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Neutrophil Gelatinase-Associated Lipocalin Discloses Acute Tubular Injury after Surgery in Canine Patients

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Abstract

Canine patients needing surgical intervention are at risk of developing acute kidney injury, whose clinical manifestation it's late. The search for early diagnostic methods revealed the Neutrophil gelatinase-associated lipocalin (NGAL) as a novel biomarker for detecting acute tubular injury (ATI). Considering the hypothesis that dogs with cancer are more likely to develop ATI, related to the different interventions throughout the surgical process, a prospective longitudinal study (from the preoperative to the immediate postoperative period) of 17 clinically stable dogs indicated for surgical procedure. The serum creatinine, urinary density, and urinary protein-to-creatinine ratio, which are traditional indicators for kidney disease, the serum, and urine NGAL biomarkers, were evaluated. The sample collections were performed three times: 12 to 24 hours before surgery, 48 hours, and 96 hours after surgery. The results showed a significant increase in urinary NGAL in 11 of 17 dogs, 48 hours after surgery, while traditional markers showed no significant change, and no related clinical signs were observed. Thus, confirming the study's hypothesis, NGAL proved helpful for the early detection of silent events of attacks on renal tubular integrity.

Keywords: NGAL; Kidney injury biomarker; Dogs

Introduction

Acute kidney injury (AKI), which initially affects the renal tubules, is characterized by rapid deterioration of renal function caused by nephrotoxic substances or renal ischemia. Although there is a possibility of recovery, success requires a timely diagnosis. However, the serum concentration of creatinine (sCr), a traditional marker to detect AKI, takes a long time to manifest. On the other hand, the biomarker Neutrophil gelatinase-associated lipocalin (NGAL) can detect active tubular injury (ATI) from two hours after the start of the inducing event [1,2] which shows the precocity of NGAL in comparison to the traditional markers. The NGAL is a glycoprotein with low molecular weight (25D) expressed in the kidney and many other organs [3]. Under normal conditions, the tubular synthesis of NGAL is low [4]. However, during active tubular injury, however, synthesis increases, and reabsorption decreases, which results in increased urinary NGAL excretion [5]. The kidneys have an intrinsic physiological mechanism of protection and regulation of renal blood flow, mediated by eicosanoid derivatives, the prostacyclin [6]. However, it may not be possible to maintain this feature under critical conditions or in the case of pre-existing chronic kidney disease (CKD). Kidney injury by ischemia can occur by any factor that provokes renal blood perfusion alterations and cellular death [7]. In veterinary

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medicine, few studies related to changes in serum NGAL (sNGAL) and urinary NGAL (uNGAL) in patients with cancer or other equally severe conditions [8]. However, in more recent studies in dogs with CKD, increases in NGAL, compared to a control group, were identified [5,9,10]. Dogs with carcinoma, or lymphoma, had higher values of uNGAL and sNGAL compared to the control group [10]. Other factors that can inhibit or neutralize the renal physiological defense include drugs and surgical procedures. Non-steroidal anti-inflammatory drugs (NSAIDs), even at therapeutic doses, can cause ischemic kidney injury [11]. Drugs for inhalation anesthesia can also pose a risk [12], as can the surgery procedure itself [2]. Assuming that surgical patients may be under many simultaneous risk factors, it is plausible to consider that they may develop kidney damage. Thus, we conducted a prospective longitudinal study to identify ATI in dogs at risk related to surgical and postoperative events using NGAL evaluation.

Methods

Preliminary information

Before the execution phase of the present study, the project was approved by the CEUA – Ethics Committee on the Use of Animals of FCAV-Unesp, campus of Jaboticabal/SP (Protocol number: 4369/20). The inclusion of dogs was made with the free and informed consent of the respective owners.

The dogs were referred to the Service of Veterinary Oncology at the Teaching Veterinary Hospital of the São Paulo State University - Jaboticabal campus, Brazil.

Over a period of four months (August to November 2021), 26 dogs referred to a Teaching Veterinary Hospital, went scheduled for soft tissue surgery were enrolled in the study.

As a criterion for inclusion the dogs should be adult dogs, >1 year of age, with at least 5 kg of body mass, without restriction of breed or gender, referred for soft tissue surgery.

For exclusion, the criteria were postoperative complications, confirmation of urinary tract infection or withdrawal of the owner were excluded.

Renal evaluation

In addition to the exams already carried out, the dogs were evaluated by the Nephrology and Urology Service to identify possible pre-existing urinary diseases.

Obtaining and preparing samples for evaluation of markers

In this study, the variables analyzed, which comprise the NGAL from urine (uNGAL), NGAL from serum (sNGAL), serum creatinine (sCr), urinary protein-to-creatinine ratio (UP/C), and urine specific gravity (USG), were measured at three different moments. The first sample collection was performed 12 to 24 hours before surgery (T0). A second sample was obtained 48 hours after the anesthesia period (T48), and the third at 96 hours after (T96). Serum and urine supernatant samples were stored at -80 degrees for NGAL assays.

Laboratory analyzes

Assays for sNGAL and uNGAL dosage were performed with the specific kit for dogs (DOG NGAL ELISA (KIT 043RUO – Bioporto Diagnostics, Hellerup, Denmark). The procedure was performed according to the manufacturer's recommendations. The read process was done by SectraMax M3 microplate reader, using a wavelength of 450nm.NGAL. The Ungal values obtained were adjusted based on the respective values of uCr. [uNGAL (pg/mL)/uCr (mg/mL) = uNGAL (pg/mg)].

Statistic

The results were evaluated by descriptive statistics and correlation tests (Spearman) were also performed for continuous variables (conventional markers and NGAL). $P \leq 0.05$ was adopted as significant values. All analysis and graphs were performed using the statistic program GraphPad Prism version 9.3.1 for Windows, GraphPad Software, San Diego, California USA.

Results

Of the 26 dogs initially recruited, 17 fully met the study's inclusion criteria. Thirteen had cancer and four underwent surgery for other reasons. The data on the physical and clinical characteristics of the dogs are described in table 1. A complementary information about cellular type tumor is presented in table 2. Among the patients included in the study (Table 1), four of them did not have cancer, but underwent a surgical procedure and were medicated with NSAIDs. Three of them for ocular surgery and one for castration. However, two of them had increased NGAL. Regarding the possible influences of surgical procedures and postoperative treatment on serum and urinary NGAL concentrations, most patients (11 out of 17) had a marked increase in NGAL (T48) and 10 out of these 17 patients had the increase in the uNGAL, probably related to ATI (Figure 1). However, only five showed an increase in sNGAL, among which, four were coincident with the increases in uNGAL. To consider the increases relevant, a minimum of twice the values obtained in the first evaluation was established as a criterion (Table 3). The Spearman r test, applied as correlation matrix, for all continuous variables, with the respective repetitions (T0, T48, and T96) showed some statistically significant results (mainly for uNGAL vs UP/C). However, these results are not clinically consistent (Tables 4,5 and 6).

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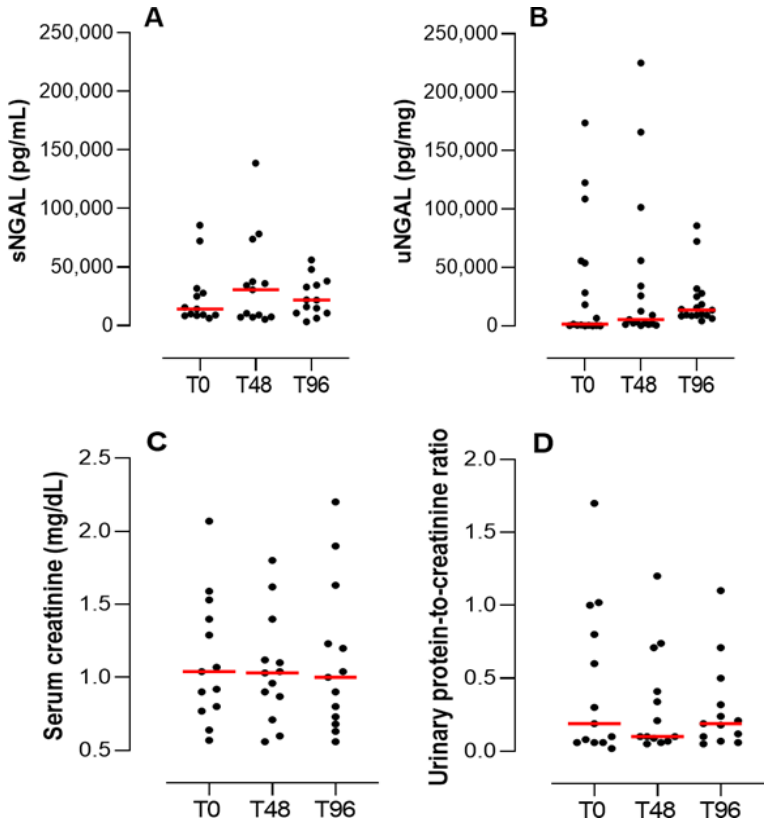

Figure 1: Scatter graphs of the analyzed variables obtained in three times: T0 (basal - before surgery), T48 (48 hours after surgery end), and T96 (96 hours after surgery end). The red lines indicate the median values.

Table 1: Physical and clinical characteristics of the 17 dogs evaluated, describing anesthetic duration and ant-inflammatory treatment used after surgery.

ID	Breed	M (n=6)	F (n=11)	Age (year)	BW (kg)	KD (n=5)	cancer (n=13)	IA (min.)	NSAID (n =12)
1	Labrador retriever		X	5	25,7	X	X	150	
4	MB		X	14	38		X	240	
6	MB	X		12	25		X	270	
16	MB		X	13	5,4		X	245	
24	Teckel		X	8	8,8	X	X	190	
2	Labrador retriever	X		9	39	X	X	40	X
9	MB		X	10	30		X	180	X
12	French bulldog	X		10	14,5		X	90	X
17	MB		X	9	17,3		X	90	X
18	Siberian Husky		X	9	33	X	X	190	X
20	Cocker spaniel	X		5	15		X	140	X
23	English bulldog		X	8	25		X	215	X
25	MB		X	10	15		X	139	X
5	MB	X		10	41			36	X
10	Dachshund		X	10	10,1	X		90	X
14	Dachshund	X		2	6,9			38	X
26	MB		X	10	16,6			98	X

ID: patient identification; M: male; F: female; MB: mixed breed; BW: body weight; KD: kidney disease; IA: inhalation anesthesia; NSAID: nonsteroidal anti-inflammatory drug;

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Table 2: Biopsies results of Cellular type tumors found in the 13 dogs with cancer.

Dog ID	Cellular type
1	round cell tumor
9	round cell tumor
20	round cell tumor
17	round cell tumor
6	round cell tumor
12	round cell tumor
23	epithelial cell tumor
24	epithelial cell tumor
25	epithelial cell tumor
2	mesenchymal cell tumor
4	mesenchymal cell tumor
16	epithelial cell tumor, round cell tumor
18	epithelial cell tumor, mesenchymal cell tumor

Table 3: NGAL increase rates (at list 2 times of T0 values) in 11 out of 17 dogs after 48 hours surgery (T0 vs T48).

Dog ID	uNGAL	sNGAL
	Times of increase	
1	13	-
4	2	-
6	18	-
10	2	2
12	10	2
14	3	-
16	-	2
17	8	-
18	3	3
23	3	-
25	11	2

IC: confidence interval. CV: coefficient of variation, uNGAL: Urine neutrophil gelatinase-associated lipocalin, sNGAL: Serum neutrophil gelatinase-associated lipocalin, sCr: serum creatinine; USG: urine specific gravity; UP/C: urinary protein-to-creatinine ratio

Table 4: Descriptive statistics data of the variables uNGAL (pg/mg), sNGAL (pg/mL), sCr (mg/dL), UP/C and urinary specific gravity (pre-surgery, 12 to 24 hours. n=17.

		uNGAL	sNGAL	sCr	UP/C	USG
Minimum		153	4215	0.6	0.02	1.007
percentile 25%		419,5	9001	0.8	0.07	1.022
Median		1598	13490	1	0.19	1.034
percentile 75%		54756	26449	1.4	0.98	1.04
Maximum		173836	85877	2.1	1.7	1.055
CI (95%)	Inferior	6428	9958	0.91	0.2	1.025
	Superior	60946	33653	1.32	0.73	1.038
CV (%)		157%	106%	36%	110%	1.25%

IC: confidence interval. CV: coefficient of variation, uNGAL: Urine neutrophil gelatinase-associated lipocalin, sNGAL: Serum neutrophil gelatinase-associated lipocalin, sCr: serum creatinine; USG: urine specific gravity; UP/C: urinary protein-to-creatinine ratio

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Table 5: Descriptive statistical data of the variables uNGAL (pg/mg), sNGAL (pg/mL), sCr (mg/dL), UP/C and urinary specific gravity (2nd collection; 48 hours post-surgery, under treatment). n=17.

		uNGAL	sNGAL	sCr	UP/C	USG
Minimum		412	2837	0.6	0.05	1.003
percentile 25%		2255	7513	0.8	0.08	1.015
Median		5412	16302	1	0.21	1.037
percentile 75%		44992	36811	1.15	0.56	1.046
Maximum		225034	138795	1.8	1.3	1.065
IC (95%)	lower	2496	7631	0.9	0.09	1.015
	Superior	34116	35916	1.1	0.41	1.046
CV (%)		169%	115%	31%	113%	1.85%

IC: confidence interval. CV: coefficient of variation, uNGAL: Urine neutrophil gelatinase-associated lipocalin, sNGAL: Serum neutrophil gelatinase-associated lipocalin, sCr: serum creatinine; USG: urine specific gravity; UP/C: urinary protein-to-creatinine ratio

Table 6: Descriptive statistical data of the variables uNGAL (pg/mg), sNGAL (pg/mL), sCr (mg/dL), UP/C and urinary specific gravity (3rd collection; 96 hours post-surgery, under treatment). n=17.

		uNGAL	sNGAL	sCr	UP/C	USG
Minimum		143,0	3388	0,60	0.05	1.009
percentile 25%		572,5	8884	0.7	0.1	1.022
Median		3766	16180	1	0.19	1.035
percentile 75%		30481	33878	1.2	0.41	1.045
Maximum		112747	56069	2.2	1.6	1.062
IC (95%)	Inferior	681,0	10871	0.7	0.1	1.024
	Superior	30292	33090	1.2	0.32	1.045
CV (%)		152%	75%	42%	122%	1.45%

IC: confidence interval. CV: coefficient of variation, uNGAL: Urine neutrophil gelatinase-associated lipocalin, sNGAL: Serum neutrophil gelatinase-associated lipocalin, sCr: serum creatinine; USG: urine specific gravity; UP/C: urinary protein-to-creatinine ratio

Discussion

Following the course and outcome of the mediate postoperative period, our results showed increases in NGAL values in 11 of the 17 dogs evaluated, which may be related to the occurrence of acute tubular injury. However, no clinical or laboratory signs were identified beyond those expected for the conditions of each patient. Although, it is already known that conventional markers manifest late [1,7,13]. As observed in many studies, creatinine is not an efficient marker to detect ATI as fast as necessary for early medical assistance. Among the evaluated dogs, five had pre-existing kidney disease and even then, there was no clinic sign. It is known that in cancer disease, neoplastic tissues can express high amounts of NGAL, in dogs [10]. In our study, from 13 dogs with cancer, seven had increased uNGAL at the first evaluation (T0). This result shows that uNGAL increase may occur independently of other factors such as surgery, medication, or chemotherapy in dogs with cancer. Although the evaluated patients underwent similar inhalation anesthesia, surgery and postoperative treatment, the medical condition of each patient differed substantially. Established kidney disease can also lead to increased NGAL [2,4,5,8,9]. The CKD is a progressive condition by consequence of sequential acute injuries (AKI) episodes of variable magnitude, especially in IRIS 3 and 4, where the intrinsic physiological mechanisms of protection and regulation of renal blood flow are probably not working properly [14]. Among the patients, five had pre-existing kidney disease and uNGAL presumably increased in the first sample collection. Inhalation anesthetics, including isoflurane and sevoflurane, which were used in the patients in the present study, may cause decreased renal perfusion depending on the administered dose [5,12]. Furthermore, patients were treated with non-steroidal anti-inflammatory drugs. Although therapeutic doses have been used, there is the possibility of side effects or adverse effects. The NSAID administered primarily inhibits COX-2, but also COX-1. It is known that dogs are particularly more sensitive to possible adverse drug effects, especially if there are comorbidities [11] as is the case in this study. The uNGAL data are more specific than sNGAL for detecting AKI [1]. When ischemic factors cause tubule injuries, the amounts of NGAL increase in the serum and urine due to the excretion of the molecule by the tubular cells [15]. However, NGAL is not only secreted by

the renal tubules, but also in organs other than the kidneys [5]. Circulating NGAL is filtered in the glomeruli, reabsorbed in the proximal tubules, and excreted into the lumen in the loop of Henle [5]. Therefore, sNGAL makes up the pull eliminated in the urine. However, the results of the present study did not show a significant correlation between the values of sNGAL and uNGAL. Descriptive statistics revealed very high coefficients of variation (CV) for the variables sNGAL, uNGAL, indicating disparity of dog's clinical conditions. On the other hand, for the conventional markers, sCr and USG, the CV results were low confirming the uniform pattern as in the three evaluation moments. However, The CV of UP/C were high, among 17 dogs only six had proteinuria (UP/C range 1.7 to 0.6). Even more, five of these dogs had preexisting kidney disease not previously diagnosed. It was demonstrated that biomarker NGAL can be related to the proteinuria level in patients with renal disease caused by infectious factors [16]. In our study, however, the data do not corroborate the previous findings. It is very important to remember that the assays for serum and urinary NGAL were performed after the end of the three collections, which It is very important to remember that the assays for serum and urinary NGAL were performed after the end of the three collections, which concealed the results. Thus, there was no bias in postoperative care the results. Thus, there was no bias in postoperative care. Therefore, since uNGAL increases were identified in the second evaluation moment, the causal factors certainly included surgical procedures and immediate postoperative treatments.

As expected, there was an increase in postoperative uNGAL concentrations consistent with acute tubular injury, although there was no signaling of traditional markers. Some dogs already had high uNGAL values at baseline indicating that the clinical condition, itself, was causing tubular injury. However, acute tubular injury intensity is dependent of multifactorial condition as surgery, postoperative treatment, severity of pre-existing illness and bad general health.

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Author contributions statement

KAHT and MBC concepted, designed and conduced the experiment, KAHT and AMFRT acquired the data, AAJJ conduced the NGAL assay. All authors analyzed the results and reviewed the manuscript.

Additional information

Competing interests: none declared.

Ethical approval: The study was approved by the institutional ethics committee (nº 4369/20).

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