

Vincristine sulfate treatment influence on kidney function of female dogs with transmissible venereal tumor

Influência do tratamento com sulfato de vincristina na função renal de cadela com tumor transmissível venéreo

Jessyca Vanderlei de Albuquerque Souza¹ ; Fernanda Danielle Maria Gonçalves² ;
Arnaldo Cesar Oliveira Gomes Lira-Junior¹ ; Pierre Barnabé Escodro¹; Diogo Ribeiro Câmara¹;
Marcia Kikuyo Notomi¹ 

¹ Universidade Federal de Alagoas, Departamento de Medicina Veterinária, Viçosa - AL, Brazil

² Centro de Diagnóstico Veterinário, Maceió – AL, Brazil

ABSTRACT

Chemotherapy agents have some undesirable and non-selective cytostatic effects. Considering that kidneys are vulnerable to drug-induced toxicity, this study evaluated renal injury caused by vincristine sulfate (VS) in 12 female dogs diagnosed with transmissible venereal tumor (TVT). The animals were treated with VS (0.025 mg/kg IV) every 7 days for 4 weeks. During treatment, the animals were subjected to clinical examination, blood count, serum measurement of symmetric dimethylarginine (SDMA), blood urea nitrogen (BUN), creatinine, alanine aminotransferase, and alkaline phosphatase. In addition, urinalysis and urinary gamma-glutamyl transferase (GGT) measurements were performed. All parameters were determined three times: before beginning the treatment (T0), after 14 days (T1), and after 28 days (T2). During the study period, there were no changes in serum urea or creatinine levels, urine specific gravity, or persistent proteinuria. Furthermore, urinary GGT measurement did not indicate tubular lesions, and consistent elevation of SDMA was found in only one patient above the reference range. The results showed that weekly therapy with VS as a single agent for 28 days does not induce renal injury in most cases.

Keywords: Chemotherapy. Gamma-glutamyl transferase. Renal injury. Renal marker. Symmetric dimethylarginine.

RESUMO

Os agentes quimioterápicos possuem efeitos citostáticos indesejáveis e não seletivos. Considerando a vulnerabilidade renal à toxicidade induzida por drogas, este estudo avaliou a lesão renal causada pelo sulfato de vincristina (VS) em 12 cadelas com diagnóstico de tumor venéreo transmissível (TVT). Os animais foram tratados com VS (0,025 mg / kg IV) a cada sete dias, durante quatro semanas. No transcurso do tratamento, os animais foram submetidos a exame clínico, hemograma, dosagem sérica de dimetilarginina simétrica (SDMA), nitrogênio ureico sanguíneo (BUN), creatinina, alanina aminotransferase e fosfatase alcalina. Além disso, foram realizadas análises de urina e medições de gama-glutamil transferase (GGT) urinária. Todos os parâmetros foram mensurados em três tempos, antes do início do tratamento (T0), aos 14 dias (T1) e aos 28 dias (T2). Durante o período do estudo, não houve alterações nas concentrações de ureia ou creatinina séricas, na gravidade específica da urina ou proteinúria persistente. Além disso, a medição de GGT urinária não indicou lesões tubulares, e elevação consistente de SDMA foi encontrada em apenas um paciente acima do intervalo de referência. Os resultados mostraram que a terapia semanal com VS como agente único por 28 dias não induz lesão renal na maioria dos casos.

Palavras-chaves: Quimioterapia. Gama-glutamil transferase. Lesão renal. Marcador renal. Dimetilarginina simétrica.

Correspondence to:

Márcia Kikuyo Notomi
 Universidade Federal de Alagoas, Departamento de Medicina
 Veterinária
 Fazenda São Luiz
 CEP: 57700-000, Viçosa – AL, Brazil
 e-mail: marcia.notomi@vicoso.ufal.br

Received: November 18, 2021

Approved: March 4, 2022

How to cite: Souza JVA, Gonçalves FDM, Lira-Junior ACOG, Escodro PB, Câmara DR, Notomi MK. Vincristine sulfate treatment influence on kidney function of female dogs with transmissible venereal tumor. *Braz J Vet Res Anim Sci.* 2022;59:e192646. <https://doi.org/10.11606/issn.1678-4456.bjvras.2022.192646>

Introduction

Despite advances in chemotherapy treatment, drug nephrotoxicity remains a complication and sometimes limits life-saving therapy, as acute kidney injury and electrolyte imbalance are the most common renal disturbance observed (Piscitani et al., 2020). Kidneys are vulnerable to drug-induced toxicity because of large blood flow, approximately 25% of cardiac, and as a consequence, nephrons can be affected by chemotherapeutic agents, inducing glomerular disease and tubulointerstitial damage (Shati, 2019).

Vincristine sulfate (VS) has been used in chemotherapy treatments for decades, especially for lymphocytic and lymphoblastic leukemia and lymphoma (López-Gómez et al., 2018). It is the most effective and practical therapy for canine transmissible venereal tumors (TVT) (Kanca et al., 2018). Although VS is well-tolerated and effective as a chemotherapeutic drug in dogs, it has some undesirable and non-selective cytostatic effects, especially in cases of TVT (Furini et al., 2014), which is affected by the immunity of the host (Kanca et al., 2018).

Most of the VS metabolites are eliminated in feces because of biliary excretion, and patients with reduced drug elimination due to increased alkaline phosphatase (ALP) are more likely to develop neurotoxicity associated with VS administration (Sajjad, 2012). About 8–15% of VS is excreted in the urine in an unchanged form. Studies in rodents related some histopathological changes in the renal tubules (degeneration, dilation, vacuolization, and necrosis of the proximal and distal convoluted tubules) and glomeruli (congestion, thinning, and detachment of the basement membrane), enhancing oxidative stress and inflammation which may lead to activation of apoptosis and

consequent cell death, thus confirming the VS nephrotoxic effect (Sajjad, 2012; Shati, 2019).

Renal function markers, such as urea and creatinine, are considered late markers because the glomerular filtration rate decrease is clinically detectable after substantial losses of nephrons, up to 50% or more, before azotemia development (Cianciolo et al., 2016). Studies evaluating urinary gamma-glutamyl transferase (GGT) concentration as an early renal marker have reported satisfactory results in detecting tubular injury (Perondi et al., 2019). Likewise, symmetric dimethylarginine (SDMA) is an early marker of glomerular filtration rate and superior to serum creatinine because it is unaffected by muscle loss, useful for the identification and monitoring of decreased renal function in dogs (Hall et al., 2014; Nabity et al., 2015). Therefore, this study aimed to evaluate the influence of VS chemotherapy on the renal function of female dogs with TVT.

Material and Methods

This study included 12 female mongrel dogs, aged 1–6 years, with compatible clinical signs and a positive cytological diagnosis of genital TVT. No changes were identified in regional lymph nodes or metastatic processes on routine clinical examination. The patient evaluation was performed three times: before beginning the chemotherapy treatment (T0), and after 14 (T1) and 28 (T2) days of its implementation. Anamnesis, physical examination, and laboratory tests were performed on all dogs. Animals with comorbidities suspected, before or during the experiment, were excluded from this study. The animals were evaluated weekly and received intravenous (IV) 0.025 mg/kg VS, according to Ganguly et al. (2016) and Kanca et al. (2018), totaling four applications during the experiment.

Automatized blood count was performed (Hematoclin 2.8 VET - Bioclin®), with differential cell counting and morphologic evaluation under optical microscopy; serum biochemical evaluation of urea, creatinine (CRE), ALP, and alanine aminotransferase (ALT) (SX-160 - Sinnova®) was performed using commercial kits (Bioclin®). An aliquot of serum (2 mL) was stored at -20 °C following the measurement of SDMA by Idexx Laboratories. Urine samples were collected and kept under refrigeration (2–4 °C, 4 h) until evaluation (chemical, physical, and sediment analysis). Urinary specific gravity was determined using a refractometer, and urinary GGT concentration was assessed using the modified Szasz technique, corrected based on urine gravity.

Data were analyzed using the following quantitative parameters: hematological, serum biochemical (ALT, ALP, urea, creatinine, and SDMA), urinary GGT, and urinary density. For each endpoint, univariate analysis of variance was used to compare the effect of various times (T0, T1, and T2).

Results

VS treatment (0.025 mg/kg) resulted in remission of clinical signs and negative cytology in 100% of the cases after four weekly administrations. Although 83.33% of the animals were rescued from the street or semi-domiciled, clinical changes were not observed during the study period, only discrete apathy and dysorexia, in addition to the genital tumor with bloody secretion. One animal presented with vasculitis and cutaneous ulceration after the third application, receiving topical treatment and presenting a good tissue repair process.

The hematological evaluation revealed mild normochromic normocytic anemia at the time of diagnosis (T0) in 42% of the animals (n = 5). In T1 and T2, it was identified in 50% (n = 6) of the cases. Furthermore, no differences were observed when comparing red series parameters (red blood cell count, hemoglobin concentration, and globular volume) and platelet count among various times (P > 0.05). A marked leukopenia due to neutropenia was shown with significant difference between T0 × T1 (P = 0.03; P = 0.02) and T0 × T2 (P = 0.02; P = 0.01), in leukocyte and neutrophil numbers, respectively. Thrombocytopenia was detected in 92% of the animals during treatment at least once (Table 1).

During chemotherapy treatment with VS, the values of ALT, ALP, urea, and creatinine (Figure 1) remained within the reference range during all times (Table 1), without

the influence of treatment at various times (P > 0.05). A reduction in urine-specific gravity was observed, from 1046 to 1037 at T0 and T2, respectively, without a statistical difference (Table 1). Five animals (42%) presented with 1+ to 3+ of protein during urine analysis, but only one animal was positive in all time-point evaluations, whereas the other four animals presented proteinuria once. Other urinary parameters were within the normal range.

Urinary GGT concentrations were corrected by urine specific gravity, observing normalized values and normal distribution (P > 0.05) at all times (Table 2). SDMA concentrations had a normal distribution (P > 0.05) at all times and within the reference range at the beginning of the study (T0). However, a non-significant elevation of SDMA from 2 to 6 µg/dL was observed in six animals (50%) at T1 when compared with T0. Despite this, only two animals (17%) had this elevation above the reference range in T1. In one of these patients, the SDMA concentration decreased again at T2, presenting a value similar to T0, and the second one had the SDMA concentration slightly increased in T1, and this elevation was maintained at T2, indicating a kidney injury. Individual variation of up to 6 µg/dL in SDMA concentration, with no clinical correlation, was observed during treatment with vincristine (Table 2).

Table 1 – Descriptive statistical analysis with a mean (X), standard deviation (SD), and minimum (Min) and maximum (max) values of the evaluated hematological and biochemical parameters of female dogs with compatible clinical signs and a positive cytological diagnosis of genital Transmissible Venereal Tumor, treated with vincristine sulfate, at the three evaluation times

	T0			T1			T2		
	X	SD	Min-max	X	SD	Min-max	X	SD	Min-max
He (x10 ⁶)	5.88	1.04	4.61-7.69	6.61	1.08	4.21-8.18	5.28	1.01	2.54-6.30
Hg (g/dl)	12.74	2.35	9.0-17.0	11.67	1.36	9.0-14.0	11.25	2.36	5.5-13.5
VG (%)	36.77	6.92	25.9-48.3	34.42	4.22	27.0-40.8	33.56	6.78	16.0-40.0
Leu (µL)	19.09 ^{a,b}	12.15	6.2-49.9	10.42 ^a	46.25	3.9-16.3	9.56 ^b	50.46	3.2-18.4
Neu (%)	13.49 ^{a,b}	11.49	5.12-44.91	5.00 ^a	2.96	1.51-9.54	4.39 ^b	2.85	1.28-9.94
Plat (x10 ³)	168.83	159.75	44-635	170.33	113.48	44-404	179.58	129.32	24-426
ALT (UI/L)	24.33	8.26	out/39	33.00	15.50	out/66	31.58	7.15	17-44
ALP (UI/L)	53.83	32.38	6-119	44.75	23.53	13-84	45.75	21.21	dez/93
BUN (mg/dL)	14.20	5.94	6.5-25.7	14.15	4.52	8.4-22.4	14.58	4.34	8.87-24.27
Creat (mg/dL)	0.79	0.23	0.42-1.16	0.79	0.30	0.45-1.37	0.79	0.26	0.47-1.4
SDMA (µg/dL)	10.58	1.88	jul/13	11.33	3.28	ago/18	11.17	3.24	7 – 19
GGT_u (UI/L)	29.7	19.09	10.3- 68.7	26.33	11.47	13.4-49.4	25.88	14.33	10.8-55.3
USG	1046.6	12.89	1024-1060	1040.0	16.62	1918-1078	1037.4	13.0	1014-1060

He: Red blood cells; Hg: hemoglobin, VG: globular volume; Leu: leukocytes; Neu: Neutrophils; Plat: platelets; ALT: Alanine Aminotransferase; ALP: alkaline phosphatase; BUN: blood nitrogen; Creat: creatinine; SDMA: symmetrical dimethylarginine; GGT_u: urinary gamma-glutamyl transferase; USG: urinary specific gravity.

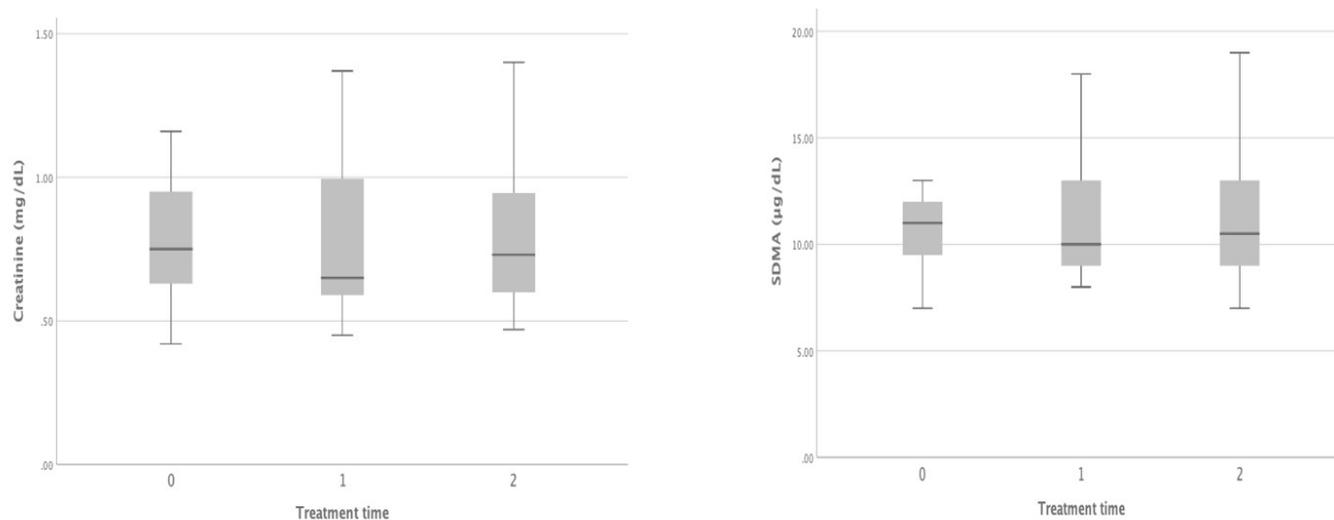


Figure 1 – Box-plot of creatinine concentrations and SDMA concentrations, maximum, minimum and mean representations, during vincristine sulfate treatment, of female dogs with compatible clinical signs and a positive cytological diagnosis of genital Transmissible Venereal Tumor, treated with vincristine sulfate, at the three evaluation times.

Table 2 – Serum creatinine, symmetric dimethylarginine (SDMA), and urinary GGT (GGTU) concentration of female dogs with compatible clinical signs and a positive cytological diagnosis of genital Transmissible Venereal Tumor, treated with vincristine sulfate, at the three assessment times

	Creatinine (mg/dL)			SDMA (µg/dL)			GGT _u (UI/L)		
	T0	T1	T2	T0	T1	T2	T0	T1	T2
1	0.98	0.55	0.61	9	13	11	21	44.4	32.6
2	1.16	1.29	1.03	11	17	11	15.1	16	13.5
3	0.72	1.37	1.4	7	9	7	23.6	26.5	21.7
4	0.73	0.6	0.73	13	11	9	24.1	25	19.3
5	1.08	1.05	0.92	11	9	14	19.6	13.4	18
6	0.77	0.65	0.72	12	10	8	10.3	17.1	10.8
7	0.5	0.7	0.55	8	9	10	46.7	20.5	18.1
8	0.57	0.58	0.59	13	10	9	50.4	30.8	39.7
9	0.69	0.65	0.75	12	18	19	13	49.4	45.8
10	0.91	0.63	0.73	10	9	13	68.7	33.5	55.3
11	0.42	0.45	0.47	11	13	13	50.4	15.5	12
12	0.92	0.94	0.97	10	8	10	14.5	23.9	23.8

Reference Value: Creatinine (0.5-1.6 mg / dL); SDMA (0-14 µg / dL); GGT_u (14-92 UI/L).

No statistical difference was identified when comparing the mean concentrations of SDMA (Figure 1).

Discussion

In canine TVT, therapeutic success is usually achieved after 2–8 VS injections in over 90% of cases (Kanca et al., 2018) In this study, four weekly applications were sufficient to achieve 100% remission in all patients. At the beginning of the study (T0), mild anemia identified in 42% of the animals is usually observed in TVT cases. However, a regenerative hypochromic microcytic anemia related to hemorrhagic processes was expected (Priyadarshini et al., 2021). Differing from this, normocytic normochromic anemia was observed due to a chronic inflammatory disease influence, which may have been aggravated by nutritional deficiencies (Cardozo et al., 2013) since some patients were stray dogs. No anemic dogs

at T0 developed a progressive decrease in blood cell count at T1 and T2, and this hematologic alteration plus the leukopenia with marked neutropenia are characteristics frequently reported in VS treatment for canine TVT (Braz & Marinho, 2021; Hantrakul et al., 2014; Kanca et al., 2018; Kumar et al., 2018). Chemotherapy has no selectivity, affecting both neoplastic and healthy tissues, causing cytopenia and even myelosuppression, which tends to be transient and fast recovered (O'Keefe & Harris, 1990). Vincristine is used for short onset improvement in platelet count, with high-dose steroids successfully applied to idiopathic thrombocytopenic purpura, unresponsive to conventional treatment in humans (Reynolds et al., 2019). Otherwise, mild thrombocytopenia was detected in 92% of the animals during treatment at least once, and an alteration was noticed in other studies with

vincristine therapy in TVT cases TVT (Hantrakul et al., 2014; Kanca et al., 2018; Kumar et al., 2018).

No significant changes in ALT and ALP concentrations were observed, indicating the absence of significant liver injury, corroborating with a study that only a transient increase in transaminases and rare hepatitis were reported after VS treatment (Grigorian & O'Brien, 2014). But in a similar study, a significant increase in liver enzymes was observed using the same drug, dose, interval, and species, with only a change in the duration of one more week (Kumar et al., 2018). Kidney injury was not identified by BUN and creatinine, whose concentrations remained within reference ranges at all times, indicating that in the case of a renal lesion, it cannot affect 75% of functional capacity. Otherwise, it would be detected if creatinine increased (Cianciolo et al., 2016). These results differ from those of Kumar et al., who identified a significant increase in urea and a slight increase in creatinine during chemotherapy of dogs with VS. Results from urine specific gravity tests present no significant gradual decrease over time (1046, 1040, and 1037), indicating a normal variation and ability to concentrate urine in 28 days.

Persistent proteinuria is an important sign of nephron disease. However, the discrete and intermittent proteinuria observed in this study could be related to preglomerular or post-glomerular causes, cardiovascular disease, tubular injury, and even early glomerular lesion⁸. As urinary GGT enzymatic activity is commonly measured in urine to diagnose nephrotoxic-induced acute renal injury and tubular cell damage (Smee et al., 2016), concentrations within the normal range at different times indicate the brush border integrity of the proximal tubule (Crivellenti et al., 2014).

Measuring SDMA, all animals presented concentrations within the reference range at T0, indicating the absence of renal function impairment. However, during the treatment with vincristine, the individual SDMA concentration presented an oscillation of up to 6 µg/dL at various times, with 83.33% of the animals within the reference values; a higher value than the Kopke et al. (2018) study, where SDMA critical difference (CD) was determined to be 1.34 µg/dL in normal dogs. CD represents the difference between two measurements from the same patient, considering a random variation rather than a true individual biological change, and despite the variation, the averages did not change in the time comparison. SDMA concentration elevation above the reference was detected in two animals. One dog returned to normal SDMA value on the next evaluation time, similar to other animals that oscillated within the reference. This variation may be due to a renal compensatory mechanism, in which the kidney uses prostaglandins vasodilatory action on afferent arterioles, increasing renal perfusion, and improving blood flow from the cortex to nephrons in

the medullary region (Lucas et al., 2019). Another patient showed a significant increase in SDMA concentration over time, characterized by the presence of renal injury without creatinine increase or urine specific gravity reduction, in agreement with Abrams-Ogg et al. (2017), who observed an increase in SDMA concentration with normal creatinine levels in dogs with lymphoma treated with VS in combination with other chemotherapeutic agents.

Not all patients exposed to nephrotoxic chemotherapeutic agents develop kidney injury. One or more of these factors combine to increase the risk of kidney injury, and the presence of several factors enhances the risk of nephrotoxicity. Other factors, including innate drug toxicity, certain host characteristics, and renal handling of the drug should be considered, as they may predispose the individual to drug-induced kidney injury (Piscitani et al., 2020). Complete remission of TVT was obtained with VS administered weekly in dogs with TVT for 28 days.

The results did not identify an alteration in renal function in serum creatinine or BUN, urine specific gravity, urinary GGT, and serum SDMA measurement in most cases. However, the progressive elevation of SDMA was detected in one patient, and it should be considered that a kidney injury might occur due to VS treatment. SDMA concentration seemed to be the earliest indication of kidney problems, although it suffered an oscillation within the reference range in treated dogs that require careful interpretation of the results.

Conclusion

The use of vincristine sulfate in dogs with TVT, as a single agent, for 28 days, proved to be safe for renal function, without kidney injury or renal filtration alteration detectable in routine exams, urinary GGT, and serum SDMA measurement, in most bitches. However, individual factors must be considered, and an injury can occur observed only by raising the SDMA.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics Statement

Approval was obtained from the Ethics and Animal Experimentation Committee of the University Federal of Alagoas (n° 63/2017).

Acknowledgements

Idexx- Brazil and Centrovét- Centro de Diagnóstico Veterinário for laboratorial analysis support. CAPES, Coordination for the Improvement of Higher Education Personnel for scholarship funding.

References

- Abrams-Ogg A, Rutland B, Levis P, Sabine V, Majeed A, Bienzle D, Linden AZ, Richardson D, Mutsaers A, Woods P. Lymphoma and symmetric dimethylarginine concentration in dogs: a preliminary study. *J Vet Intern Med.* 2017;31(5):1584-5.
- Braz PH, Marinho CP. Comparison between hematological and biochemical changes caused by conventional and metronomic chemotherapies in the treatment of canine transmissible venereal tumor. *Pesq Vet Bras.* 2021;41:e06575. <http://dx.doi.org/10.1590/1678-5150-pvb-6575>.
- Cardozo RM, Reis R, Barbosa MJB, Alcalá COR, Assis MF. Avaliação hematológica em cães errantes da região urbana de Maringá-PR. *Pubvet.* 2013;7(26):1-8. <http://dx.doi.org/10.22256/pubvet.v7n26.1645>.
- Cianciolo R, Hokamp J, Nabity M. Advances in the evaluation of canine renal disease. *Vet J.* 2016;215:21-9. <http://dx.doi.org/10.1016/j.tvjl.2016.04.012>. PMID:27198066.
- Crivellenti LZ, Mesa JS, Meirelles AÉ, Borin Crivellenti S, Mireya EG, Canola JC, Hatayde MR, Santana AE, Dantas M, Silva GE. False positivity of gamma-glutamyl transpeptidase measurement in urine. *Ren Fail.* 2014;36(4):581-4. <http://dx.doi.org/10.3109/0886022X.2014.880325>. PMID:24456228.
- Furini AAC, Guimarães PM, Silva FS, Lopes MASM, Castro KF, Atique TSC, Netto HA. Estudo da variação neutropênica pelo sulfato de vincristina em cães com TVT tratados em um hospital veterinário do noroeste paulista, Umuarama. *Arquivos Ciênc Vet Zool.* 2014;17(1):5-9.
- Ganguly B, Das U, Das AK. Canine transmissible venereal tumour: a review. *Vet Comp Oncol.* 2016;14(1):1-12. <http://dx.doi.org/10.1111/vco.12060>. PMID:23981098.
- Grigorian A, O'Brien CB. Hepatotoxicity secondary to chemotherapy. *J Clin Transl Hepatol.* 2014;2(2):95-102. PMID:26357620.
- Hall JA, Yerramilli M, Obare E, Yerramilli M, Jewell DE. Comparison of serum concentrations of symmetric dimethylarginine and creatinine as kidney function biomarkers in cats with chronic kidney disease. *J Vet Intern Med.* 2014;28(6):1676-83. <http://dx.doi.org/10.1111/jvim.12445>. PMID:25231385.
- Hantrakul S, Klangkaew N, Kunakornsawat S, Tansatit T, Poapolathep A, Kumagai S, Poapolathep S. Clinical pharmacokinetics and effects of vincristine sulfate in dogs with transmissible venereal tumor (TVT). *J Vet Med Sci.* 2014;76(12):1549-53. <http://dx.doi.org/10.1292/jvms.14-0180>. PMID:25649934.
- Kanca H, Tez G, Bal K, Ozen D, Alcigir E, Atalay Vural S. Intratumoral recombinant human interferon alpha-2a and vincristine combination therapy in canine transmissible venereal tumour. *Vet Med Sci.* 2018;4(4):364-72. <http://dx.doi.org/10.1002/vms3.119>. PMID:30117719.
- Kopke MA, Burchell RK, Ruaux CG, Burton SE, Lopez-Villalobos N, Gal A. Variability of symmetric dimethylarginine in apparently healthy dogs. *J Vet Intern Med.* 2018;32(2):736-42. <http://dx.doi.org/10.1111/jvim.15050>. PMID:29469955.
- Kumar VVVA, Kumari KN, Kumar KS, Kumar VG, Lakshman M. Effect of Vincristine chemotherapy in TVT affected dogs. *The Pharma Innovation Journal.* 2018;7(4):163-6.
- López-Gómez L, Díaz-Ruano S, Girón R, López-Pérez AE, Vera G, Herradón Pliego E, López-Miranda V, Nurgali K, Martín-Fontelles MI, Uranga JA, Abalo R. Preclinical evaluation of the effects on the gastrointestinal tract of the antineoplastic drug vincristine repeatedly administered to rats. *Neurogastroenterol Motil.* 2018;30(11):e13399. <http://dx.doi.org/10.1111/nmo.13399>. PMID:29971865.
- Lucas GNC, Leitão ACC, Alencar RL, Xavier RMF, Daher EF, Silva GBD Jr. Pathophysiological aspects of nephropathy caused by non-steroidal anti-inflammatory drugs. *J Bras Nefrol.* 2019;41(1):124-30. <http://dx.doi.org/10.1590/2175-8239-jbn-2018-0107>. PMID:30281062.
- Nabity MB, Lees GE, Boggess MM, Yerramilli M, Obare E, Yerramilli M, Rakitin A, Aguiar J, Relford R. Symmetric dimethylarginine assay validation, stability, and evaluation as a marker for the early detection of chronic kidney disease in dogs. *J Vet Intern Med.* 2015;29(4):1036-44. <http://dx.doi.org/10.1111/jvim.12835>. PMID:26079532.
- O'Keefe DA, Harris CL. Toxicology of oncologic drugs. *Vet Clin North Am Small Anim Pract.* 1990;20(2):483-504. [http://dx.doi.org/10.1016/S0195-5616\(90\)50040-6](http://dx.doi.org/10.1016/S0195-5616(90)50040-6). PMID:2180191.
- Perondi F, Lippi I, Ceccherini G, Marchetti V, Guidi G. Evaluation of urinary γ -glutamyl transferase and serum creatinine in non-azotaemic hospitalised dogs. *Vet Rec.*

2019;185(2):52. <http://dx.doi.org/10.1136/vr.104439>. PMID:31076519.

Piscitani L, Sirolli V, Di Liberato L, Morroni M, Bonomini M. Nephrotoxicity associated with novel anticancer agents (aflibercept, dasatinib, nivolumab): case series and nephrological considerations. *Int J Mol Sci.* 2020;21(14):4878. <http://dx.doi.org/10.3390/ijms21144878>. PMID:32664269.

Priyadarshini N, Das DP, Panda SK, Samal L. Transmissible venereal tumours (TVT) in bitches: a haematological, biochemical and histopathological study. *J Entomol Zool Stud.* 2021;9(1):490-3. Priyadarshini N, Das DP, Panda SK, Samal L. Transmissible venereal tumours (TVT) in bitches: a haematological, biochemical and histopathological study. *J Entomol Zool Stud.* 2021;9(1):490-3. <http://dx.doi.org/10.22271/j.ento.2021.v9.i1g.8194>.

Reynolds SB, Hashmi H, Ngo P, Kloecker G. Rescue therapy for acute idiopathic thrombocytopenic purpura unresponsive to conventional treatment. *BMJ Case Rep.*

2019;12(1):bcr-2018-227717. <http://dx.doi.org/10.1136/bcr-2018-227717>. PMID:30642865.

Sajjad H. Study of nephrotoxic effects of vincristine-treatment in mice. *Ann Biol Res.* 2012;3(5):2304-6.

Shati AA. Sub-chronic administration of vincristine sulphate induces renal damage and apoptosis in rats via induction of oxidative stress and activation of Raf1-MEK1/2- Erk1/2 signal transduction. *Int J Morphol.* 2019;37(1):273-83. <http://dx.doi.org/10.4067/S0717-95022019000100273>.

Smee NM, Pohlman LM, Vahl C, et al. Effect of storage time and temperature on canine urine enzymes N-acetyl- β -D-glucosaminidase (NAG) and γ -glutamyl transpeptidase (GGT). *Int J Appl Res Vet Med.* 2016;14(1):114-21.

Financial support: Fapeal (Alagoas State Research Support Foundation) for granting the scholarship, to Idexx for support in SDMA analysis.