasma platys*, *Hepatozoon canis* and *Ehrlichia canis* cause hematological alterations such as anemia, pancytopenia, and thrombocytopenia (Thrall, 2007; Vilela et al., 2013), which may change the values of laboratory tests in the studied dogs. The lack of this evaluation presents a limitation of this study.

Changes in the erythrogram of dogs infected with *L. infantum* were observed in this study, in the form of mild to moderate anemia and thrombocytopenia. However, the pathogenesis of anemia in CanL includes additional mechanisms such as reduced erythropoietin synthesis due to renal failure (Paltrinieri et al., 2016). Anemia may also result from the parasite's invasion of the bone marrow, which induces inflammation that may contribute to a decrease in erythrocyte production (Da Costa-Val et al., 2007). Anemia is usually present in normocytic and normochromic forms with a non-regenerative character (Freitas et al., 2012). Thrombocytopenia is a common finding in dogs with CanL and occurs due to vasculitis caused by circulating immune complexes,

thrombocytopoiesis disorders, and PL destruction (Solano-Gallego L., et al., 2009; De Carvalho Nicolato et al., 2013).

Hyperproteinemia is caused by hyperglobulinemia, which is associated with the activation of B lymphocytes and high antibody production (Freitas et al., 2012; Paltrinieri et al., 2016; Maia and Campino, 2018). A decrease in the production or renal loss of AL was also observed in this study, but the increase in GL levels was very expressive, causing the levels of PROT to increase significantly during CanL (Giunchetti et al., 2008; Solano-Gallego, et al., 2009). As for urinalysis, in the present study, proteinuria was the most frequent alteration in animals with CanL to both mild or severe degrees, as described by Amusategui et al. (2003) and Bonfanti and Zatelli (2004).

Glomerulonephritis, tubulointerstitial nephritis, and nephropathy are common in CanL (De Oliveira Frazilio et al., 2018). Initially, glomerulonephritis may manifest as asymptomatic proteinuria (Koutinas and Koutinas, 2014; Paltrinieri et al., 2016), but with its progression, excretion dysfunction can occur in the presence of an increase or decrease in the glomerular filtration rate (GFR) and systemic hypertension (Plevraki et al., 2006; Cortadellas et al., 2008). An increase in GFR associated with hypertension can amplify glomerulopathy, resulting in the progression of CKD (Koutinas and Koutinas, 2014). End-stage CKD is a severe manifestation of disease progression and the leading cause of death in CanL. The two main parameters used to classify the degree of kidney disease in CanL, according to (Solano-Gallego et al., 2011; Laia et al., 2011), are UPC, used as a marker of glomerular pathology, and serum CR level, used as a marker of renal excretion (Torrent et al., 2018).

In 2019, IRIS included SDMA levels in the CKD staging of chronic kidney disease. SDMA is methylated arginine produced by cellular catabolism correlated with CREAT and GFR (Nabity et al., 2015). Several studies have demonstrated that SDMA levels are elevated in dogs with CKD (Hall et al., 2014; Nabity et al., 2015). Studies have suggested that SDMA levels also increase earlier than serum CR levels (Hall et al., 2014; Nabity et al., 2015) because its production is not influenced by the loss of muscle mass. SDMA showed a moderate correlation with UR and PH in T0. An increase in SDMA, UR, PH, and CR and a reduction in UR is expected as there is a worsening of renal excretion and a decrease in TGF.

There is a paucity of data on the behavior of SDMA in dogs naturally infected with *Leishmania* sp., with only four studies reported to date (Pardo-Marín et al., 2017; De Oliveira Frazilio et al., 2018; Torrent et al., 2018; Giapitzoglou et al., 2020). The serum concentration of SDMA is elevated mainly in azotemic dogs with severe proteinuria and decreased UR. D (Giapitzoglou et

al., 2020). SDMA correlated with CR and UR. D, a result like that obtained by (Giapitzoglou et al., 2020). A study carried out by (Torrent et al., 2018) found different results, without observing a correlation between SDMA and CR.

SDMA was also inversely correlated with the red RBC and HC. Kidneys perform numerous metabolic functions, including their contribution to erythropoiesis. Several factors contribute to anemia in patients with reduced GFR and CanL. The reduction of erythropoietin of renal origin is an important cause, but other mechanisms, such as anemia of inflammatory origin, the influence of uremic toxins on the survival time of the erythroid lineage, cofactor deficiency, blood loss due to various bleeding events, and parasitic destruction (Babitt and Lin, 2012; Fiocchi et al., 2017; Lippi et al., 2021).

There was a significant and progressive increase in the number of dogs with SDMA levels above the reference values (IRIS, 2023) as the stage progressed. The expected result of disease progression between stages demonstrated that SDMA could be used to classify CanL severity (Giapitzoglou et al., 2020). A different result was found by Torrent et al. (2018), who found no significant difference in the comparison between the stages (Solano-Gallego et al., 2011).

In this study, two dogs classified in stage IIa (non-proteinuric and non-azotemic) with elevated SDMA levels were observed. (Cortadellas et al., 2008) pointed out that dogs with CanL and UPC levels between 0.2 and 0.5 may have a decrease in GFR. Dogs with CanL may have some renal perfusion impairment secondary to hypovolemia, which could cause an increase in SDMA concentration without an association with proteinuria or renal azotemia. Torrent et al. (2018) suggested that dogs with CanL and changes in SDMA concentration without proteinuria should be carefully examined for pre-renal causes of impaired renal perfusion.

UPC was shown to be an earlier diagnostic sign of acute kidney injury (AKI) concerning SDMA in dogs naturally infected with *L. infantum* observed in this study, which is in line with other studies (Pardo-Marín et al., 2017; Torrent et al., 2018; Giapitzoglou et al., 2020). Glomerulonephritis is the main pathological event of nephropathy in CanL, and proteinuria is the primary laboratory alteration. However, glomerular pathology can cause a reduction in GFR and an increase in the serum concentrations of SDMA and CR (Koutinas and Koutinas, 2014; Paltrinieri et al., 2016)

When evaluated as prognostic markers and predictors of kidney injury and kidney failure, the UPC and SDMA levels did not show a statistically significant correlation. Pardo-Marín et al. (2017) identified a reduction in the concentration of UPC, however, they did not observe a decrease in the SDMA levels in dogs after leishmanicidal treatment. In this study, we observed a cor-

relation between SDMA (T0) and CR. U (T1) levels; however, as the dogs examined in this study underwent a treatment with immunotherapy, an improvement in GFR may have occurred after the reduction of the injury caused by CanL. Gouveia et al, 2021 found that immunotherapy with vaccines was the most effective treatment for negative serology in the ELISA and IFAT tests after the treatments compared to other treatments in the study. The decrease in the result in the serology may suggest a reduction in the production of antigen-antibody complexes influencing the GFR.

An increase in PROT and GL levels, associated with a decrease in AL levels, has been observed in several studies (Artan et al., 2006; Sales et al., 2017). This increase in PROT and the presence of hyperglobulinemia are considered to be among the most common alterations in CanL and may be associated with increased levels of anti-*Leishmania* antibodies, especially in severe stages of the disease (Castro et al., 2012; Freitas et al., 2012). However, hypoalbuminemia observed in animals may be due to the migration of albumin to the extravascular environment, with the formation of edema, a widespread clinical change in CanL (Silva, 2007; Freitas et al., 2012) and/or associated with an inflammatory process and/or albuminuria (Pierantozzi et al., 2013; Proverbio et al., 2016). Given the processes that occur during CanL due to the association of GL with the stimulation of B lymphocytes (Vieira et al., 2021), we can explain why the serum concentration of GL and PROT (T0) had a significant correlation with LE (T1). AL levels did not significantly correlate with any of the biomarkers.

The development of glomerulopathies not only leads to complications resulting from the accumulation of uremic toxins and fluid and electrolyte imbalances, but also causes systemic arterial hypertension, aggravating the clinical picture of patients and possibly compromising other organs, such as the heart (Schiffrin et al., 2007). In this study, seventeen (28%) dogs were identified with SBP above 160 mmHg. Out of these, seven (41%) were non-azotemic and non-proteinuric. Cortadellas et al. (2008) observed that 49.5% (52 / 105) of the dogs had some degree of renal disease, and 61.5% (32 / 52) of these dogs were diagnosed with systemic hypertension (SH). Moreover, SH also was diagnosed in 2% of dogs without renal disease. Braga et al. (2015) found that all dogs with hypertension had histopathological and laboratory evidence of glomerular disease. However, there was no statistically significant correlation between elevated BP and the severity of glomerular lesions.

This study found that SBP (T0) correlated with LE count (T1). CanL glomerulonephritis can form large antigen-antibody complexes, inciting inflammation and overloading of glomerular capillaries, resulting in obstruction that can further elevate glomerular pressure (Harrison et al., 2012). There is also

evidence that inflammatory cells accumulate in the perivascular region of the kidney (Theuer et al., 2002) also contributing to hypertension.

Unlike SBP, data in the literature cite the destruction of LE in uremic patients (Minnaganti and Cunha, 2001; Cohen and Hörl, 2012; Pahl et al., 2010). In this study, predictive factors of LE (T0) and HC (T1) concerning UR were observed through an inverse correlation. Uremia is associated with hematological abnormalities such as hemostatic, granulocytic, lymphocytic, and PL disorders, mainly caused by chemotaxis, phagocytosis, and oxidative abnormalities. Some diseases related to antigen presentation have also been reported in uremic patients (Minnaganti and Cunha, 2001; Cohen and Hörl, 2012) demonstrated that patients with renal failure had impaired body defense. Pahl et al. (2010) reported that the number of B lymphocytes and their ability to produce antibodies was reduced in patients with uremia. A study conducted on dogs infected with *Leishmania sp.*, showed more intense hematological alterations, such as profound anemia, thrombocytopenia, and leukopenia, characterizing pancytopenia associated with bone marrow hypoplasia or aplasia as a result of the invasion of the microorganism and immune-mediated processes (Paltrinieri et al., 2016). Hyperplasia and peripheral cytopenia can partially be attributed to the increased destruction of mature blood cells in the periphery. There are also morphologic features indicative of differentiation blockage and dyserythropoietic changes in the erythroid precursors (Poulaki et al., 2021)

HC (T0) also showed an inverse correlation with serum PROT (T1). Thus, the presence of leukopenia and anemia may indicate an initial process of immunosuppression and an increase in parasite load, which may lead to a change in hyperglobulinemia, an initial process of renal injury, and disease progression.

PH concentrations were also moderately correlated with UR and PTN levels. A study demonstrated an essential relationship between PH and proteinuria. When analyzing the renal protective response, it was found that for a reduction in proteinuria, a reduction in protein intake was not necessary, but a reduction in PH, either in its urinary excretion or serum concentration. Patients with low PH levels had the most significant decrease in proteinuria, regardless of UR excretion (a factor used to estimate protein intake) (Di Iorio et al., 2013).

Based on all findings and discussion it can be concluded that the most associated parameters are PL and SBP. In order to interpret the results and analyze the predictive factors and prognostic markers, we analyzed Figure 1 and isolated four patients who presented a negative evolution, starting from classification in stage IIa at T0 to stage IV at T1. Two patients developed thrombo-

cytopenia and systemic arterial hypertension at T0. Neither of the patients had proteinuria, azotemia, or hyperglobulinemia and had SDMA < 14µg/dL. The authors pointed out the importance of evaluating systemic hypertension and thrombocytopenia in dogs with *L. infantum* infection. The other two patients exhibited no changes in laboratory test results at T0. New studies should be carried out in order to evaluate PL and SBP as predictive factors and prognostic markers.

## CONCLUSIONS

PL was the primary physiological biomarker to demonstrate value as a predictive factor and prognostic marker being related to biomarkers of kidney injury as PH and UPC. It has been shown that SBP, LE, HC, PH and UR. D are predictive or prognostic markers in dogs infected with *L. infantum*. There was no advantage of SDMA over UPC for assessing kidney injury in dogs infected with *L. infantum* at the time of the study. SDMA and UPC didn't show significance as predictive or prognostic markers in dogs infected with *L. infantum*. It is concluded that the evaluation of thrombocytopenia is important in the evaluation of CanL, and its reversal corresponds to an improvement in the prognosis.

## Author's Contributions

FSM and APCV: data collection, analysis and experimental design. They conducted the gathered the necessary data and performed statistical analysis on the results; assisted in data interpretation and manuscript preparation. They contributed equally to the interpretation of the experimental results, helped in drafting the manuscript, and critically reviewed the content for accuracy and clarity. JCCV and VMR contributed to the theoretical framework and literature review.

## Competing interest

The author(s) declare that they have no competing interests.

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Received: 03.06.2023.

Accepted: 20.11.2023.