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Endotoxic kidney injury in Beagle dogs assessed by serum creatinine and symmetric dimethylarginine, and urinary neutrophil gelatinase-associated lipocalin and clusterin

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ABSTRACT

Sepsis of Gram negative bacterial origin results in lipopolysaccharide-induced endotoxemia. This often leads to acute kidney injury (AKI) and its recognition remains a challenge and delays treatment. As renal damage occurs before a rise in serum creatinine is detected, new early biomarkers of kidney injury need to be explored. The aim of this study was to determine changes in serum parameters of renal function and urine biomarkers of renal injury. This was a descriptive study. Endotoxemia was induced intravenously in six anaesthetized Beagles (T1). To achieve normotension, dogs received fluids (T2), followed by a continuous infusion of noradrenaline and dexmedetomidine or 0.9% NaCl (T3). Ten minutes later, the dogs received fluids (T4) and noradrenaline and dexmedetomidine or 0.9% NaCl in a crossover manner (T5). At each timepoint, blood and urine were collected for serum creatinine, urea, symmetric dimethylarginine, urine protein/creatinine (UPC) ratio, urine neutrophil-gelatinase-associated lipocalin (U-NGAL), U-NGAL/creatinine ratio, urine clusterin (U-clusterin) and U-clusterin/creatinine ratio. Data were analyzed using a mixed-effect model taking into account time and stage of veterinary AKI (VAKI). Three of six dogs had a VAKI stage ≥1; one with anuria and elevated creatinine. Serum creatinine ($P < 0.001$), U-NGAL/creatinine ratio ($P = 0.01$) and U-clusterin/creatinine ratio increased over time ($P < 0.01$). The UPC ratio (mean (range) 0.68 (0.35–2.3) versus 0.39 (0.15–0.71) $P < 0.01$) and U-NGAL (3164 pg/mL (100–147,555) versus 100 (100–14,524), $P = 0.01$) were higher in VAKI stage ≥1 versus stage 0, respectively. Endotoxemia induced VAKI stage ≥1 in half of the dogs. Repeated measurement of selected parameters could detect AKI early.

1. Introduction

Sepsis is characterized by a systemic inflammatory response of the organism to an infectious insult, usually of bacterial origin, that can lead to extensive tissue damage and multiple organ dysfunction. One of the most common complications of sepsis is the development of acute kidney injury (AKI) (Ali et al., 2007; Umbro et al., 2016). Sepsis-induced AKI is diagnosed in 40–50% of human patients in intensive care units (Gómez and Kellum, 2016) and increases the mortality rates by six to eight-fold. In veterinary medicine, sepsis-related AKI was reported to occur in 12% of dogs with septic abdomen (Kenney et al., 2010). The mortality rate in humans and animals with AKI is around 50% (Cho et al., 2018), possibly due to the difficulty of early diagnosis.

The most widely used scales for the diagnosis and classification of AKI in dogs today are veterinary International Renal Interest Society (IRIS) (Cowgill, 2016) and the Veterinary Acute Kidney Injury (VAKI) guidelines (Thoen and Kerl, 2011). Both classifications rely heavily on serum creatinine, although acute changes in its concentration lag behind both the development and recovery of kidney injury (Prowle, 2013). Creatinine remains the main criterion for classifying AKI mostly because of its wide availability. However, the opportunity for an early and effective treatment may have passed by the time AKI is detected with the IRIS or VAKI classification. Therefore, several biomarkers of early kidney injury were explored in plasma and urine of dogs (Boyd et al., 2019;

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García-Martínez et al., 2012; Lee et al., 2012; Monari et al., 2020; Peris et al., 2020), rats (Mohamed et al., 2017) and humans (Fuchs and Hewitt, 2011; Lane et al., 2020; Pianta et al., 2017; Shemin and Dworkin, 2011; Siew et al., 2011) including neutrophil-gelatinase-associated lipocalin (NGAL) and clusterin.

NGAL is a 25 kDa protein covalently bound to neutrophil gelatinase whose expression is markedly increased following injury to the renal tubular epithelium (Mishra et al., 2004). In azotemic dogs, plasma NGAL and urinary NGAL/creatinine ratio (U-NGAL/creatinine) were increased compared with those dogs without azotemia (Steinbach et al., 2014). Increased U-NGAL was detected in a hemorrhagic dog model as early as 2 h after reperfusion (Davis et al., 2016). U-NGAL is shown to have a high sensitivity and specificity to distinguish AKI from non-AKI in human patients (Nickolas et al., 2008). However, the specificity of U-NGAL to distinguish AKI in septic humans is low (Shapiro et al., 2010) as neutrophils increase circulating NGAL during systemic inflammation (Mårtensson and Bellomo, 2014; Otto et al., 2015). The specificity of U-NGAL to discriminate AKI in septic dogs could not be demonstrated, however U-NGAL values were higher in septic than in control dogs (Cortellini et al., 2015).

Clusterin is a glycoprotein consisting of two 40 kDa subunits linked by disulphide groups (Blaschuk et al., 1983). In monkeys receiving a nephrotoxic dose of gentamicin, urinary clusterin (U-clusterin) increased earlier than serum creatinine (Gautier et al., 2016). Although U-clusterin was specific for tubular injury in one study (Hidaka et al., 2002), it is currently considered a renal biomarker not restricted to a particular region of the nephron (Dieterle et al., 2010). To the authors' knowledge, no studies have investigated over time changes of the above two biomarkers of renal damage in the urine of septic dogs.

The aim of this study was to determine whether *Escherichia coli* lipopolysaccharide (LPS)-induced endotoxemia can lead to AKI in Beagle dogs within hours, as evidenced by serum renal function parameters and urinary biomarkers NGAL and clusterin. We hypothesized that 30% of dogs will develop AKI, with urinary concentrations of NGAL and clusterin being significantly different between dogs with and without AKI.

2. Materials and methods

Ethical committee approval was obtained from the Swiss authorities of the Canton of Zurich (ZH244/17, approved 30 March 2018). Six purpose-bred Beagle dogs (three intact females and three intact males) with a median age of 7.4 years (range: 5–9.8) and a body weight of 13.7 kg (range: 11.4–17.9) were included in the study. The dogs were scheduled for euthanasia under anesthesia due to another study (ZH057/17), with no impact on our study. A year ago, dogs were enrolled in a parasitological study where they were infected with *Dirofilaria repens* and treated for it. During the anesthesia of the current study, for purposes related to the aforementioned project, dogs also underwent CT and MRI, cardiac output monitoring via thermodilution, and microcirculation measurement using side stream dark field camera. Based on physical examination, complete blood cell count, serum biochemistry, blood gas and urinalysis, all dogs were considered healthy at the time of enrolment. The animals were treated according to the principles of the Swiss government guidelines.

2.1. Preanesthetic preparation and anesthesia

Before the procedure, the dogs were fasted for 12 h, with free access to water. They were premedicated with methadone 0.2 mg/kg intramuscularly (Methadone; Streuli Pharma). An intravenous (IV) catheter (Vasofix 20G; Braun) was aseptically placed in a cephalic vein and anesthesia was induced with propofol to effect (Propofol 1%; Fresenius Kabi). The dogs were intubated and anesthesia was maintained with sevoflurane (Sevorane; AbbVie, Switzerland) with end-tidal concentration 1.8 vol% in an oxygen-air mixture (inspiratory oxygen 60%). Ringer's acetate (Ringer's Acetate; Fresenius Kabi) was administered at 5 mL/kg/h IV. Anesthesia monitoring included heart rate, blood pressure, respiratory rate, tidal volume, peripheral oxygen saturation and end-tidal carbon dioxide concentration. A constant rate infusion (CRI) of fentanyl (Fentanyl; Sintetica) was administered at 5 μg/kg/h. Urinary catheterization with Foley catheter (8Fr, 55 cm, MILA) was performed in all dogs.

After completion of the preparatory phase, baseline blood and urine samples were collected as described below and 1 mg/kg of *Escherichia coli* LPS endotoxin (Lipopolysaccharides from *Escherichia coli* O111:B4; Sigma-Aldrich) diluted in 20 mL saline (NaCl 0.9%; Braun) was injected IV over 10 min. After induction of endotoxemia the dogs were resuscitated with 30 mL/kg Ringer's acetate IV given over 30 min and then the noradrenaline infusion was titrated (initial rate of 0.05 μg/kg/min IV) to achieve a mean arterial pressure (MAP) of 85–90 mmHg. In addition, the dogs received a 0.5 μg/kg/h dexmedetomidine CRI or a 0.9 % NaCl infusion at the same volume for the purpose of aformentioned study. After discontinuation of the CRIs and a washout period of 10 min, the entire treatment including the 30 mL/kg Ringer's acetate bolus was repeated in a crossover procedure (Fig. 1). The order of treatments was randomized by drawing lots from an envelope and the researchers were blinded to treatment.

Blood and urine samples were collected before endotoxin administration (T0), at the end of endotoxin administration (T1), at the end of the first fluid therapy (T2), after reaching MAP of 85–90 mmHg in the first phase (T3), at the end of the second fluid therapy (T4) and after reaching MAP of 85–90 mmHg in the second phase of the study (T5) as shown in Fig. 1.

Urine collected through the urinary catheter (by complete emptying of the urinary bladder) was quantified and a sample was sent for direct analysis of protein, creatinine, dipstick assessment and sediment analysis. Creatinine was measured by enzymatic colorimetric assay (Jaffé method), urea by kinetic test, UPC by turbidometry, urine specific gravity by refractometry and renal biomarkers and urine analysis were measured and analyzed at the in-house laboratories of the University of Zurich. A sample was frozen at −80 °C for batch analysis (12 months later) of U-NGAL and U-clusterin using commercially available ELISA kits (Dog NGAL ELISA Kit; BioPorto Diagnostics (Nabity et al., 2012) and Canine Clusterin ELISA, BioVendor (García-Martínez et al., 2012), respectively). The analysis of U-NGAL and U-clusterin were performed according to the manufacturer's instructions. For U-NGAL, samples were diluted 1:500 and if the concentrations were below the standard range, measurements were repeated with a dilution of 1:100. For U-clusterin, samples were diluted 1:10 and if the concentrations were below the standard range, measurements were repeated with a 1:3 dilution. The ratios of urinary protein, U-NGAL and U-clusterin to creatinine were calculated. Blood was drawn, centrifuged and the serum used to measure creatinine and urea concentrations. The leftovers were frozen at −80 °C for batch analysis (12 months later) of symmetric dimethylarginine (SDMA) in a commercial laboratory (IDEXX Diavet).

As IRIS classification takes into account changes of creatinine within 48 h and our study had a duration of only a few hours we chose VAKI classification. We defined each dog as being in VAKI stage 0, 1, 2 or 3 at each time point based on serum creatinine concentrations or creatinine increase (Thoen and Kerl, 2011). Briefly, the criteria for defining VAKI stage 0 were defined as creatinine rise <150% from baseline, stage 1 as creatinine rise of 150–199% from baseline or creatinine rise of 26.5 μmol/L (0.3 mg/dL) from baseline, stage 2 as a creatinine increase of 200–299% from baseline and stage 3 as creatinine increase of ≥300% from baseline or an absolute creatinine concentration > 354 μmol/L (4.0 mg/dL).

2.2. Statistical analysis

Data was explored with descriptive statistics and the mixed-effect model analysis. The concentrations of U-NGAL and U-clusterin below

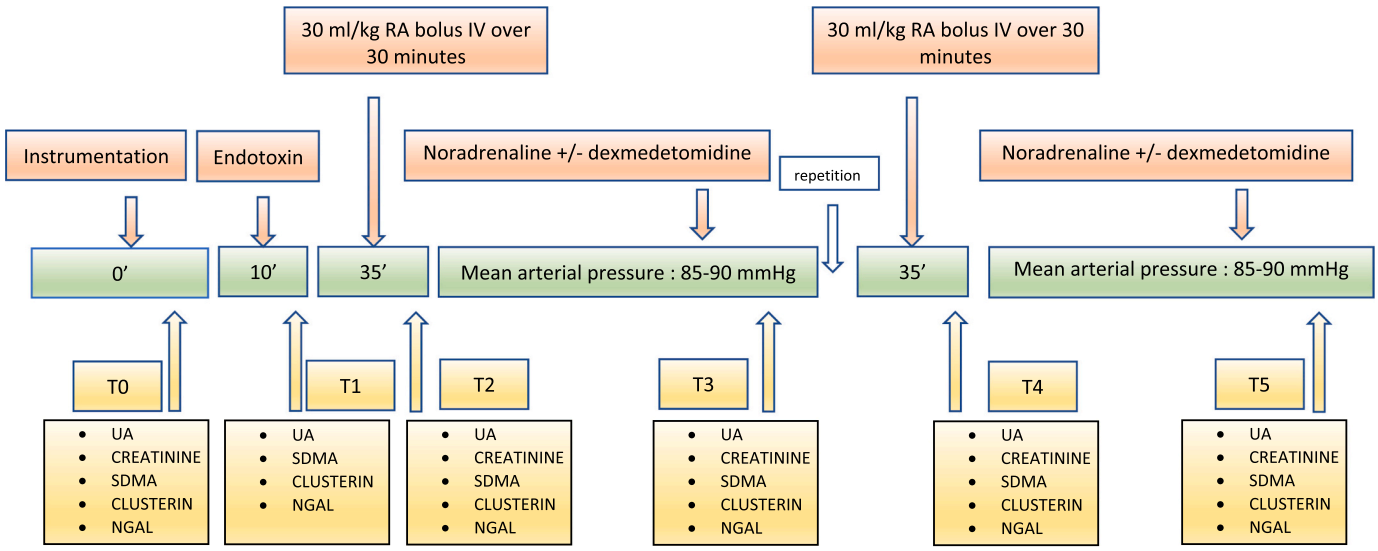

Fig. 1. Schematic presentation of study protocol.

the detection limit of the assay were arbitrarily replaced by a value of 400 pg/mL and 12 ng/mL, respectively, corresponding to the lower limits of quantification. For U-clusterin, concentrations above the detection limit even after additional dilution were replaced by a value of 640 ng/mL, the highest concentration measured. Serum renal function parameters creatinine, urea and SDMA, and the urinary biomarkers U-NGAL and U-clusterin, as well as the UPC ratio of dogs that met the criteria for VAKI stage ≥1 were compared with those with VAKI stage 0 using a mixed-effect model taking into account time, VAKI stage and their interaction. Statistical analysis was performed using Prism 9 (GraphPad Software). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Samples and VAKI stages

A total of 59 blood samples and 161 urine samples were collected from the six dogs and analyzed. In one dog (#6) no blood sample was available at T0 and T3 for SDMA. In the same dog, no urine sample was available at T2 and in another dog (#5) at T2 and T4 due to anuria.

Urine analyses revealed no casts or glucosuria however in all dogs there was presence of blood in the urine.

Based on serum creatinine, urea and SDMA concentrations, as well as urinalysis and UPC ratio, none of the dogs were affected by nephropathy at baseline (T0). In addition, serum creatinine and urea concentrations remained within the reference interval in all dogs at all time points, except for the former which increased transiently in one dog (#5, at T5). Based on the increased serum creatinine concentrations over time, VAKI was induced in three of the six dogs after endotoxin administration (#1, #5 and #6; Fig. 2); VAKI stage 1 from T2 and T3 onwards (#6 and #1) and VAKI stage 2 from T3 onwards (#5).

3.2. UPC ratio, urine output, urine specific gravity and creatinine clearance

The UPC ratio was above 0.5 in all dogs except one (#2), in which the highest value was 0.49 (Fig. 3). The UPC ratio was higher in dogs with VAKI stage ≥1 versus those with VAKI stage 0 (Table 1) ($P < 0.01$). After endotoxin administration, the median urine output was 1.24 mL/kg/h (range: 0.08–2.46) in dogs with AKI and 1.66 mL/kg/h (range: 1.24–11.65, $P = 0.5$) in those without AKI. In five of the six dogs urine output remained 1–2 mL/kg/h after endotoxin administration, whereas in one dog (#5, with VAKI stage 2) urine output decreased and developed into anuria. Urine specific gravity was <1.030 in four of the six dogs at the end of anesthesia; all dogs received intravenous fluids. Endogenous creatinine clearance was calculated ((urine creatinine x

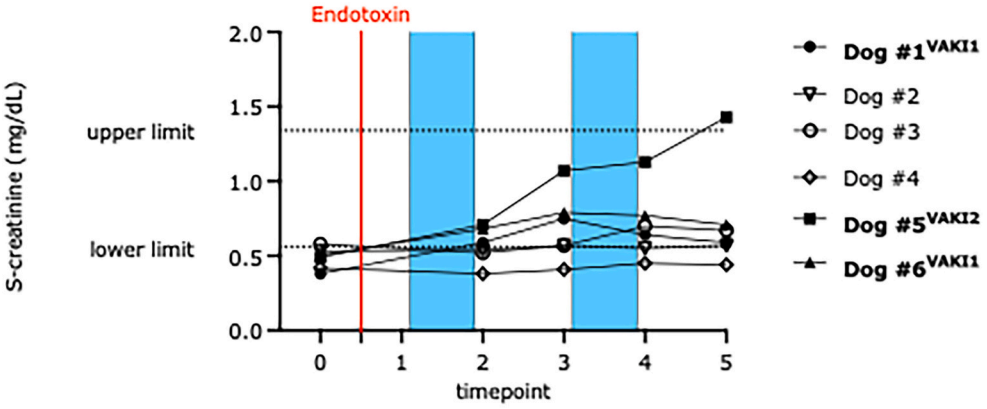

Fig. 2. Serum creatinine concentrations (mg/dL) in the 6 dogs treated with LPS at the 5 different timepoints.
Legend: S-, serum. Shaded area represents the administration of a 30 mL/kg Ringer's acetate bolus over 30 min. Vertical endotoxin line represents the point of endotoxin administration. Dogs that were diagnosed with AKI according to VAKI classification (serum creatinine increase of ≥26.5 μmol/L or increase ≥150% from baseline) stage ≥1, are marked with bold font and VAKI stage in superscript. The reference interval for serum creatinine is marked with interrupted lines and marked with upper and lower limit. Timepoint T0 represents the baseline before endotoxin, T1 is the end of endotoxin administration (no sample taken at this timepoint), T2 is the end of the first fluid therapy, T3 is the timepoint when mean arterial pressure (MAP) of 85–90 mmHg was achieved in the first phase, T4 is the end of the second fluid therapy and T5 is the timepoint when MAP of 85–90 mmHg was achieved in the second phase of the study.

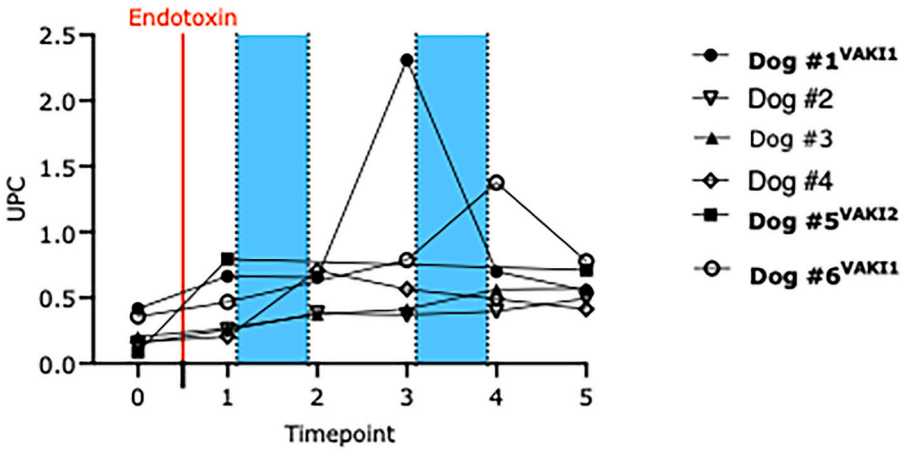

Fig. 3. Urine protein-creatinine ratio in the 6 dogs treated with LPS at the 6 different timepoints.
Legend: UPC- urine protein creatinine ratio. Shaded area represents the administration of a 30 mL/kg Ringer's acetate bolus over 30 min. Vertical endotoxin line depicts the endotoxin administration. Dogs that were diagnosed with AKI according to VAKI classification (serum creatinine increase of ≥26.5 μmol/L or 150% from baseline) stage ≥1, are marked with bold font and VAKI stage in superscript. There is a missing value for dog #6 at T3 (sample unavailable). T0 represents the baseline before endotoxin administration, T1 is the end of endotoxin administration, T2 is the end of the first fluid therapy, T3 is the timepoint when mean arterial pressure (MAP) of 85–90 mmHg was achieved in the first phase, T4 is the end of the second fluid therapy and T5 is the timepoint when MAP of 85–90 mmHg was achieved in the second phase of the study.

Table 1
Serum creatinine and SDMA concentrations, UPC ratio, U-NGAL concentrations, U-NGAL/creatinine ratio, U-clusterin concentrations and U-clusterin/creatinine ratio in the 6 dogs (3 with VAKI stage 0 and 3 with VAKI stage ≥1).

	VAKI stage 0 ($n = 3$)	VAKI stage ≥1[b] ($n = 3$)	Time effect	VAKI effect	Time×VAKI effect
Serum creatinine			$P < 0.01$[a]	$P = 0.39$	$P = 0.46$
(μmol/L)	48 (34–62)	63 (34–126)			
(mg/dL)	0.54 (0.38–0.7)	0.71 (0.38–1.43)			
Serum SDMA (μg/dL)	9 (8–13)	13 (8–19)	$P = 0.36$	$P = 0.16$	$P = 0.06$
UPC ratio	0.39 (0.15–0.71)	0.68 (0.35–2.3)	$P = 0.08$	$P < 0.01$[a]	$P = 0.11$
U-NGAL			$P < 0.01$[a]	$P = 0.01$[a]	$P < 0.01$[a]
(pg/mL)	100 (100–14,524)	3164 (100–147,555)			
(ng/mL)	0.1 (0.1–14.52)	3.2 (0.1–147.55)			
U-NGAL/creatinine ratio			$P = 0.01$[a]	$P = 0.09$	$P = 0.02$[a]
(pg/mg)	18.92 (6.83–2986)	343.9 (14.81–22,176)			
(ng/mg)	0.02 (0.01–2.99)	0.3 (0.02–22.17)			
U-clusterin			$P < 0.01$[a]	$P = 0.06$	$P = 0.02$[a]
(ng/mL)	120.7 (20.88–245.9)	203.4 (10–640)			
U-clusterin/creatinine ratio			$P < 0.01$[a]	$P = 0.39$	$P = 0.46$
(ng/mg)	11.8 (3.56–164.2)	35.3 (0.78–197.1)			

Legend: SDMA, symmetric dimethylarginine; UPC, urine protein/creatinine; U-NGAL, urinary neutrophil-gelatinase-associated lipocalin; VAKI, veterinary acute kidney injury; U-, urinary. [a]Significant *P*-values ($P < 0.05$). [b]Dogs with serum creatinine concentrations increased by 26.5 μmol/L or > 150% from baseline were grouped as VAKI stage ≥1. Values are presented as median and range.

urine volume) /serum creatinine concentration)) and reported as creatinine clearance/min/kg (Cockcroft and Gault, 1976). In VAKI dogs creatinine clearance was 0.001 mL/min/kg (0–0.014) and in non-VAKI dogs 0.004 mL/min/kg (0.001–0.014, $P = 0.1239$).

3.3. Effect of time on blood and urine parameters, and VAKI stages

Values for serum creatinine, serum SDMA, UPC ratio, U-NGAL, U-NGAL/creatinine ratio, U-clusterin and U-clusterin/creatinine ratio are shown in Table 1. There was a significant effect of time on the increase in serum creatinine ($P < 0.01$), U-NGAL ($P < 0.01$), U-NGAL/creatinine ratio ($P < 0.05$), U-clusterin ($P < 0.01$) and U-clusterin/creatinine ratio ($P < 0.01$). U-NGAL ($P < 0.05$) was significantly higher in dogs with VAKI stage ≥1 versus VAKI stage 0. There was a significant effect of time × VAKI on the increase in U-NGAL ($P < 0.01$), U-NGAL/creatinine ratio ($P = 0.02$) and U-clusterin ($P = 0.02$) in VAKI stage ≥1 versus VAKI stage 0.

3.4. Comparison of VAKI stages

Serum SDMA concentrations were within the reference interval in all dogs, except in the dog that was diagnosed with VAKI stage 2 that developed anuria and creatinine above the reference interval (dog #5) (Fig. 4). Concentrations of U-NGAL were approximately 10 times higher in dogs with VAKI stage 1 (dog # 1, #6) than the highest concentration reached in dogs with VAKI stage 0 (dog # 4), i.e., > 140,000 vs. > 14,000, respectively (Fig. 5). The U-NGAL/creatinine ratio in dogs with VAKI stage 1 (dog # 1 and #6) were about 4–7 times higher than the highest ratio reached in dogs with VAKI stage 0 (dog # 4); 22,175 pg/mg, 12,506 pg/mg, versus 2985 pg/mg, respectively (Fig. 6). U-clusterin concentrations were above the detection limit (640 ng/mL) in dogs with VAKI stage 1 (dog # 1 and #6), versus the highest concentration obtained in dogs with VAKI stage 0 (dog # 3), i.e. > 640 ng/mL versus 245 ng/mL, respectively (Fig. 7). All dogs reached the highest U-clusterin/creatinine ratio (ng/mg) at the last time point (T5), except dog #5, which was anuric and for which no values were therefore available (Fig. 8). In 18 samples of NGAL and one sample of clusterin the values were below the lower limit of quantification and in four samples clusterin was above the upper detection limit.

4. Discussion

In this study, administration of LPS to Beagle dogs caused AKI according to the VAKI classification in half of the dogs, with one of them reaching serum creatinine and SDMA concentrations above the reference interval and anuria. Serum creatinine concentrations, U-NGAL/creatinine ratio and U-clusterin/creatinine ratio increased over time. The interaction between time and VAKI was associated to the increase in

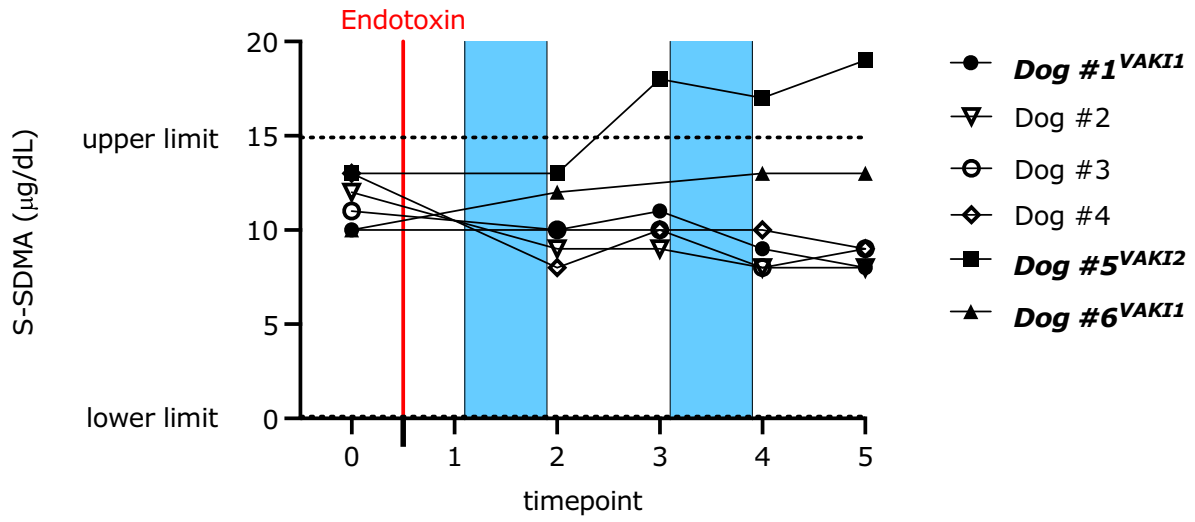

Fig. 4. Serum SDMA concentrations (µg/dL) in the 6 dogs treated with LPS at the 5 different timepoints.
Legend: S- SDMA, serum symmetric dimethylarginine. Shaded area represents the administration of a 30 mL/kg Ringer's acetate bolus over 30 min. Vertical endotoxin line depicts the endotoxin administration. Dogs that were diagnosed with AKI according to VAKI classification (serum creatinine increase of ≥26.5 µmol/L or 150% from baseline) stage ≥1, are marked with bold font and VAKI stage in superscript. The interrupted lines represent the upper and lower limit of reference interval for SDMA. There is a missing value for dog #6 at T3 (sample unavailable). T0 represents the baseline before endotoxin administration, T1 is the end of endotoxin administration, T2 is the end of the first fluid therapy, T3 is the timepoint when mean arterial pressure (MAP) of 85–90 mmHg was achieved in the first phase, T4 is the end of the second fluid therapy and T5 is the timepoint when MAP of 85–90 mmHg was achieved in the second phase of the study.

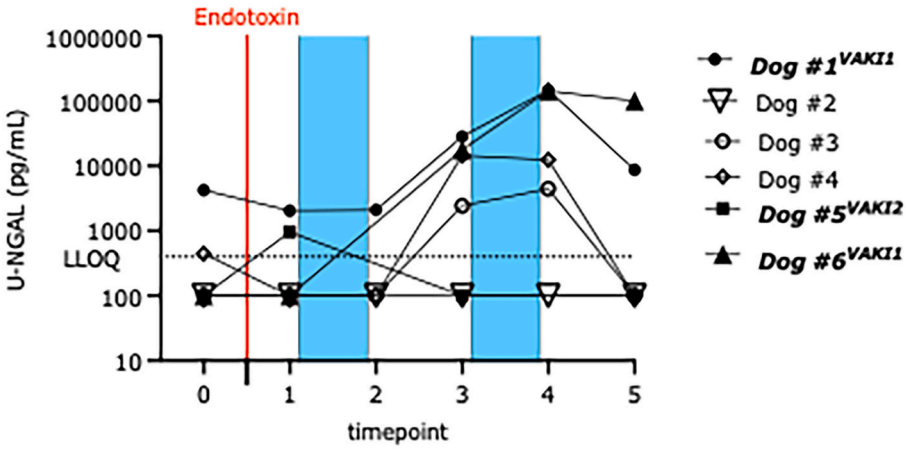

Fig. 5. U-NGAL concentrations (pg/mL) in the 6 dogs treated with LPS at the 6 different timepoints.
Legend: LLOQ, lower level of quantification; U-NGAL, urine neutrophil-gelatinase-associated lipocalin. Shaded area represents the administration of a 30 mL/kg Ringer's acetate bolus over 30 min. Vertical endotoxin line depicts the endotoxin administration. Dogs that were diagnosed with AKI according to VAKI classification (serum creatinine increase of ≥26.5 umol/L or 150% from baseline) stage ≥1, are marked with bold font and VAKI stage in superscript. The interrupted line at U-NGAL 400 pg/mL represents the LLOQ. There are missing values for dog #6 at T2 (sample unavailable) and dog #5 at T2 and T4 due to anuria. T0 represents the baseline before endotoxin administration, T1 is the end of endotoxin administration, T2 is the end of the first fluid therapy, T3 is the timepoint when mean arterial pressure (MAP) of 85–90 mmHg was achieved in the first phase, T4 is the end of the second fluid therapy and T5 is the timepoint when MAP of 85–90 mmHg was achieved in the second phase of the study.

the U-NGAL/creatinine ratio in dogs with VAKI stage ≥1 versus stage 0. The UPC ratio was higher in dogs with a VAKI stage ≥1 versus stage 0.

AKI occurred in 50% of the dogs within 3 h after endotoxin exposure. The frequency is similar to observations in humans, where sepsis-induced AKI was found in approximately 50% of intensive care patients (Ali et al., 2007). In dogs, AKI occurred in 12% of cases with clinical septic abdomen (Kenney et al., 2010), in 26% with acute pancreatitis (Gori et al., 2019) and in 63% with heat stroke (Segev et al., 2015). More recently, using several novel biomarkers of renal injury, Nivy reported hospital-acquired AKI in 13% of non-azotemic dogs (Nivy et al., 2021). It is worth noting that in all aforementioned studies in dogs, except that of Nivy, serum creatinine was used to diagnose AKI, likely underestimating its actual prevalence.

Although we used VAKI classification, during AKI the increase in serum creatinine may be delayed by 48 h, hence its early diagnosis is easily missed. In people (Pickering et al., 2013) with critical illness and septic mice (Doi et al., 2009) creatinine synthesis decreases by 25–50%, making the diagnosis of AKI difficult. In addition, patients with AKI and sepsis are more likely to receive larger volumes of fluid, possibly leading to dilution of serum creatinine concentration. Furthermore, serum creatinine concentration usually exceeds reference values only after at least 75% of renal function is lost. A combination of the above factors was the likely reason to explain why creatinine increased above the reference range only in one dog.

Serum SDMA concentrations remained within normal limits in five of the six dogs, which is not surprising since 40% of renal function must be lost before SDMA increases. This is why SDMA is known to be an earlier marker of renal dysfunction than creatinine, where 75% of renal loss leads to elevated creatinine levels. Nonetheless, similar to creatinine, SDMA did not consistently increase in hospital-acquired AKI in dogs (Nivy et al., 2021). Furthermore, SDMA was considered only a moderate predictor for development of AKI during hospitalization (Dahlem et al., 2017). Of note, the dog that had serum SDMA above the reference range also had elevated creatinine above upper reference value.

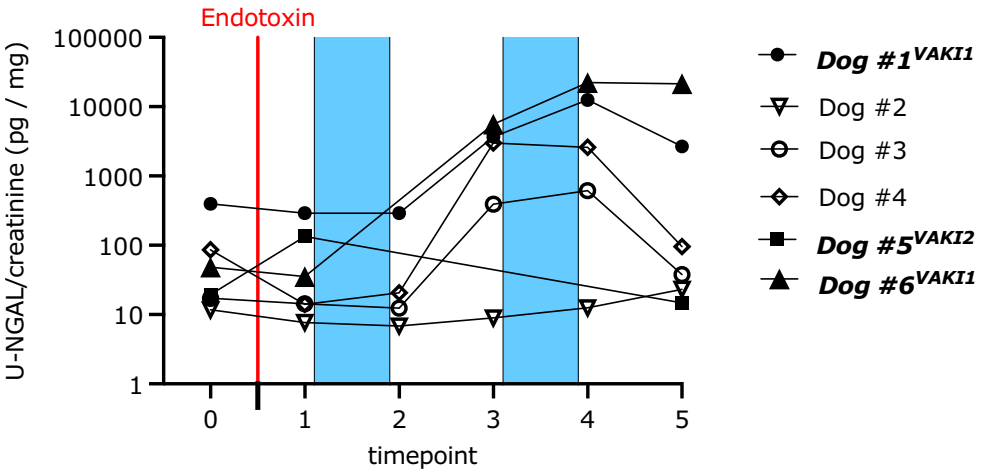

Fig. 6. U-NGAL/creatinine ratio in the 6 dogs treated with LPS at the 6 different timepoints. Legend: U-NGAL, urine neutrophil-gelatinase-associated lipocalin. Shaded area represents the administration of a 30 mL/kg Ringer's acetate bolus over 30 min. Vertical endotoxin line depicts the endotoxin administration. Dogs that were diagnosed with AKI according to VAKI classification (serum creatinine increase of ≥26.5 umol/L or 150% from baseline) stage ≥1, are marked with bold font and VAKI stage in superscript. There are missing values for dog #6 at T2 (sample unavailable) and dog #5 at T2 and T4 due to anuria. T0 represents the baseline before endotoxin administration, T1 is the end of endotoxin administration, T2 is the end of the first fluid therapy, T3 is the timepoint when mean arterial pressure (MAP) of 85–90 mmHg was achieved in the first phase, T4 is the end of the second fluid therapy and T5 is the timepoint when MAP of 85–90 mmHg was achieved in the second phase of the study.

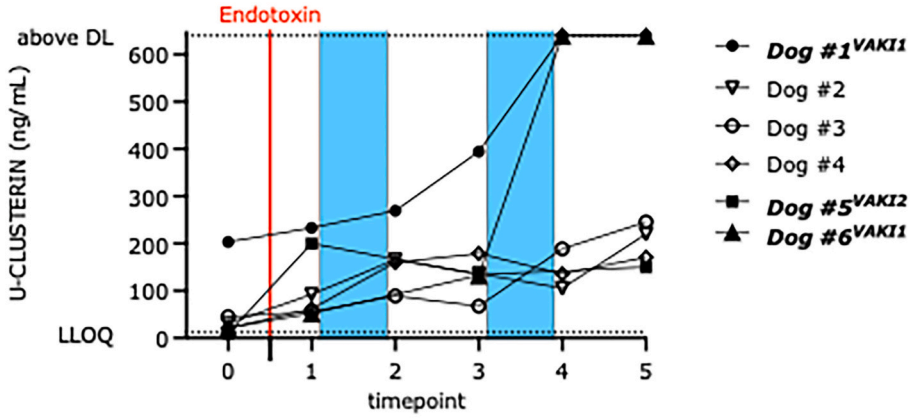

Fig. 7. U-clusterin concentrations (ng/mL) in the 6 dogs treated with LPS at the 6 different timepoints. Legend: DL, detection limit; LLOQ, lower level of quantification; U-, urinary. Shaded area represents the administration of a 30 mL/kg Ringer's acetate bolus over 30 min. Vertical endotoxin line depicts the endotoxin administration. Dogs that were diagnosed with AKI according to VAKI classification (serum creatinine increase of ≥26.5 umol/L or 150% from baseline) stage ≥1, are marked with bold font and VAKI stage in superscript. The interrupted line at urinary clusterin concentration of 12 ng/mL represents the LLOQ and at 640 ng/mL the concentration above the DL considering the applied dilutions. There are missing values for dog #6 at T2 (sample unavailable) and dog #5 at T2 and T4 due to anuria. T0 represents the baseline before endotoxin administration, T1 is the end of endotoxin administration, T2 is the end of the first fluid therapy, T3 is the timepoint when mean arterial pressure (MAP) of 85–90 mmHg was achieved in the first phase, T4 is the end of the second fluid therapy and T5 is the timepoint when MAP of 85–90 mmHg was achieved in the second phase of the study.

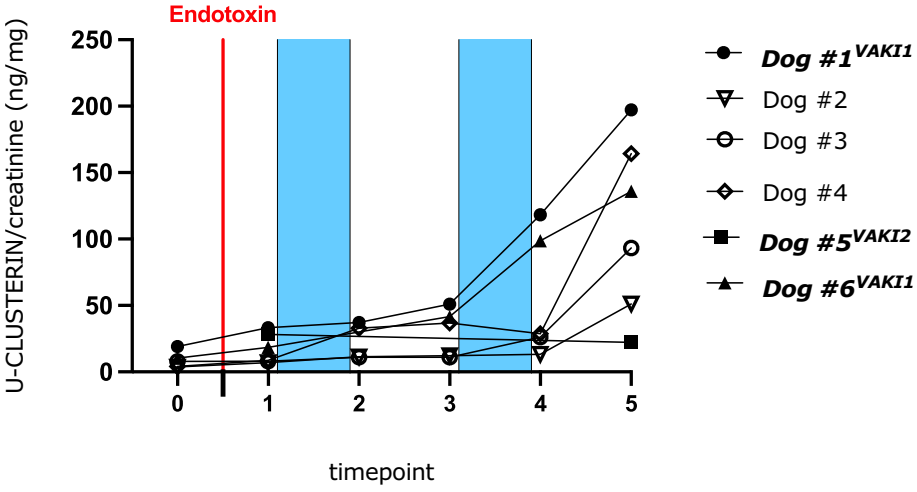

Fig. 8. U-clusterin/creatinine concentrations (ng/mg) in the 6 dogs treated with LPS at the 6 different timepoints.
Legend: U-, urinary. Shaded area represents the administration of a 30 mL/kg Ringer's acetate bolus over 30 min. Vertical endotoxin line depicts the endotoxin administration. Dogs that were diagnosed with AKI according to VAKI classification (serum creatinine increase of ≥26.5 umol/L or 150% from baseline) stage ≥1, are marked with bold font and VAKI stage in superscript. There are missing values for dog #6 at T2 (sample unavailable) and dog #5 at T2, T3 and T4 due to anuria. T0 represents the baseline before endotoxin administration, T1 is the end of endotoxin administration, T2 is the end of the first fluid therapy, T3 is the timepoint when mean arterial pressure (MAP) of 85–90 mmHg was achieved in the first phase, T4 is the end of the second fluid therapy and T5 is the timepoint when MAP of 85–90 mmHg was achieved in the second phase of the study.

The UPC ratio reached values above 0.5 in all but one dog (dog #2) indicating proteinuria. Furthermore, the UPC ratio was higher in dogs with VAKI stage ≥1 versus stage 0. Proteinuria can be due to impaired tubular processing of filtered proteins, altered vascular permeability of the glomeruli, or both (Lees et al., 2005). It is possible that LPS-induced inflammation produced proteinuria through increased glomerular permeability. A recent study (Hall et al., 2021) showed a positive correlation between the UPC ratio, the severity of dental disease and the duration of anesthesia in dogs and cats undergoing dental treatment. Therefore, both inflammation and anesthesia may have contributed to proteinuria in our study. Alternatively, proteinuria suggested tubular injury. Nonetheless, other markers of tubulopathy, such as glucosuria and the presence of casts (De Loor et al., 2013) were not observed (supplementary data).

Creatinine clearance was lower compared to reported values in healthy beagles of 3.7 ± 0.77 mL/min/kg, which may indicate that GFR in our dogs was below normal (Cockcroft and Gault, 1976). We hypothesize that endotoxemia affected renal blood flow.

To overcome the limitations of serum creatinine, U-NGAL, U-clusterin and their ratio to creatinine were studied. All of them increased over time, confirming that they are biomarkers for early detection of AKI in dogs. However, our values were much lower compared to those documented by Monari (Monari et al., 2020). We collected urine samples immediately after LPS administration and stored at −80 °C for 12 months before processing. In contrast, in the investigation by Monari, samples were collected at the time AKI was diagnosed based on creatinine concentration and stored at −80 °C for up to 6 months until assayed. The lower values observed in our series were likely due to sampling of dogs immediately after LPS, rather than the time before analysis. In fact, one study (Schuh et al., 2016) showed no changes in U-NGAL concentration after 5 years at −80 °C. In addition, IV fluid boluses might have resulted in diluted samples. This might reduce U-NGAL and U-clusterin concentrations but not their ratio to creatinine.

Noradrenaline has been shown to improve renal circulation in dogs and therefore may have influenced the results of our study (Peng et al., 2005).

The measurement of U-NGAL and U-clusterin have limitations. Systemic inflammation was shown to increase NGAL due to increased circulating neutrophils (Mårtensson and Bellomo, 2014; Otto et al., 2015). Therefore, it is possible that the inflammation induced by LPS contributed to the increase in NGAL to some extent. Nevertheless, U-NGAL was remarkably higher in dogs with VAKI stage 1 versus stage 0 (approximately 10 times), likely indicating that AKI caused its increase. In addition, Miranda (Miranda et al., 2015) showed that dexmedetomidine has a beneficial effect on microcirculation in endotoxemic hamsters. If dexmedetomidine decreases the degree of LPS-induces inflammation in dogs, the increase in U-NGAL in our study represented a true indicator of AKI. Another limitation is the use of creatinine for indexation as this has been shown to lose accuracy with changes in glomerular filtration rate i.e. AKI (Waikar et al., 2010). As we also had presence of blood in almost all urine samples on urine analysis, this might have explained high values of urinary clusterin (Yerramilli et al., 2016). We hypothesize that the presence of blood in the urine was due to inflammation and or changed glomerular permeability and not trauma, as we catheterized the urethra with soft Foley catheters without any problems. Additionally, Davis et al. reported hematuria related to AKI subsequent to severe hypotension (Davis et al., 2022).

Only in the dog with VAKI stage 2 urine output decreased and anuria was reached, whereas it was normal in the other dogs. LPS-induced inflammation probably impaired microcirculation in this dog, reducing its urine excretion.

The major limitation of this study is the limited sample size, but for ethical reasons it was not possible to include more dogs. In addition, kidney biopsies were not available and glomerular filtration rate was not measured to further characterize renal function. Another limitation is the lack of a control group. Nevertheless, each dog served as its own control before induction of endotoxemia.

5. Conclusion

VAKI occurred in half of the dogs within a few hours of endotoxin exposure. Monitoring trends in creatinine, UPC and SDMA increase during hospitalization of endotoxemic dogs is recommended to early detect and treat AKI. An increase in the U-NGAL/creatinine or U-clusterin/creatinine ratio is currently used in research and could warn of an impending AKI. However, in our study they could not distinguish between dogs with and without AKI.

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Declaration of Competing Interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rvsc.2023.104966.

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