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Concentrations and kinetics of renal biomarkers in dogs with gastric dilatation-volvulus with and without 24-h intravenous lidocaine

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Background: Gastric dilatation volvulus (GDV) can lead to organ failure including acute kidney injury (AKI). Due to its cytoprotective, antioxidant and anti-inflammatory effects, lidocaine has a potential to prevent AKI in dogs with GDV.

Design and setting: Prospective, observational cohort study in client-owned dogs with GDV.

Objective: To determine concentrations of renal biomarkers for AKI in dogs with GDV with and without intravenous (IV) lidocaine therapy.

Methods: Thirty-two dogs were randomized to receive either IV lidocaine (2 mg/kg, followed by a lidocaine constant rate infusion at a dose of 50 μg/kg/min over 24 h; $n = 17$) or no lidocaine ($n = 15$). Blood and urine samples were taken at admission (T_0) (only blood), during or immediately after surgery (T_1), and 24 (T_{24}) and 48 (T_{48}) h after surgery. Plasma creatinine (pCr), plasma neutrophil gelatinase-associated lipocalin (pNGAL), urinary NGAL (uNGAL), uNGAL to creatinine ratio (UNCR), and urinary gamma-glutamyl transferase to creatinine ratio (uGGT/uCr) were evaluated. Biomarker concentrations were compared between dogs with and without IV lidocaine and the course of each marker was determined in comparison to its admission value.

Results: In the entire population, a significantly higher pCr at T_0 (median, 95 μmol/L, interquartile range, 82–105) compared with T_1 (69 μmol/L, 60–78), T_{24} (63 μmol/L, 52–78), and T_{48} (78 μmol/L, 65–87) ($P < 0.001$) was found. Plasma NGAL increased significantly between T_0 (5.66 ng/mL, 3.58–7.43) and T_{24} (7.50 ng/mL, 4.01–11.89) ($P = 0.006$) and T_{48} (9.86 ng/mL, 5.52–13.92) ($P < 0.001$), respectively. Urinary NGAL increased significantly between T_1 (0.61 ng/mL, 0.30–2.59) and T_{24} (2.62 ng/mL, 1.86–10.92) ($P = 0.001$) and T_{48} (4.79 ng/mL, 1.96–34.97 ($P < 0.001$), respectively. UNCR increased significantly between T_1 (0.15 μg/mmol, 0.09–0.54) and T_{24} (1.14 μg/mmol, 0.41–3.58) ($P = 0.0015$) and T_{48} (1.34 μg/mmol, 0.30–7.42) ($P < 0.001$), respectively. Concentrations of uGGT/uCr increased significantly from T_0 highest at T_{24} (6.20 U/mmol, 3.90–9.90) and significantly decreased at T_{48} (3.76 U/mmol, 2.84–6.22) ($P < 0.001$). No significant differences in any renal biomarker concentration were found between dogs with and without IV lidocaine therapy.

Conclusion and clinical relevance: Plasma NGAL, uNGAL and UNCR remained increased up to 48 h post-surgery. No evidence of lidocaine-associated renoprotection was found.

KEYWORDS

AKI, renal biomarker, canine gastric torsion, ischemia-reperfusion, organoprotection, lidocaine

Introduction

Gastric dilatation volvulus (GDV) is an acute and life-threatening condition in large breed dogs. Common consequences are impaired gastric blood supply, obstructive and distributive shock, decreased systemic tissue perfusion and ischemia (1, 2). The most severe complications associated with GDV are ischemic reperfusion-injury (IRI), systemic inflammatory response syndrome (SIRS), and multiple organ dysfunction syndrome (MODS), including cardiac arrhythmias and acute kidney injury (AKI) (2–9). AKI, detected by creatinine-based criteria, has been found to occur in 2.3 and 8% of dogs with GDV, and has been reported to be a significant risk factor for death (3, 8).

Lidocaine is a potent local anesthetic and class Ib antiarrhythmic agent. It also acts as a scavenger of reactive oxygen species and as an inflammatory modulator in both human and veterinary patients and counteracts several pathways in IRI (10, 11). In dogs with experimentally induced gastric dilation, lidocaine treatment was cardio- and gastroprotective and reduced gastric tissue damage (12). Bruchim et al. (3) found a significant decrease in the incidence of AKI and cardiac arrhythmias, and in length of hospitalization in GDV dogs receiving early intravenous (IV) lidocaine, followed by a constant rate infusion (CRI) over 24 h. However, no randomized-controlled trial has evaluated the renoprotective effect of IV lidocaine in GDV dogs.

Neutrophil gelatinase-associated lipocalin (NGAL), a protein expressed during proximal tubular epithelial cell injury, is used as a biomarker in blood and urine to detect early-stage AKI (13–16). NGAL has been found to be one of the earliest and most consistently generated proteins in ischemic and nephrotoxic AKI in human and dog models (17, 18). Urinary NGAL (uNGAL) seems to be more sensitive than plasma NGAL (pNGAL) (19–23), and uNGAL-to-creatinine ratio (UNCR) has been shown to be a specific and sensitive marker for naturally occurring AKI in dogs, although it is also increased in chronic kidney disease (CKD) (24, 25). Urinary gamma-glutamyl transferase (uGGT) is a brush border enzyme located primarily in the metabolically active proximal renal tubule. Higher uGGT to creatinine (uCr) ratios (uGGT/uCr) were found in dogs with AKI and CKD compared with healthy dogs (24, 25).

We conducted an open-label, randomized-controlled trial to evaluate concentrations of plasma creatinine (pCr) and two novel renal biomarkers (pNGAL, uNGAL, UNCR, and uGGT/uCr) in dogs with GDV with and without IV lidocaine. The first objective was to determine renal biomarker concentrations up to 48 h after surgery. The second objective was to compare these biomarkers between dogs with and without IV lidocaine administration. The first hypothesis was that dogs with GDV have increased concentrations of renal biomarkers compared to healthy dogs. The second hypothesis was that dogs receiving IV lidocaine have significantly lower concentrations of renal biomarkers in the post-surgical period compared to dogs not receiving lidocaine, indicating renoprotection.

Materials and methods

Study design and settings

Our study was a single center, prospective, randomized, non-blinded, parallel-group clinical trial. It was conducted in the Small Animal Hospital of the Vetsuisse-Faculty of the University of Bern using dogs diagnosed with GDV from August 2017 until September 2018. This study was approved by the Federal Veterinary Service of Switzerland (BE69/17). The patients were treated according to the National Council of Animal Care. Owners signed an informed consent at the time of recruitment. The study is reported in line with the CONSORT statement (26).

Subjects

All dogs with GDV were eligible for the study. A diagnosis of GDV was based on history and clinical examination findings and was confirmed by abdominal radiographs. Exclusion criteria were known history of kidney disease and administration of nephrotoxic drugs (such as non-steroidal analgesic drugs) within 14 days before inclusion in the study. Some data from the dogs in the non-lidocaine group were used in a previous study (9). Additionally, 18 healthy dogs, which were part of a previous study, served as a healthy control group for pCr, uNGAL and UNCR (23).

Randomization

Enrolled dogs were randomized to either receive (LIDO group) or not receive (NO-LIDO group) IV lidocaine over a period of 24 h. Randomization was performed by using the permuted block technique (27). A block size of 6 subjects was chosen (3 dogs receiving lidocaine, 3 dogs not receiving lidocaine) and the order of this assignment was random (sealed slips in an envelope).

Abbreviations: AKI, acute kidney injury; APPLE, acute patient physiologic and laboratory evaluation; CRI, constant rate infusion; GDV, gastric dilatation and volvulus; IRI, ischemic reperfusion-injury; IV, intravenous; NGAL, neutrophil gelatinase-associated lipocalin; pCr, plasma creatinine; pNGAL, plasma neutrophil gelatinase-associated lipocalin; uCr, urine creatinine; uGGT, urinary gamma-glutamyl transferase; uGGT/uCr, urinary gamma-glutamyl transferase to creatinine ratio; UNCR, urinary neutrophil gelatinase-associated lipocalin to creatinine ratio; uNGAL, urinary neutrophil gelatinase-associated lipocalin; VAKI, veterinary acute kidney injury.

TABLE 1 Criteria of different stages of the VAKI scoring system (29).

VAKI stage	Criteria
Stage 0	Creatinine increase by <50% from baseline
Stage 1	Creatinine increase by 50–99% from baseline
	OR creatinine increase of 26.5 μmol/L (0.3 mg/dL) from baseline
Stage 2	Creatinine increase by 100–199% from baseline
Stage 3	Creatinine increase of ≥200% from baseline
	OR an absolute creatinine value >354 μmol/L (4.0 mg/dL)

Stage 0, no AKI, stage 1–3 AKI.

Interventions

General treatment protocol

All GDV dogs underwent standardized stabilization, anesthesia, surgery, and post-operative monitoring as described in a previous study by the same institution (9). For cardiovascular stabilization, supplemental oxygen and IV isotonic balanced and buffered crystalloids (Plasma-Lyte A®, Baxter AG, Switzerland) was administered at the clinician's discretion. Initial analgesia was provided by either IV methadone (0.2 mg/kg; Methadon Streuli®, Streuli Pharma AG, Switzerland), or a bolus of fentanyl (5 μg/kg; Fentanyl Curamed®, Actavis Switzerland AG, Switzerland) followed by a CRI of fentanyl at a rate of 5 μg/kg/h. In dogs with severe gastric distension, transcutaneous gastrocentesis for gastric decompression with a 14- or 16-gauge needle was performed after initiation of fluid therapy. Blood pressure was evaluated with oscillometry. The administered IV fluid volumes during initial stabilization, and intra- and post-operative periods were recorded throughout the study. To evaluate mentation, a mentation score (28) was performed every 8 h by trained ICU personal.

Lidocaine treatment protocol

Dogs assigned to the LIDO group received 2 mg/kg lidocaine (Lidokain 2% Streuli®, Streuli Pharma AG, 8730 Uznach, Switzerland) IV, over 15 min parallel to IV fluid therapy but prior to any other intervention. Afterwards, a lidocaine CRI was started at a dose of 50 μg/kg/min, which was continued over 24 h, unless there was a medical reason to stop (e.g., atrioventricular block) or prolong (e.g., sustained ventricular tachycardia) administration. Dogs assigned to the NO-LIDO group did not receive any lidocaine during the entire study period, unless they developed ventricular tachycardia with subsequent cardiovascular compromise. Those dogs were excluded from the study and received antiarrhythmic therapy as needed.

Patient data and illness severity scores

Baseline data collected at the time of admission included patient demographics (age, breed, sex, and body weight) and clinical data (mentation, heart rate, respiratory rate, rectal temperature, and systolic blood pressure). An acute patient physiologic and laboratory evaluation ($APPLE_{fast}$) score (28) was compiled at admission (T_0), 24 (T_{24}) and 48 h (T_{48}) after surgery. The presence and degree of AKI was evaluated by using the veterinary acute kidney injury (VAKI) staging system as previously described (29). This staging system is based on absolute and relative changes in pCr levels over time (Table 1). For calculation of the VAKI stage, admission (T_0) pCr concentrations were used as a baseline and the highest post-surgery (T_{24} or T_{48}) pCr concentration as the second creatinine concentration for each patient.

Sampling and laboratory analysis

Venous blood samples were obtained at admission (prior to any therapeutic intervention, T_0), immediately after surgery (T_1), and at 24 ± 4 h (T_{24}) and 48 ± 4 h (T_{48}) post-surgery. At T_0, T_{24}, and T_{48}, blood was collected in a 1.3 ml K2-EDTA tube (K2-EDTA Sarstedt AG, Switzerland) and a 9 ml heparin tube (Li-Heparin LH/1.3, Sarstedt AG, Switzerland) for analyses of hematological (Advia® 2120i, Siemens Healthcare Diagnostics AG, Switzerland) and biochemical (Cobas® c501, Roche Diagnostics (Schweiz) AG, 6343 Rotkreuz, Switzerland) variables, and lactate (RAPIDPoint® 500; Siemens Healthcare AG, Switzerland), necessary for calculating the $APPLE_{fast}$ score. An aliquot (0.5 ml) of the heparinized plasma was used for biochemical analyses. The remaining plasma was aliquoted and stored in a −80°C freezer within 1 h of blood collection until batch analyses of pNGAL. At T_1, only a heparin sample was collected for pNGAL immediately after surgery or, for dogs euthanized intraoperatively, immediately prior to euthanasia.

Urine was collected during surgery by cystocentesis or immediately after surgery (T_1) by aseptic catheterization, and at T_{24} and T_{48} by voided midstream sample. During the daytime, an aliquot of each fresh urine sample was submitted to the laboratory within 2 h for analyses of refractometric USG, dipstick semiquantitative chemistry (Combur 9®, Roche Diagnostics (Schweiz) AG, 6343 Rotkreuz, Switzerland), sediment examination, measurement of urine creatinine (uCr) concentration, and uGGT/uCr. The uGGT activity was measured with an enzymatic method (GGT-2, Roche Diagnostics (Schweiz) AG, 6343 Rotkreuz, Switzerland) and uCr was measured using an enzymatic assay (CREP2, Roche Diagnostics (Schweiz) AG, 6343 Rotkreuz, Switzerland) on a biochemistry analyzer (Cobas® c501, Roche Diagnostics (Schweiz) AG, 6343 Rotkreuz, Switzerland). If samples were obtained after laboratory opening hours, refractometric USG and dipstick semiquantitative chemistry were performed immediately. Samples were refrigerated (4°C) overnight, and biochemical and sediment examination was performed the next day. Pyuria was defined as ≥5 leucocytes per high power field and results of all urinary biomarkers were excluded if pyuria was detected.

Aliquots of urine samples were centrifuged twice at 1,500 g for 5 min and the supernatant frozen at −80°C within 1 h after sampling for later batch analysis of uNGAL. Urinary NGAL and pNGAL concentrations were determined using a sandwich ELISA (KIT 036, BioPorto Diagnostics, Gentofte, Denmark) following the manufacturer's instructions using 96-well ELISA plates precoated with a mouse monoclonal antibody against dog NGAL. Briefly, 100 μL of each standard or diluted plasma and urine samples were inoculated into the wells in duplicates. After incubation and washing, another mouse monoclonal antibody labeled with biotin was added to the samples to detect bound NGAL. Horseradish peroxidase-conjugated streptavidin and a color forming substrate were used to develop the assay after another incubation and washing. The absorbance was measured at 450 nm. Samples that showed results

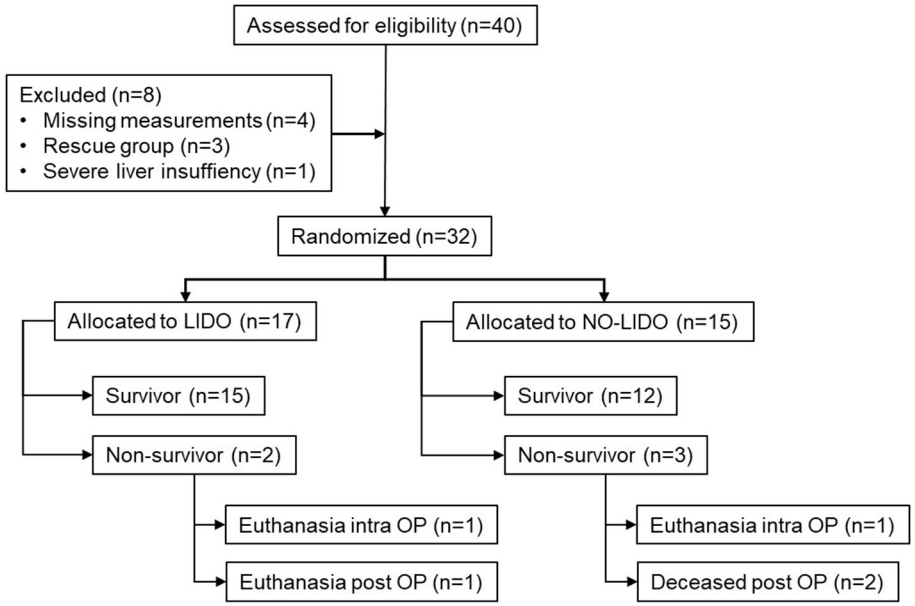

FIGURE 1
Inclusion and exclusion of 40 dogs presented with GDV into the study cohort. LIDO, GDV dogs treated with lidocaine; NO-LIDO, GDV dogs treated without lidocaine; rescue group, dogs initially enrolled in the NO-LIDO group but needed lidocaine due ventricular tachycardia; intra OP, intra-operatively, post OP, post-operatively.

out of range were remeasured with higher dilutions until a value in the measurable range was obtained or until a dilution of 1:900 was attained. The UNCR was calculated by dividing uNGAL [ng/dl] and urine creatinine concentration [mmol/l] and by multiplying by 100 (30). Results from blood and urine samples from a previous study evaluating pNGAL, uNGAL, and UNCR in healthy dogs using the same ELISA kit were available for use as a historical healthy control group (23).

Statistical analyses

Statistical analyses were done using commercial statistical software (MedCalc®, Version 20.110, MedCalc statistical software, 8400 Ostend, Belgium). Shapiro-Wilk tests were used to assess normal distribution. As some data were not normally distributed, all data are reported as median with range and/or interquartile range (IQR). Statistical differences of quantitative variables between groups were examined with Mann-Whitney rank sum tests. Significance of differences between the time points within the groups were assessed using Wilcoxon rank sum tests. Categorical variables between groups were analyzed using Chi-squared or Fisher's exact tests. Euthanized and naturally deceased patients were summarized in one non-survivor group for statistical analysis. Statistical significance was defined as $P \leq 0.05$ and all tests were two-tailed. To counteract the issue of multiple comparisons, Bonferroni corrections were used.

Results

Cohort characteristics and outcome

Forty dogs presented with GDV during the study period. After exclusion of 8 dogs, 32 dogs were enrolled in the study, of which 17 were allocated to the LIDO group and 15 to the NO-LIDO group (Figure 1). Demographic and clinical baseline data are presented in Table 2. The following breeds were represented in the LIDO group: German Shephard ($n = 3$), Great Dane ($n = 5$), and one each of Border Collie, Briard, Bernese Mountain Dog, Dalmatian, Doberman Pinscher, Eurasier, Golden Retriever, Wirehaired Pointing Griffon, Labrador Retriever. In the NO-LIDO group, the following breeds were represented: mixed breed ($n = 5$), St. Bernard ($n = 2$), Weimaraner ($n = 2$), and one each of Great Dane, Golden Retriever, Labrador Retriever, Spanish Mastiff, Newfoundland, and Standard Poodle. The overall mortality was 15.6% (Figure 1), and no significant difference in outcome was found between the two groups ($P = 0.529$). The median volume of crystalloids given between T_0 and T_1 was 91 ml/kg (IQR, 65–133) in the LIDO group and 81 ml/kg (IQR, 64–105) in the NO-LIDO group. The median volume of crystalloids given between T_1 and T_{24} was 67 ml/kg (IQR, 53–85) in the LIDO group and 58 ml/kg (IQR, 49–81) in the NO-LIDO group. The median volume of crystalloids given between T_{24} and T_{48} was 49 ml/kg (IQR, 41–64) in the LIDO group and 48 ml/kg (IQR, 45–52) in the NO-LIDO group. No significant difference in the volume of fluid administered was found at any time point between the groups.

VAKI staging was only possible in the 27 survivors. All but one patient (96.3%), met the criteria for VAKI stage 0, and one patient (LIDO group) had VAKI stage 1. In this dog, pCr increased by 39 µmol/L between T_0 and T_{24}, whereby pCr was within the reference range at all time points.

Plasmatic renal biomarkers

No significant difference in concentrations of pCr and pNGAL was found between the healthy control group and GDV dogs at T_0 (Table 3). At T_1, dogs with GDV had significantly lower pCr and

TABLE 2 Demographic and clinical baseline data (median, min–max) in 32 dogs randomized to the LIDO or NO LIDO group.

Characteristic	LIDO ($n = 17$)	NO-LIDO ($n = 15$)	*P*-value
Age (years)	8.7 (1.5–13.6)	7.6 (2.1–14.5)	0.745
Sex (*n*)			n/a
Female, intact	3	1	
Female, neutered	1	6	
Male, intact	4	4	
Male, neutered	9	4	
Body weight (kg)	36.9 (25.0–80.1)	34.9 (17.3–86.6)	0.763
Mentation score	1 (0–3)	0 (0–3)	0.166
Heart rate (bpm)	140 (72–200)	140 (88–210)	>1
Rectal temperature (°C)	38.5 (37.4–39.8)	38.4 (37.8–39.5)	>1
Respiratory rate (bpm)	40 (24–100)	44 (20–120)	>1
SBP (mmHg)	150 (99–198)	152 (103–194)	>1
$APPLE_{fast}$	22 (15–33)	20 (10–34)	0.375
Transcutaneous gastrocentesis	10	12	0.204
Δt clinicals signs (min)	120 (60–320)	120 (60–540)	0.929
Δt T_0–surgery (min)	102 (30–159)	82 (46–112)	0.064
Blood lactate (RI: 0.42–2.10 mmol/L)	T_0 2.92 (1.72–6.80)	T_0 2.77 (1.35–10.38)	>1
	T_1 1.48 (0.93–5.38)	T_1 1.34 (0.55–3.21)	>1
Gastric wall changes	Mild: 10	Mild: 9	0.730
	Moderate: 5	Moderate: 3	
	Severe: 2	Severe: 3	

Mentation score (28): 0, Normal; 1, able to stand unassisted, responsive but dull; 2, can stand only when assisted, responsive but dull; 3, unable to stand, responsive; 4, unable to stand, unresponsive; SBP, systolic blood pressure assessed with oscillometry (SunTech® Vet20™ blood pressure monitor, Morrisville, NC, USA); Δt clinicals signs, duration of clinical signs prior to fluid therapy in minutes; Δt T_0–surgery; duration of time between admission and surgery in minutes; gastric wall changes according to reference (9).

pNGAL (Table 3). No significant difference in pCr and pNGAL was found between GDV dogs with and without lidocaine at any sampling point (Table 4). In the entire population, a significantly higher pCr at T_0 compared with T_1, T_{24}, and T_{48} ($P < 0.001$), and a significant increase of pNGAL between T_0 and T_{24} ($P = 0.006$), and between T_0 and T_{48} ($P < 0.001$) was found (Table 3; Figure 2).

Urinary renal biomarkers

Compared to controls, uNGAL was significantly higher in dogs with GDV at T_{24} and T_{48} (Table 3). The UNCR was significantly lower at T_1 in dogs with GDV compared to the healthy control group (Table 3).

No significant difference in uNGAL, UNCR, and uGGT/uCr measurements was found at any time point between GDV dogs with and without lidocaine (Table 4). In the entire population, a significant increase in uNGAL concentrations between T_1 and T_{24} ($P = 0.001$) and T_1 and T_{48} ($P < 0.001$), and a significant increase of UNCR from T_1 to T_{24} ($P = 0.0015$) and from T_1 to T_{48} ($P < 0.001$) was found. Concentrations of uGGT/uCr were highest at T_{24}, and significantly decreased between T_{24} and T_{48} ($P < 0.001$) (Figure 2, Table 3). For technical reasons, not all of the intended urine samples could be obtained from some patients.

Discussion

This study evaluated concentrations plasma and urinary renal biomarkers in dogs with GDV and compared the effect of early IV lidocaine therapy followed by a 24-h CRI on these renal biomarkers in a randomized-controlled manner.

The main findings of this study are a significant increase of pNGAL, uNGAL, and UNCR in both groups at T_{24} and T_{48} compared to T_0 or T_1, respectively, whereas pCr significantly decreased. During the entire study period, pNGAL, uNGAL and UNCR revealed highest values at T_{48}. As biomarker measurement was not continued beyond 48 h, the duration of increased biomarkers cannot be assessed with our data. However, only uNGAL at T_{24} and T_{48} was significantly higher than historical healthy controls. Furthermore, a significant change in uGGT/uCr over time was not evident, except a significant decrease between T_{24} and T_{48} in the NO-LIDO group. Contrary to the authors' hypothesis, no significant differences in urinary and plasmatic renal biomarkers were found between GDV dogs with and without 24-h IV lidocaine therapy.

AKI is described as a complication of GDV in dogs and its incidence may be underestimated in the critical care setting (3, 8, 31). As the kidney receives 20% of the cardiac output, it is vulnerable to global hypoperfusion, and circulatory shock is one potential factor responsible for renal injury (2, 32). Furthermore,

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TABLE 3 Median (IQR) of plasma and urinary renal markers in dogs with GDV and 18 healthy control dogs.

Variable	Time point	GDV dogs	Historical healthy control group	*P*-value
Plasma creatinine (μmol/L)	T_0	95 (82–105)	83.5 (69–94)	0.219
	T_1	69 (60–78)*		0.044
	T_{24}	63 (52–78)*		0.015
	T_{48}	78 (65–87)*		0.95
pNGAL (ng/mL)	T_0	5.66 (3.58–7.43)	10.68 (4.60–12.30)	0.061
	T_1	4.42 (3.35–7.30)		0.026
	T_{24}	7.50 (4.01–11.89)*		>1
	T_{48}	9.86 (5.52–13.92)*		>1
uNGAL (ng/mL)	T_1	0.61 (0.30–2.59)	0.2 (0.2–1.25)	0.608
	T_{24}	2.62 (1.86–10.92)†		<0.001
	T_{48}	4.79 (1.96–34.97)†		<0.001
UNCR (μg/mmol)	T_1	0.15 (0.09–0.54)	2.11 (1.32–7.45)	<0.001
	T_{24}	1.14 (0.41–3.58)†		0.153
	T_{48}	1.34 (0.30–7.42)†		0.711
uGGT/uCr (U/mmol)	T_1	5.28 (3.12–14.09)	n/a	n/a
	T_{24}	6.20 (3.9–9.90)	n/a	n/a
	T_{48}	3.76 (2.84–6.22)§	n/a	n/a

pNGAL, plasma urinary neutrophil gelatinase-associated lipocalin; uNGAL, urinary NGAL; UNCR, urinary NGAL/urine creatinine ratio; uGGT/uCr, urinary gamma-glutamyl transferase to creatinine ratio; T_0, at admission; T_1, immediately after surgery (plasma marker) or during surgery by cystocentesis or immediately after surgery by urinary catheterization (urine marker); T_{24}, 24 ± 4 h post-surgery; T_{48}, 48 ± 4 h post-surgery; *values differing significantly from T_0 ($P < 0.05$, Bonferroni corrected); †values differing significantly from T_1 ($P<0.05$, Bonferroni corrected); §values differing significantly from T_{24} ($P < 0.05$, Bonferroni corrected); P-value, level of significance for comparison between the healthy control group and GDV dogs (Bonferroni corrected).

general anesthesia, fluid therapy, surgical trauma, blood loss, and repositioning of the stomach leads to congestive nephropathy, IRI, damage induced by pro-inflammatory cytokines, and oxidative stress in dogs with GDV (1, 3, 33). Renal ischemic injury causes damage to the glomerulus, renal tubular cells, and renal vasculature (32). During ischemic AKI, ATP depletion results in cytoskeletal changes in epithelial and endothelial cells, causing disruption of function, and a decrease in glomerular filtration rate. Furthermore, apoptosis and necrosis are major mechanisms of cell death that have important roles in ischemia (34, 35). Two previous studies, assessing the incidence of AKI in dogs with GDV, reported an incidence of 8 and 2.3%, respectively, using serum creatinine concentration as a marker (3, 8). However, serum creatinine concentration is less sensitive than some novel renal biomarkers, among them NGAL, which may result in delayed recognition of renal damage (36). Indeed, NGAL was found to increase 24–72 h earlier than serum creatinine and is considered an early marker for ischemic and nephrotoxic renal injury in both people (14, 18) and dogs (19, 21).

In the present study, the significant increase in pNGAL at 24–48 h after surgery compared to T_0 is probably a sign for renal tubular injury. However, NGAL can be also released from neutrophils and epithelial cells from various tissues (37). Therefore, the origin of the measured pNGAL in dogs in the present study cannot be clearly attributed to the kidney (37). The decrease of pNGAL (and pCr) at T_1 is most likely due to hemodilution after fluid resuscitation. Compared to pNGAL, uNGAL seems to be a more sensitive marker in dogs (19). In a previous study, post-surgery pNGAL did not detect AKI, whereas uNGAL was significantly higher in dogs with AKI at 12 h post-surgery (19). In the present study, uNGAL was significantly increased at T_{24} and T_{48} comparted to T_1 and was also significantly higher compared to the healthy controls. Increased uNGAL is a consequence of lower reabsorption of uNGAL at the proximal tubulus and/or higher expression of uNGAL mRNA in the tubuli, and indicates renal tubular injury (19, 21, 23, 36, 38).

Although the increase in pNGAL and uNGAL was mild, it is probably a sign of stressed or injured tubular cells, which persisted for at least 48 h after surgery. However, increased release of NGAL of non-renal origin can occur in systemic inflammatory states, such as GDV, and may have contributed to elevated levels found in our study (39). Differentiation between molecular forms of NGAL to determine their origin has been performed in human medicine but such assays are not available for dogs (40). When comparing NGAL concentrations of our study to previous studies in dogs with AKI or chronic kidney disease, values reported in previous studies were higher than those found in our study from dogs with GDV (23, 30, 41). In a previous study, pNGAL concentration <13.4 ng/ml excluded AKI with a sensitivity of 90%, and pNGAL >64 ng/ml was 100% specific for AKI (23). We found pNGAL >13.4 ng/ml in 6 GDV dogs at T_{24}, and 11 dogs at T_{48}, but pNGAL concentrations >64 ng/ml were not found in any dog at any time.

As uGGT does not pass the glomerulus and urinary values are of tubular origin if the glomerulus is intact, uGGT ratio might be a better marker of AKI than uNGAL in states of systemic inflammation like GDV. In humans, measurement of uGGT/uCr resulted in detection of 22% additional patients with AKI compared with plasma creatinine concentration alone in a population of ICU patients (42). Studies in dogs that have evaluated uGGT/uCr as a

TABLE 4 Median (IQR) of plasma and urinary renal markers in dogs with GDV treated with lidocaine (LIDO group) or without lidocaine (NO-LIDO group).

Variable	Time point	LIDO group	*n*	NO-LIDO group	*n*	*P*-value
Plasma creatinine (RI: 52–117 μmol/L)	T_0	87 (78–99)	17	97 (90–113)	15	0.236
	T_1	68 (58–78)	17	75 (66–78)	15	>1
	T_{24}	63 (52–82)	15	62 (54–71)	12	>1
	T_{48}	70 (65–85)	15	81 (66–86)	12	>1
pNGAL (ng/mL)	T_0	5.66 (3.56–6.72)	17	4.50 (3.78–10.16)	15	>1
	T_1	4.69 (2.90–7.34)	17	4.19 (3.47–7.11)	15	>1
	T_{24}	7.50 (3.71–11.38)	15	6.98 (4.85–13.14)	12	>1
	T_{48}	8.41 (5.52–11.88)	15	10.76 (5.68–14.12)	12	>1
uNGAL (ng/mL)	T_1	1.22 (0.39–3.75)	16	0.40 (0.21–2.06)	13	0.287
	T_{24}	4.57 (1.57–25.41)	12	2.62 (2.06–5.81)	11	>1
	T_{48}	8.91 (1.34–33.51)	14	4.68 (2.59–38.68)	10	>1
UNCR (μg/mmol)	T_1	0.19 (0.12–1.23)	16	0.10 (0.05–0.49)	13	0.374
	T_{24}	1.79 (0.43–8.83)	12	0.61 (0.42–1.79)	11	>1
	T_{48}	2.97 (0.42–7.36)	13	1.03 (0.19–8.96)	10	>1
uGGT/uCr (U/mmol)	T_1	5.06 (3.36–14.04)	14	6.08 (2.66–28.84)	15	>1
	T_{24}	6.24 (4.70–10.43)	12	5.62 (3.90–8.93)	12	>1
	T_{48}	5.55 (3.43–7.29)	12	3.38 (2.67–3.96)	12	>1

RI, reference interval; pNGAL, plasma urinary neutrophil gelatinase-associated lipocalin; uNGAL, urinary NGAL; UNCR, urinary NGAL/creatinine ratio; uGGT/uCr ratio, urinary gamma-glutamyl-transferase to creatinine-ratio; T_0, at admission; T_1, immediately after surgery (plasma marker) or during surgery by cystocentesis or immediately after surgery by urinary catheterization (urine marker); T_{24}, 24 ± 4 h post-surgery; T_{48}, 48 ± 4 h post-surgery; P-value, level of significance between the LIDO and NO-LIDO group for each parameter (Bonferroni corrected).

marker of AKI are inconclusive. In dogs with gentamycin-induced nephrotoxicity, a 2- to 3-fold increase of baseline uGGT/uCr ratio within 24 h indicated early onset of AKI (25). Other recent studies show variable discriminatory ability between dogs with AKI from dogs with chronic kidney disease and healthy controls (24, 43). In addition, the uGGT/uCr ratio has been shown to have significant within-day variations, which may be more pronounced in dogs with AKI compared with healthy dogs (25, 44, 45). In the present study no relevant changes in uGGT/uCr over time were found. One reason may be a lower sensitivity of uGGT compared to NGAL-based markers. Furthermore, the inflammatory component may have led to a non-renal increase in NGAL.

The incidence of AKI in dogs with GDV using the VAKI staging system varied in our study depending on the time point used as a baseline. We used pCr at T_0 as the baseline value (before fluid resuscitation), resulting in a diagnosis of AKI in only one dog (3.7%, VAKI stage 1). If assessed using pCr at T_{24} as the baseline (24 h after fluid resuscitation and hemodilution) and T_{48} as the second time point, the incidence VAKI stage 1 was 14.8% (4–3 dogs in the LIDO group, 1 dog in the NO-LIDO-group). In general, comparing biomarkers of assumed parenchymal kidney injury against a creatinine-based reference method that imperfectly estimates functional kidney impairment is problematic (46). Indeed, dilution or concentration of pCr in fluid-loaded or hemoconcentrated patients may lead to under- or overestimation of AKI assessed by creatinine-based criteria. At time T_0, most dogs were probably hemoconcentrated, and at T_1 they were hemodiluted due to aggressive fluid resuscitation. Therefore, VAKI is of questionable suitability for the diagnosis of AKI in dogs with GDV. Moreover, other creatinine-based AKI classifications systems, such as Risk–Injury–Failure–Loss–Endstage renal disease (RIFLE) and Acute Kidney Injury Network (AKIN) are likely of no greater utility (47). Regarding the use of ratios of urinary biomarkers to creatinine, uCr excretion is probably not constant in dogs with an unsteady hemodynamic state like GDV dogs. Under non-steady-state conditions, such as shock and subsequent high fluid rates, uCr excretion changes over time. Unless the considered biomarker behaves exactly like creatinine (i.e., filtered, secreted to some extent, normally not absorbed), the ratio is influenced by differences in uCr excretion (46). Normalization of biomarkers by uCr ratios may, therefore, result in both underestimation and overestimation of the biomarker excretion depending on the clinical context (46).

Lidocaine is a well-known local anesthetic and antiarrhythmic drug. Lidocaine CRI further prevents the intraoperative nociceptive response in dogs due to its analgesic potential, thereby reducing the intraoperative use of opioids (i.e., fentanyl) without causing clinically significant hemodynamic instability (48). In addition, lidocaine exhibits cytoprotective effects, as it exhibits antioxidant actions, such as reduction in the formation of reactive oxygen species and lipid peroxidation, and anti-inflammatory actions (10, 11). Suggested mechanisms for these effects include the inhibition of transcellular Na^+/Ca^{2+} exchange and subsequent reduction of intracellular Ca^{2+} accumulation during ischemia, scavenging of radicals, diminished release of superoxide from granulocytes, and decreased immune cell activation, migration into ischemic tissue, and subsequent cell death and endothelial damage (3, 10). In rats with diabetic nephropathy, renoprotective effects such as decreased

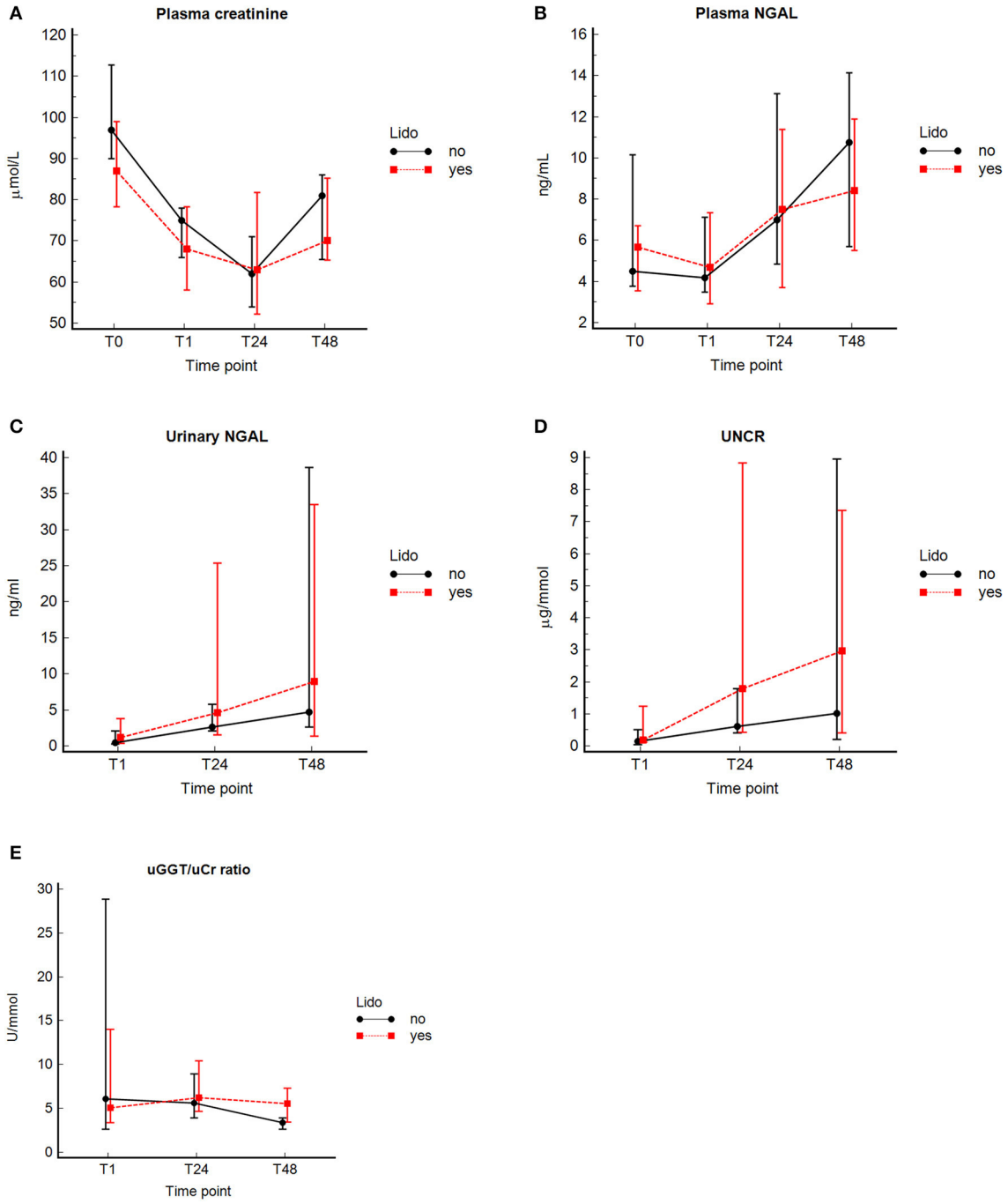

FIGURE 2
Concentrations (median and interquartile range) of pCr, pNGAL, uNGAL, uGGT/uCr, and UNCR at the different time points in 32 dogs with GDV with and without lidocaine. Median (central value marker-dot) and interquartile range (error bars) of concentrations of plasma creatinine (pCr) **(A)**, plasmatic neutrophil gelatinase-associated lipocalin (pNGAL) **(B)**, urinary NGAL (uNGAL) **(C)**, uNGAL to urine creatinine ratio (UNCR) **(D)**, and urinary gamma-glutamyl transferase to urine creatinine ratio (GGT/uCr) **(E)** in dogs with lidocaine and without lidocaine; T_0, at admission (prior any therapeutic intervention); T_1, immediately after surgery (plasma marker), or during surgery by cystocentesis or immediately after surgery by urinary catheterization (urine marker); and at 24 ± 4 (T_{24}), and 48 ± 4 h (T_{48}) post-surgery.

inflammation and oxidative stress, and improved oxygenation and renal functions were found (49). Experimental studies found gastric and cardiac organoprotection by IV lidocaine in rats and dogs (12, 50, 51). Bruchim et al. found that in dogs with spontaneous GDV, early IV lidocaine bolus followed by a 24-h CRI post-admission significantly decreased the occurrence of AKI, cardiac arrhythmias, and the length of hospitalization (3). However, these results cannot be directly compared with our results because the study designs differed and only one dog (LIDO group) developed VAKI stage 1 in our study population. Nevertheless, we did not find any significant difference in plasmatic or urinary renal biomarkers between GDV dogs with and without lidocaine treatment and we could not confirm any renoprotective potential based on our study results.

There are some limitations in this study. The cohort size of dogs with GDV was small, which could entail type 2 errors. Because the study ended after 48 h, an increase in pCr or other renal biomarkers shortly after the end of the study cannot be excluded. Furthermore, institutional reference ranges for NGAL (pNGAL, uNGAL, UNCR and uGGT/uCr) were not available, and results in GDV dogs from the present study were compared to values from a small historical healthy control group from a study performed in 2014 (except for uGGT/uCr) (30). Since the ELISA kit used in the control group was from the same manufacturer but not from the same LOT number, the comparability of the renal biomarker values between GDV dogs and a historical control group is questionable. In addition, dogs of the control group were neither age nor breed matched to the GDV dogs. Because of possible age- and breed-related differences in the concentrations of renal biomarkers or the risk of developing AKI, matching on breed and age would have been advantageous to exclude the influence of these risk factors (52).

Conclusion

The results of this study show that pNGAL, uNGAL, and UNCR significantly increased during the 48-h period post-surgery, whereas pCr did not. This suggests stressed or injured tubular cells and a potentially higher incidence of AKI in dogs with GDV than previously described, which is likely underestimated when pCr concentrations alone are evaluated. Administration of lidocaine did not affect concentrations of renal markers in dogs with GDV.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by Animal Experiment Committee of the Swiss Federal Veterinary Office (registration number BE 69/17). Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

AL: sample collection, study design, laboratory analyses, data analysis, and preparation of the manuscript. AB: sample collection and data analysis. TF and SS: preparation of the manuscript. LP and EM: laboratory analyses and preparation of the manuscript. K-NA: study design, sample collection, data analysis, and preparation of the manuscript. All authors contributed to, read, and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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