Neutrophil Gelatinase-Associated Lipocalin in Dogs With Sepsis Undergoing Emergency Laparotomy: A Prospective Case–Control Study

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Background: Neutrophil gelatinase-associated lipocalin (NGAL) is an early indicator of acute kidney injury (AKI) in dogs and its use has not been evaluated in dogs with sepsis.

Animals: Fifteen dogs with sepsis requiring laparotomy (study dogs) and 10 dogs undergoing surgery for intervertebral disc disease (control dogs).

Objective: To determine whether NGAL increases in dogs with sepsis undergoing emergency laparotomy and whether it is correlated with development of AKI and survival.

Methods: Longitudinal study conducted at a referral teaching hospital. Serum neutrophil gelatinase-associated lipocalin (sNGAL), urinary NGAL normalized to urinary creatinine concentration (UNCR), and serum creatinine concentration were measured at 4 time points (admission, after anesthesia, and 24 and 48 hours postsurgery). Development of AKI (increase in serum creatinine concentration of 0.3 mg/dL) and in-hospital mortality were recorded. Linear mixed-model analysis was employed to assess differences between groups over time. Mann–Whitney U-test was performed for comparison of continuous variables between groups and Chi square or Fisher's exact tests were used to assess correlation between discrete data.

Results: Serum NGAL and UNCR were significantly higher in study dogs across all time points ($P = .007$ and $P < .001$, respectively) compared with controls. Urinary NGAL normalized to creatinine in the study group was not significantly different between survivors (n = 12) and nonsurvivors (n = 3). Dogs that received hydroxyethyl starch had significantly higher UNCR across all time points ($P = .04$) than those that did not.

Discussion—Conclusion: Serum neutrophil gelatinase-associated lipocalin and UNCR are increased in dogs with sepsis requiring emergency laparotomy. Additional studies are needed to evaluate its role as a marker of AKI in this population.

Key words: Acute kidney injury; Canine; Neutrophil gelatinase-associated lipocalin.

Acute kidney injury (AKI) is defined as a sudden injury to the renal parenchyma and reduction in renal function, regardless of underlying cause.[1] In critical illness and in sepsis, AKI has been identified as one of the main contributors to morbidity and mortality in human patients.[2,3]

The incidence of AKI in hospitalized septic dogs managed in an intensive care setting has been reported as 12% and the in-hospital mortality rate for dogs with AKI varies from 54% to 86%.[4,5]

No consensus has yet been reached in veterinary medicine, however, with regard to criteria that should

be used to define kidney injury. There are several emerging classification systems available, including veterinary AKI staging system (VAKI) and risk, injury, failure, loss of kidney function, and end-stage kidney disease-like criteria.[4,6]

The main pitfalls of the current systems are the use of insensitive and poorly specific markers, such as serum creatinine concentration or urine output. Serum creatinine concentration is not linearly related to glomerular filtration rate (GFR) and can be influenced by factors other than renal function such as muscular mass and hydration state.[7] Glomerular filtration rate is the gold standard for the diagnosis of renal disease, but this method, aside from not being readily clinically available, only detects reduction in renal function and not cellular injury, thus, leading to delayed recognition of disease.[1,8]

Abbreviations:

ACE	angiotensin-converting enzyme
AKI	acute kidney injury
AKIN	acute kidney injury network
APPLE	acute patient physiologic and laboratory evaluation score
MODS	multiple organ dysfunction syndrome
NGAL	neutrophil gelatinase-associated lipocalin
NSAID	nonsteroidal anti-inflammatory drug
RIFLE	risk, injury, failure, loss of kidney function, and end-stage kidney disease
SIRS	systemic inflammatory response syndrome
UNCR	urinary NGAL normalized to creatinine
VAKI	veterinary acute kidney injury

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Since the 1990s, there has been a perceived need to identify early markers of AKI in order to identify at-risk patients. To this end, many biomarkers, such as N-acetyl-β-D-glucosaminidase, kidney injury molecule-1 (KIM-1), and neutrophil gelatinase-associated lipocalin (NGAL) have been studied in human and canine patients.[7,8]

Neutrophil gelatinase-associated lipocalin is a 25 kDa protein, belonging to the lipocalin family. It is covalently bound to gelatinase and secreted by neutrophils and is normally expressed at low concentrations in the kidney, lung, trachea, and gastrointestinal tract.[9,10] It is freely filtered through the glomerulus and re-absorbed in the proximal tubule, such that healthy individuals usually have very low urinary excretion of NGAL.[11] Its production is highly upregulated when epithelial cell damage occurs. In clinical studies in people and dogs, its concentration in urine increases 24–72 hours before serum creatinine concentration, which enables earlier detection of AKI.[9,12,13] Neutrophil gelatinase-associated lipocalin can be measured in plasma, serum or in urine and it usually is expressed as an absolute concentration. In some studies, urinary NGAL has been normalized to urinary creatinine concentration in an attempt to decrease variation associated with changes in urine volume.[12,14,15]

Plasma and urine NGAL is increased in people with sepsis and studies have shown an inconsistent ability for plasma or serum NGAL to detect AKI in humans with severe sepsis or septic shock.[16,17]

The ability of urinary NGAL to predict AKI recently has been evaluated in veterinary medicine. A number of clinical studies, published in the last few years, have shown a significantly higher urinary NGAL concentration in dogs with AKI compared to healthy dogs.[12–14]

The aim of this study was to evaluate the usefulness of both serum NGAL and urine NGAL normalized to urinary creatinine (UNCR) for detecting AKI in dogs with sepsis undergoing emergency laparotomy compared to dogs with intervertebral disc disease undergoing surgery. It was hypothesized that an increase in urinary NGAL concentration would be associated with the development of AKI, longer time to discharge, increased morbidity (assessed by the acute patient physiologic and laboratory evaluation score [APPLE] full scoring system and multiple organ dysfunction syndrome [MODS] criteria), and higher mortality.

Material and Methods

The study was a prospective investigation performed on client-owned dogs. The protocol was approved by the Ethics and Welfare Committee of the Royal Veterinary College, London (URN 2012 1155) and informed owner consent was obtained before enrolling each dog in the study.

The study group included dogs with sepsis that were to undergo emergency laparotomy. Dogs initially were recruited if they fulfilled systemic inflammatory response syndrome (SIRS) criteria (Table 1).[18] Dogs were included in the analysis if they fulfilled criteria for sepsis (ie, the presence of SIRS and confirmed or suspected infection).[19] Infection was defined as the presence of intracellular bacteria on cytological examination or microbiologi-

Table 1. Systemic inflammatory response syndrome (SIRS) criteria used for inclusion of dogs within the study group; SIRS was defined if patients fulfilled 2 or more criteria.[18]

Variable	SIRS criteria fulfilled if
Heart rate	>120/min
Respiratory rate	>20/min
Body temperature	<38.1°C or >39.2°C
White blood cell count	<6 × 10³ or >16 × 10³/μL
Increased band neutrophils with normal neutrophil count	3%

cal evidence of infection in the abdominal fluid or aspirates from an abscess. Dogs with azotemia (defined as plasma or serum creatinine concentration >1.57 mg/dL [138 μmol/L]) on presentation, or a history of chronic kidney disease, were excluded from the study.

A control group of systemically healthy dogs undergoing emergency surgical intervention for intervertebral disk disease (IVDD) also was recruited.

Admission Data

Clinical data were collected on admission and included signalment, disease process, heart rate, respiratory rate, rectal temperature, systolic blood pressure, whole blood lactate concentration, and serum creatinine concentration.

Hospitalization Data

Before or during the course of hospitalization, administration of vasopressors, hydroxyethyl starches, blood products, human serum albumin, and potentially nephrotoxic drugs (eg, nonsteroidal anti-inflammatory drugs [NSAID], angiotensin-converting enzyme inhibitors) were recorded.

Occurrence of AKI, azotemia, hypotension, and survival were recorded as categorical data. Acute kidney injury was defined as an increase in serum creatinine concentration of 0.3 mg/dL (26.4 μmol/L) within 48 hours, in keeping with the Acute kidney injury network criteria.[4] Serum creatinine concentration was measured on admission, postanesthesia and 24 and 48 hours postsurgery. Hypotension was defined as a systolic Doppler pressure <90 mmHg or a mean invasive arterial pressure <60 mmHg. The APPLE full score on admission was calculated for each dog when data were available.[20] Multi-organ dysfunction syndrome was defined as ≥2 organ systems having signs of dysfunction.[5]

Sampling and Sample Processing

Blood and urine samples were taken from dogs in both groups for NGAL and creatinine measurements. Samples were taken at 4 time points: (I) on admission, (II) after anesthesia (within 2 hours postanesthesia), and (III) 24 and (IV) 48 hours postoperatively. Because ours was a clinical study performed in the United Kingdom on nonexperimental animals, only residual volumes of blood taken from samples for clinical need could be used.

Urine samples were collected by voiding or from an indwelling urinary catheter, and an aliquot of each fresh urine sample was submitted to the laboratory for urinalysis (refractometric urine specific gravity [USG], dipstick semiquantitative chemistry, and sediment examination) and urine protein-to-creatinine ratio (UP:C) measurement.[a] Pyuria was defined as ≥5 white blood cells per high power field.[21] Aliquots of the urine and serum samples were centrifuged at 400 × g for 5 minutes and the supernatant frozen

at −70°C within 1 hour (urine) and 8 hours (serum) for later batch analysis.

Serum creatinine concentrations were determined from stored frozen serum samples using a biochemical analyzer.[a] Serum NGAL was measured on the same day using the same thawed samples. All samples thawed for NGAL analysis were refrozen within 1 hour to allow for re-analysis if required.

Urine and serum NGAL concentrations were determined using a sandwich ELISA,[b,c] following the manufacturer's instructions.[d] Urine and serum samples initially were diluted 1 : 200 using the diluent provided in the kit and the assay was performed according to the manufacturer's instructions. When necessary, dilution of the samples was adjusted (1 : 20 to 1 : 10,000) to ensure that the NGAL concentration fell on the linear part of the standard curve. Samples requiring further dilution were thawed and frozen a maximum of 3 times. This thaw–refreeze process has been shown not to affect NGAL stability.[15] Urinary NGAL concentrations were normalized by the urinary creatinine concentration (mg/dL) to express results as UNCR. The final normalized urinary NGAL (UNCR) is expressed as ng/mg.

A dual set of pooled quality control samples (high and low concentrations) also were included within each plate for assessment of interassay and intra-assay variability.

Statistics

Statistical analyses were performed in SPSS[e] and figures were drawn using Graphpad Prism.[f] Continuous data were presented as median (range) in the original scale because some of the data have been log-transformed in the statistical analysis and the sample size was small.

Repeated measurement of UNCR and serum neutrophil gelatinase-associated lipocalin (sNGAL) were analyzed in a linear mixed-effects model (LMM) taking time, group (sepsis versus control dogs), colloids, AKI, MODS, hypotension, and blood product requirement as potential fixed effects and dog as random effect. Potential 2-way interactions between categorical fixed factors and time were assessed. A continuous variable was log-transformed if its residuals from LMM analysis were skewed to the right.

Although many continuous data were normally distributed, admission data between clinical course and treatment groups and survivors were compared using the Mann–Whitney U-test because of the small sample size. Similarly, Spearman's correlation coefficient was used to measure the linear correlation between the 2 sets of continuous data to avoid the potential influence of outliers. The association between categorical variables was analyzed using chi-squared or Fisher's exact test if the contingency table contained a value <5. Statistical significance was set at $P < .05$.

Results

Recruitment and Admission Characteristics

Twenty-one dogs were enrolled in the study group and 10 dogs were enrolled in the control group between June 2012 and August 2013. One dog in the study group subsequently was excluded because no urine had been stored for presurgery NGAL analysis. Twenty dogs initially were included in the study. Five of these were removed because they did not fulfill criteria for sepsis. Four of these dogs had gastric dilatation and volvulus and 1 had severe gastroenteritis. The final study group therefore was represented by 15 dogs. Of these dogs, 13 had septic peritonitis and 2 had intra-abdominal abscessation.

Admission variables and days of hospitalization were compared between the study and the control groups and reported in Tables 2 and 3.

Clinical Course and Treatment

All dogs, in both the study and control groups, had blood pressure recorded over the course of the entire anesthetic period, and the study population had periodic blood pressure measurements made during hospitalization (Tables 2 and 3). Eleven of 15 dogs in the study group were hypotensive (4 pre-operatively and 10 intra-operatively). Two of 3 nonsurvivors were hypotensive in the immediate postoperative period and required vasopressor therapy. Blood pressure was not measured on admission for any of the dogs in the control group because they were considered to be cardiovascularly stable based on clinical examination.

None of the dogs in the control group developed AKI, whereas 2/15 (13%) dogs in the study group met AKI criteria. but did not become azotemic (serum creatinine concentration increased by 0.57 mg/dL [50 μmol/L] and 0.65 mg/dL [57 μmol/L] in 24 hours). These 2 dogs later were euthanized because of clinical deterioration. No difference was identified between the incidence of AKI in the study and control groups ($P = .5$; Table 3).

In the study population, 12 dogs (80%) survived to discharge, 1 dog died. and 2 were euthanized after clinical deterioration. Nine dogs (90%) in the control group were discharged, with 1 being euthanized because of persistent paraplegia (Table 3).

Serum NGAL Results

Intra-assay and inter-assay variability for sNGAL was 2.0% and 10.4% for the high concentration quality control, and 7.7% and 19.3% for the low concentration quality control, respectively.

No significant difference was identified for time-group interaction and among times, but sNGAL was significantly higher in the study population compared to the control population ($P = .007$; Fig 1 and Table 4). A time-colloid administration interaction was present ($P = .01$). The cause of significance seemed to derive mainly from the difference between the time points within the noncolloid group, and there was no difference between the colloid group at all time points (Table 4). There was no association between AKI, MODS development, hypotension and survival, and sNGAL (Table 4).

UNCR Results

There was no time-group interaction and no difference among times for UNCR, but the study group had significantly higher UNCR than did the control dogs at all measured time points ($P < .001$). No association was detected between the UNCR and development of AKI ($P = .25$) or MODS ($P = .49$; Fig 2 and Table 4). Four of the 30 dogs (2 in each group) had pyuria. There was

Table 2. Measurements taken at admission between study and control patients.

	Study	Control	P value
Heart rate (bpm)	128 (70–190)	120 (80–172)	1
Respiratory rate (rpm)	36 (20–100)	40 (20–80)	.69
Temperature (°C)	39.2 (37.8–40.7)	38.8 (36.9–39.4)	.36
White Blood cells (10^9)	17.3 (2.2–47.8)	–	–
Lactate (mg/dL) (R.I. 0–22.5)	11.7 (6.3–99) (n = 15)	16.2 (16.2–18) (n = 3)	.2
Doppler systolic blood pressure (mmHg)	130 (60–170)	–	–

R.I., Reference Interval.

Measurements are expressed as median (range) and statistical significance was set at $P < .05$.

Table 3. Pre-admission treatment, clinical course and treatment received in the study and in the control dogs.

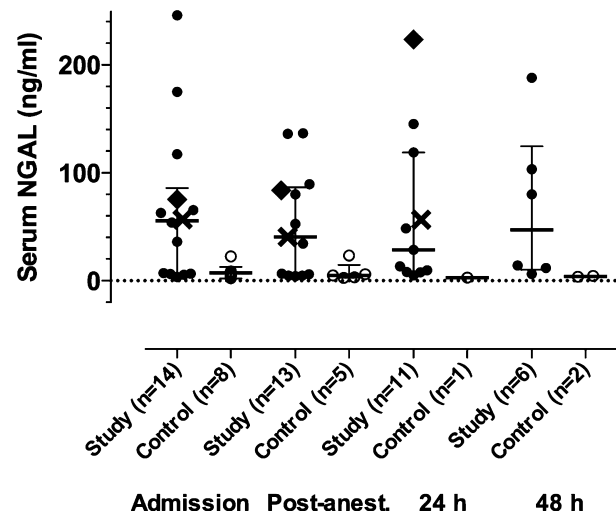|	Study	Control	P value
Admission characteristic			
Previous surgery (5 days)	5/15 (33%)	0/10 (0%)	.61
Fluid therapy prior admission (crystalloids)	13/15 (86%)	1/10 (10%)	**< .01**
Nonsteroidal anti-inflammatory medications	4/15 (27%)	6/10 (60%)	.12
Clinical course			
Hypotension	11/15 (73%)	0/10 (0%)	**< .01**
APPLE score	28 (8–38) (14/15)	–	–
MODS	4/15 (27%)	0/10 (0%)	.12
AKI	2/15 (13%)	0/10 (0%)	.5
Survival to discharge	12/15 (80%)	9/10 (90%)	.62
Treatment			
Blood products	5/15 (33%)	0/10 (0%)	.06
Human serum albumin	3/15 (20%)	0/10 (0%)	.25
Hydroxyethyl starch (Voluven®)	9/15 (60%)	0/10 (0%)	**< .01**
Days Hospitalization	7 (1–11)	12.5 (1–11)	.42
Days ICU	3 (1–11)	0 (0–0)	**< .01**

APPLE score was calculated.[20] MODS (Multi-Organ Dysfunction Syndrome) was defined as dysfunction of at least 2 organs.[5] AKI (Acute Kidney Injury) was defined as an increase of 0.3 mg/dL (26.5 μmol/L) from baseline.[4]

Significant differences are highlighted (bold; significance set at a P value ≤.05).

Fig 1. The medians and interquartile range of serum neutrophil gelatinase-associated lipocalin at different times between the control and the study group are represented by the bar and the whiskers with all the patients displayed in a scattered plot manner. The 2 acute kidney injury patients are represented by x and ♦ on the graph. Serum neutrophil gelatinase-associated lipocalin concentrations were significantly higher across all time points in the study group.

no association between UNCR and the presence of pyuria at any of the time points ($P = .55$). None of the dogs in the control group was diagnosed with a urinary tract infection (UTI). One dog in the study group was diagnosed with a UTI, and this dog also had prostatic abscessation and septic peritonitis.

No time-colloids administration interaction was identified and no difference among time points was detected. Dogs that received hydroxyethyl starch[g] (12/30), however, had significantly higher UNCR compared to dogs that did not across all time points ($P = .04$; Fig 3 and Table 4), with the 2 dogs that developed AKI receiving colloids, either pre-operatively or intra-operatively.

All dogs in the control group received NSAIDs after anesthesia, whereas none of the dogs in the study group received NSAIDs after admission. No time-NSAID administration interaction and no difference among

times was detected, but dogs in the study group that did not receive NSAIDs before admission (n = 11) had significantly higher UNCR ($P = .04$; Table 4).

There was a significant association between UNCR and days spent in the intensive care unit (ICU; Table 5), but no correlation was found between UNCR and duration of hospitalization (admission, $P = .5$; post-anesthesia, $P = .3$; 24 hours, $P = .34$; 48 hours, $P = .09$).

No significant difference in UNCR was detected between dogs that survived to discharge and those that did not ($P = .08$).

Correlation

There was a moderate correlation between the UNCR and sNGAL on admission (r_s 0.76) and postanesthesia (r_s 0.82; Table 4). No correlation was found between UNCR and urinary pH and USG.

There was a correlation between UNCR and UP:C (admission, r_s 0.61; postanesthesia, r_s 0.92; 24 hours, r_s 0.87; 48 hours, r_s 0.75), but no correlation was found

Table 4. Differences in sNGAL and UNCR for each single category and differences between time and time-category interaction.

	Category	Category	Time	Time-category interaction
sNGAL	Sepsis	**P = .007**	P = .13	P = .95
	AKI	P = .28	P = .24	P = .83
	Hypotension	P = .49	P = .23	P = .33
	Survival	P = .09	P = .25	P = .34
	NSAIDs	P = .12	P = .23	P = .47
	Colloids	P = .72	P = .16	**P = .01**
	MODS	P = .49	P = .24	P = .57
	Blood products	P = .96	P = .24	P = .43
UNCR	Sepsis	**P < .001**	P = .29	P = .12
	AKI	P = .25	P = .1	P = .5
	Hypotension	P = .55	P = .08	P = .75
	Survival	P = .08	P = .11	P = .19
	NSAIDs	**P = .04**	P = .08	P = .21
	Colloids	**P = .04**	P = .12	P = .07
	MODS	P = .49	P = .08	P = .44
	Blood products	P = .21	P = .1	P = .07

AKI, acute kidney injury; NSAID, nonsteroidal anti-inflammatory drug; MODS, Multi-organ dysfunction syndrome; UNCR, urinary NGAL normalized to creatinine.

Significant differences are highlighted (bold; significance set at a P value < .05)

between UNCR and APPLE full score at any of the time points (Table 4).

Discussion

Our study focused on detecting the effect of sepsis on sNGAL and UNCR and to assess their changes over the course of hospitalization. The control population was chosen to compare the effect of general anesthesia and surgery on NGAL in comparison to the septic dogs undergoing emergency laparotomy. This group was also chosen because it theoretically allowed timed and noninvasive urine sampling because of the routine placement of urinary catheters or bladder expression.

Sepsis has been shown to be associated with development of AKI secondary to ischemia-reperfusion injury, damage induced by pro-inflammatory cytokines and oxidative stress and tubular dysfunction leading to decreased glomerular filtration and tubular reabsorbtion dysregulation.[22] Neutrophil gelatinase-associated lipocalin is secreted by neutrophils and it is therefore logical to expect an increase in its concentration in inflammatory states. In particular, during sepsis, NGAL expression increases, not only in the kidney but also in leukocytes and liver. Therefore, higher
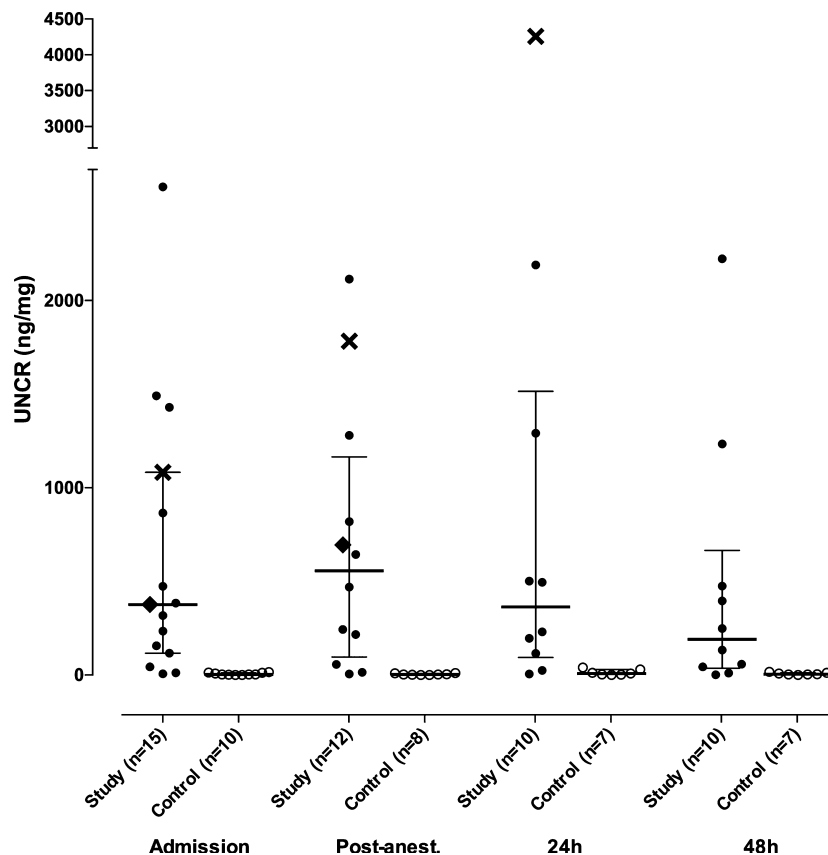
Fig 2. The medians and interquartile ranges of urinary neutrophil gelatinase-associated lipocalin to creatinine ratio (UNCR) at different times between the control and the study group are expressed by the bar and the whiskers, respectively, with the single patients displayed in a scatter plot manner. The 2 patients with acute kidney injury have been identified with x and ♦. The study group had significantly higher UNCR across all the time points (P < .001).
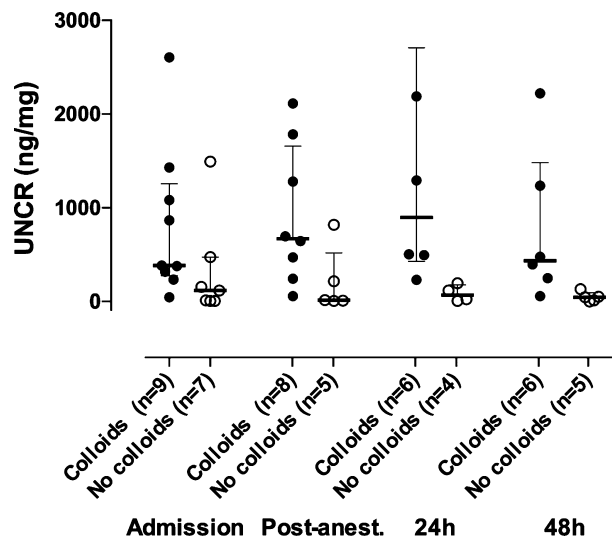
Fig 3. The medians and interquartile ranges of urinary neutrophil gelatinase-associated lipocalin to creatinine ratio (UNCR) at different times between patients that received colloids and patients that did not are expressed by the bar and the whiskers, respectively, with the single patients displayed in a scatter plot manner. Statistical significance was set at $P < .05$. Dogs that received colloids had significantly higher UNCR concentration across all time points.

NGAL both in blood and in urine are expected, regardless of AKI.[11] A study in human patients showed that sNGAL was increased in patients with sepsis, severe sepsis, and septic shock, but was not able to detect AKI in this population, especially in the sicker categories.[16] The results of the present study confirm that sNGAL was also increased in dogs with sepsis compared to a control population. However, sNGAL was not associated with mortality or correlated with the severity of the disease, as assessed by the APPLE scoring system or MODS development, suggesting that this marker may not be a useful clinical severity predictor.

The UNCR differed between the study and control groups across all time points was evaluated ($P < .001$). The median UNCR concentration in the study group

postanesthesia was as high as 114 times (admission) and 185 times higher than in the control group, highlighting the marked difference between the 2 groups. However, similar to sNGAL, UNCR was not associated with mortality, suggesting that this marker may not be useful to assess the severity of disease.

A study in people showed that septic AKI patients had higher urinary NGAL than nonseptic AKI patients.[23] Both sNGAL and UNCR were not associated with AKI development over the course of hospitalization in this study. This finding may be a consequence of the inability of NGAL to distinguish between the presence of AKI and systemic inflammation in septic dogs, and as a result may suggest that NGAL is a poor marker of AKI in this population of dogs. The lack of significance to detect AKI, however, could have been a result of the small population size and thus indicate a type II error.

Neutrophil gelatinase-associated lipocalin increases 3 hours after ischemic renal injury in people undergoing cardio-pulmonary bypass and peaks at 36 hours in critically ill patients developing AKI during hospitalization.[24] However, NGAL has been shown to increase up to 72 hours earlier than serum creatinine concentration in the event of AKI. Therefore, this study may not have detected an increase in serum creatinine concentration if it occurred after the 48 hours in sample, potentially leading to AKI recognition being missed in some patients.[10,23] This time window was selected based on evidence in human patients showing that adverse outcome is associated with increased serum creatinine concentration within 48 hours, and the immediate postoperative period was deemed most appropriate to detect eventual development of AKI.[13,25]

The increase in UNCR in dogs with sepsis in our study perhaps represents sub-clinical AKI, not detected by the VAKI system, or may have been related to an increase in serum creatinine concentrations that occurred after 48 hours.[4] The increase in serum creatinine concentration used to define AKI in our study was considered to be a sensitive way of identifying these dogs. However, serum creatinine concentration may not be altered in the event of tubular injury, indicated by an increase in NGAL production, because the GFR of

Table 5. Spearman's correlation (r_s) between UNCR and UP:C, serum creatinine, sNGAL, day in ICU and APPLE score.

	UP:C	Serum creatinine	sNGAL	Days ICU	APPLE score
UNCR admission	$UP:C_{adm}$ $P < .001$; r_s 0.61	$Creat_{adm}$ $P = .17$	$sNGAL_{adm}$ $P < .01$; r_s 0.76	$P < .001$; r_s 0.7	$P = .44$
UNCR post	$UP:C_{post}$ $P < .001$; r_s 0.92	$Creat._{post}$ $P = .03$; r_s 0.54	$sNGAL._{post}$ $P < .01$; r_s 0.82	$P < .001$; r_s 0.77	$P = .71$
UNCR 24	$UP:C_{24}$ $P < .001$; r_s 0.87	$Creat._{24}$ $P = .11$	$sNGAL_{24}$ $P = .07$	$P < .001$; r_s 0.79	$P = .08$
UNCR 48	$UP:C_{48}$ $P = .001$; r_s 0.75	$Creat._{48}$ $P = .15$	$sNGAL._{48}$ $P = .33$	$P < .001$; r_s 0.77	$P = .2$

sNGAL, serum neutrophil gelatinase-associated lipocalin; APPLE, acute patient physiologic and laboratory evaluation score; UNCR, urinary NGAL normalized to creatinine.

Statistical significance was set at $P < .05$.

the affected nephrons may not be altered or overall renal compensation may occur in other nephrons. Conversely, tubular injury, detected by an increase in NGAL concentration, may lead to a decrease in glomerular function as a consequence of tubular flow obstruction and tubulo-glomerular interaction.[26] In this situation, NGAL may not be correlated with GFR, and its increase does not consistently reflect a change in serum creatinine concentration.

It is unlikely that the difference in UNCR between the 2 groups was a consequence of altered passage of NGAL across the glomerular filtration barrier. In fact, although there was an association between UP:C and UNCR, sNGAL, and UNCR were only moderately correlated on admission and post anesthesia. It appears more likely that in severe inflammation, renal derangements lead to decreased re-absorptive capacity in the proximal tubules or upregulation of NGAL production at the level of the thick ascending loop, distal tubule, and collecting ducts.[10]

One study showed that, in dogs undergoing surgery, urinary NGAL was significantly higher in dogs with AKI 12 hours postoperatively, whereas plasma NGAL was not able to detect AKI.[13] Another study in dogs with a variety of diseases, including heatstroke and snake envenomation, showed that UNCR on admission was significantly higher in dogs with AKI (grade I to V) and detected AKI earlier than the increase in plasma creatinine concentration.[12] However, 9 of 15 dogs (60%) in our study had UNCR results on admission higher than the cutoff of 238 ng/mg established by a previous study for distinguishing nonazotemic AKI grade I patients from dogs with related renal or urinary conditions.[12] However, the pathophysiology of AKI in sepsis is substantially different from other causes of AKI, such as ischemia or toxic damage, and NGAL expression is upregulated in inflammatory conditions, such as infection.[21,22] Of the 9 dogs with UNCR >238 ng/mg, only 2 developed AKI and none of them developed azotemia. This cutoff may therefore not be useful in dogs with sepsis and additional studies are needed to establish a cutoff for this population.

Both UNCR and sNGAL were not significantly associated with time to discharge, but were associated with ICU stay. Timing of discharge and ICU hospitalization are clinician-dependent and financially based decisions and these factors might have influenced this result. In addition, our control population was represented by patients with IVDD, which have predictably long hospitalization times.

Patients that did not receive NSAIDs before admission had higher UNCR compared to patients that did across all time points. This difference may have been simply a consequence of more severe disease in dogs not receiving NSAIDs, because there currently is no evidence that NSAIDs may induce urinary NGAL downregulation.

An interesting finding was the significant difference in UNCR between the dogs that received colloids and dogs that did not. The higher UNCR in dogs that received colloids was consistent across all 4 time points, and UNCR was increased before surgery although only 4 of the 9 dogs were given colloids on admission, whereas 4 received them intra-operatively and 1 postoperatively. This difference may simply reflect a difference in severity of illness, because colloids typically are used more often in dogs with more serious signs of hypoperfusion and potentially more severe inflammation. This may have induced an upregulation of NGAL secretion and an increase in UNCR independent of colloid administration.[11]

In people, hydroxyethyl starches are associated with an increased risk of kidney injury, and recent studies have highlighted an association between the use of hydroxyethyl starches (including pentastarch and tetrastarch) and kidney injury or renal replacement therapy requirement.[27,28] Usually, an increase in uNGAL is expected to occur at least 3 hours after the renal injury.[11] If these artificial colloids induced AKI in the dogs of our study, a difference at the pre-anesthesia sample would not be expected, because most patients did not receive hydroxyethyl starches until intra-operatively or postoperatively. The results of our study should therefore be interpreted with caution.

One limitation of this study is that there were 21/100 urine samples and 40/100 serum samples missing. Analysis of all of the samples may have added further information to our analysis and allowed better interpretation of the data. The absence of results, in addition to the small number of cases enrolled, therefore may have caused the analysis to be underpowered.

A second limitation involves the choice of the control group. The median admission data for this group was not significantly different from that of the study group, leading to a potential confounding overlap within the population (Tables 1 and 2). However, the underlying diseases affecting the 2 groups were substantially different, being localized in the control dogs and systemic and pro-inflammatory in the study patients. Additional studies may benefit from the use of more stringent SIRS criteria, allowing clear clinical distinction between the control and the study group on admission.

In addition, our study monitored serum creatinine concentration for a 48-hour period. Because NGAL may increase up to 72 hours earlier than the serum creatinine concentration in the event of renal injury, some patients with AKI may have been missed.[11,24]

A further limitation includes the clinician-based decision for administration of hydroxyethyl starches, blood products or institution of vasopressor therapy, rendering objective interpretation of these data or assessment of the severity of illness difficult. It is also currently unknown whether drugs, such as human serum albumin, may affect serum and urinary NGAL release and tubular re-absorption, interfering with the results of the study.

Additional prospective studies with larger populations of dogs with sepsis and use of additional urinary markers are warranted to assess the association between NGAL and inflammation, sepsis and AKI.

Footnotes

Acknowledgment

References

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