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ORIGINAL STUDY

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Prospective evaluation of novel biomarkers of acute kidney injury in dogs following cardiac surgery under cardiopulmonary bypass

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Abstract

Objective: To assess the occurrence of acute kidney injury (AKI) in dogs undergoing cardiac surgery under cardiopulmonary bypass (CPB) and explore associations between traditional and novel serum and urinary biomarkers.

Design: Prospective cohort study conducted between July 2018 and April 2019.

Setting: University teaching hospital.

Animals: Nineteen dogs undergoing cardiac surgery under CPB with preoperative serum creatinine <140 μmol/L (<1.6 mg/dl).

Interventions: Blood and urine samples were obtained at 4 time points: preoperatively following general anesthesia induction, immediately postoperatively, and 2 and 4 days postoperatively (T_1, T_2, T_3, and T_4). AKI was defined as an increase in serum creatinine ≥26.4 μmol/L (≥0.3 mg/dl) above baseline within 48 hours. Serum creatinine, C-reactive protein (CRP), symmetric dimethylarginine (SDMA), inosine, beta-aminoisobutyric acid (BAIB), urinary clusterin (uClus), and urinary cystatin B (uCysB) were measured. Data were log-transformed (log_{10}) when appropriate and assessed using linear mixed-effects models.

Measurements and Main Results: AKI occurred in 3 of 19 dogs (15.8%, 95% confidence interval: 0.047–0.384). Inosine increased at T_2 (adjusted mean ± standard error: 53 ± 5.6) in all dogs, and then gradually decreased. Log_{10}uCysB increased at T_2 (2.3 ± 0.1) in all dogs and remained high. Log_{10}CRP and log_{10}uClus increased significantly at T_3 (1.9 ± 0.1 and 3.6 ± 0.1, respectively) in all dogs and remained increased. There was a significant positive association between serum creatinine and SDMA ($P < 0.001$, estimate ± standard error: 0.06 ± 0.00), between log_{10}CRP and log_{10}uClus ($P < 0.001$, 0.35 ± 0.08), between SDMA and creatinine as well as between SDMA and

Abbreviations: AKI, acute kidney injury; BAIB, beta-aminoisobutyric acid; CPB, cardiopulmonary bypass; CRP, C-reactive protein; GFR, glomerular filtration rate; MMVD, myxomatous mitral valve disease; NGAL, neutrophil gelatinase-associated lipocalin; SDMA, symmetric dimethylarginine; uClus, urinary clusterin; uCysB, urinary cystatin B; UOP, urinary output.

BAIB ($P < 0.001$, 11.1 ± 0.83 and $P < 0.001$, 1.06 ± 0.22, respectively) for all dogs at all time points.

Conclusions: Inosine and uCysB concentrations changed in all dogs immediately following a surgery under CPB and may indicate tubular injury. Further studies are required to ascertain the usefulness of those biomarkers in early detection of AKI.

KEYWORDS
AKI, BAIB, clusterin, CPB, creatinine, CRP, cystatin B, inosine, SDMA

1 | INTRODUCTION

Hospital-acquired acute kidney injury (AKI) can increase morbidity and mortality. A recent meta-analysis reported overall mortality to be as high as 45% among dogs with AKI.[1] Prompt recognition is of paramount importance as rapid correction of any inciting cause, targeted monitoring, and treatment may promote renal recovery. AKI is one of the most severe complications observed in people following cardiac surgery. It is reported that between 18% and 30% of pediatric and adult patients sustain an AKI following a cardiac surgery under cardiopulmonary bypass (CPB) depending on the diagnostic criteria used.[2–5] Cardiac surgery under CPB is currently performed on dogs in a number of small animal centers across the world but the frequency of an associated-AKI in dogs is unknown.

Current identification of AKI in azotemic and nonazotemic dogs is based on an increase in serum creatinine by ≥26.4 μmol/L (≥0.3 mg/dl) with or without reduction in urine output.[6] Although commonly used as a marker of kidney function, serum creatinine can be affected by biological variability.[7–9] Additionally, the anticipated delay between onset of kidney injury and any detectable increase in serum creatinine concentrations limits the use of serum creatinine as a very early marker of AKI.[7] For the above reasons, novel specific biomarkers of kidney injury, which allow early detection of kidney injury in a timely manner, are desirable. Some of the biomarkers of interest in this context include symmetric dimethylarginine (SDMA), inosine, beta-aminoisobutyric acid (BAIB), urinary clusterin (uClus), and urinary cystatin B (uCysB). SDMA has been widely investigated as an endogenous functional marker of kidney disease in cats and dogs.[10–12] Changes in serum inosine, a structural marker of proximal tubular injury, have been documented in a rodent model of ischemic renal injury and in a preliminary experimental canine study.[13,a] Based on some preliminary studies, some authors speculated that changes in blood BAIB concentrations, a cationic end product of the pyrimidine degradation pathway, could be associated with renal function.[14,15] There is also a growing interest in urinary biomarkers. Kidney-specific uClus has been shown to be a promising marker of kidney disease in dogs with leishmaniasis- and gentamicin-induced kidney injury.[16,17] Additionally, increase in uClus and uCysB concentrations has been recently documented in dogs envenomated by *Vipera berus berus* suggesting a potential of those biomarkers in early detection of kidney injury.[18] C-reactive protein (CRP) has been considered an indicator of inflammation in dogs but there is also a growing amount of evidence to suggest that CRP may be a factor in development and progression of AKI.[19–22]

The aims of this study were to prospectively assess the frequency of postoperative AKI in dogs undergoing cardiac surgery under CPB using routinely available serum creatinine as per IRIS guidelines, and describe changes in concentrations of novel biomarkers for up to 4 days following the surgery in this population of dogs. Furthermore, this study aimed to determine any association between concentrations of novel biomarkers and serum creatinine, SDMA, and CRP.

It was hypothesized that concentrations of urinary biomarkers (uClus and uCysB) and serum inosine expressed in units will increase within 48 hours following cardiac surgery under CPB and that those changes would precede any significant changes in serum creatinine or SDMA concentrations. It was also hypothesized that changes in renal biomarkers concentrations will be positively correlated with an increase in CRP reflecting a systemic inflammatory state.

2 | MATERIALS AND METHODS

2.1 | Animals

Client-owned dogs presenting to a single small animal referral center and scheduled to undergo cardiac surgery under CPB between July 2018 and April 2019 were prospectively enrolled into the study following the owners' informed consent. Each dog had a full cardiac assessment performed prior to the surgery by a board-certified cardiologist. Dogs diagnosed with myxomatous mitral valve disease (MMVD) were further classified into stages as per previously published guidelines.[23] Suitability of the dog for the open-heart surgery was evaluated jointly by a cardiologist and a cardiac surgeon.

Dogs were excluded if any of the following conditions were met: weight below 5 kg, death during the surgical procedure or whilst still on CPB, preoperative PCV ≤18%, preoperative azotemia defined as serum creatinine ≥140 μmol/L (≥1.6 mg/dl), preoperative serum creatinine value not available, and missing data defined as more than 1 value not available for any measured biomarker of kidney function for reason other than postoperative death.

Breed, weight and age on the day of surgery, sex, primary diagnosis, type of surgical procedure performed, length of general anesthesia,

surgery and aortic cross-clamping, average urinary output (UOP) measured in ml/kg/h over the first 12 hours postoperatively, and mortality were recorded. Development of AKI was defined as an increase in serum creatinine by ≥26.4 μmol/L (≥0.3 mg/dl) between any 2 of the time points and above the patient's baseline over 48 hours.[6] Patients with AKI were further stratified into grades according to previously published International Renal Interest Society (IRIS) guidelines with the highest grade reached during the study period being reported.[6]

Treatment that the dogs were receiving prior to surgery was not standardized. Premedication, general anesthetic, and postoperative treatment were also not standardized and the choice of treatment protocol was dependent on attending clinicians' preferences (anesthetist, perfusionist, cardiac surgeon, criticalist). All animals received blood products during or following cardiac surgery under CPB; these included fresh frozen plasma, whole blood, or packed red blood cells and neither the type nor the volume transfused was standardized. The choice of blood product was at the discretion of the attending clinician.

2.2 | Sampling and samples preparation

Ethical approval for the study was given by the Clinical Research and Ethical Review Board at the Royal Veterinary College (URN: M2017 0130-2). Only residual blood and urine samples were collected as part of this study. Blood samples were obtained via direct venipuncture, a central venous catheter, or a long stay catheter, depending on the level of instrumentation. The timing of sampling for blood collection was based on an existing protocol for routine monitoring of biochemical and electrolyte parameters in dogs undergoing cardiac surgery under CPB at the study institution. Blood and urine samples were obtained at 4 time points: T_1, preoperatively, following induction of GA but prior to the surgery and CPB; T_2, on the day of the surgery, following the procedure and within 6 hours from transfer to the intensive care unit; T_3, 2 days after the surgery; T_4, 4 days after the surgery. No additional sampling for the purpose of this study was permitted under the ethical approval terms.

Urine samples were obtained via an indwelling urinary catheter or via free catch collection, depending on the dog's level of instrumentation. All dogs had an indwelling urinary catheter placed immediately prior to surgery, with the catheter removed within the first 24 hours postoperatively as per routine postoperative care protocol. Time of removal was at the cardiac surgeon's discretion.

At each time point, residual serum samples for measurement of creatinine, CRP, SDMA, inosine, and BAIB were obtained. Urine was also obtained at the given time points for measurement of uClus and uCysB. Blood samples were centrifuged within 30–60 minutes of collection (Relative Centrifugal Force, RCF: 9742 × *g* for 2 minutes[b]) and serum was separated. Of the separated serum, 0.25 ml was immediately transferred to a citrate tube and swirled to rehydrate the citrate. Citrated serum was allowed to stand for 10–15 minutes in room temperature. Urine samples were centrifuged (RCF: 219 × *g* for 2 minutes[c]) and supernatant collected. Remaining serum, citrated serum, and urine supernatant samples were stored in a temporary freezer at −20°C and were moved within 48 hours of preparation to a long storage freezer (−80°C) where they remained for a maximum period of 8 months until shipment to the laboratory. Following the enrolment period, blood and urine samples were shipped on dry ice to an international commercial laboratory[d] in 2 separate batches, where they were confirmed to be frozen upon arrival and analyzed immediately upon thawing.

2.3 | Biomarker analysis

Citrated serum was used to measure inosine, and remaining blood biomarkers were measured in serum. SDMA (μmol/L [μg/dl]) was analyzed using liquid chromatography–mass spectrometry as previously described.[24] Serum CRP (mg/L [μg/ml]) was measured on a clinical biochemistry analyzer using the species-specific Gentian Canine CRP Reagent kit. Serum creatinine (μmol/L [mg/dl]), inosine (units), and BAIB (μg/dl) were measured using liquid chromatography–mass spectrometry assays. Inosine was initially measured in μg/dl and converted into units according to the following conversion formula: (1/inosine (μg/dl)) × 1000. As inosine expressed in μg/dl is expected to reduce secondary to kidney injury, the reporting in units was performed for ease of comparison with other biomarkers that are all expected to increase secondary to renal injury. uCysB (ng/ml) and uClus (ng/ml) were measured using research ELISA assays currently in development at IDEXX Laboratories.[e] The ELISA for clusterin measures the kidney-specific isoform and does not detect the isoforms present in blood.[14]

2.4 | Statistical analysis

Commercial software was used to perform all statistical tests.[f] Where the biomarker value was below the level of quantification, this value was substituted with the value equal to the detection limit. Normality for numerical data was assessed using Shapiro–Wilk test and values were reported as mean ± standard deviation or median (range), as appropriate. Percentage change from baseline (T_1) was calculated for each biomarker and time point. Adjusted means ± standard error were calculated for all time points and each biomarker.

Associations between kidney biomarkers and serum creatinine, SDMA, or CRP were analyzed using a linear mixed-effects model to account for repeated measures from the same dogs. Skewed data were log-transformed ($\log_{10}$) prior to further analysis. Only biomarkers that were found to have $P < 0.1$ in the univariable analysis were then evaluated in the multivariable model and manual stepwise backward elimination method was used to remove nonsignificant biomarkers until all remaining biomarkers were significant at $P < 0.05$. P-value < 0.05 was defined as statistically significant.

Percentage change from baseline was calculated manually for all dogs and for each biomarker in their original values using the following formula: [(adjusted mean at T_2, T_3, or T_4 − adjusted mean at T_1)/adjusted mean at T_1] × 100 = % change. Obtained results were then plotted on a single graph for descriptive representation of the change of biomarkers values from baseline.

3 | RESULTS

A total of 41 dogs underwent cardiac surgery under CPB during the study period. Nineteen dogs were enrolled into the study with 22 dogs excluded due to the following reasons: weight <5 kg ($n = 16$), missing data ($n = 2$), preoperative azotemia ($n = 1$), inappropriate sample storage ($n = 1$), preoperative serum creatinine value not available ($n = 1$), lack of owner's consent ($n = 1$).

Breeds included Cavalier King Charles Spaniel or a crossbreed of ($n = 7$), Chihuahua ($n = 3$), Labrador Retriever ($n = 2$), Beagle ($n = 2$), and 1 of each of the following: Border Collie, Boston Terrier, Boxer dog, Cocker Spaniel, and Havanese dog. There were 6 male entire dogs, 6 male neutered dogs, 5 female neutered dogs, and 2 female entire dogs enrolled. Median age at the time of surgery was 8.5 years (range: 0.6–12.1). Median preoperative weight of dogs was 10.0 kg (range: 5.6–23.4).

The primary diagnosis in 15 dogs was MMVD (stage C [$n = 13$], stage D [$n = 2$]), mitral valve dysplasia ($n = 1$), and tricuspid valve dysplasia ($n = 3$). One dog with tricuspid valve dysplasia was additionally diagnosed with a common atrium. Mitral valve repair was performed in 16 dogs and tricuspid valve repair in 3 dogs. The dog diagnosed with a common atrium additionally underwent repair of the atrial septal defect under the same surgery. Mean length of general anesthesia was 339.1 ± 62.0 minutes ($n = 17$). Mean length of surgery was 182.6 ± 23.9 minutes ($n = 16$). Mean length of aortic cross-clamping was 74.9 ± 12.8 minutes ($n = 18$). Mean length of hospitalization following surgery was 9.1 ± 3.5 days ($n = 19$).

TABLE 1 Values recorded for each acute kidney injury (AKI) case and calculated adjusted means ± standard errors for all non-AKI cases

						Non-AKI cases	
Biomarker	**Units**	**Time point**	**AKI case 1**	**AKI case 2**	**AKI case 3**	**Adjusted mean**	**Standard error**
Serum creatinine	μmol/L (mg/dl)	1	130.3 (1.5)	81.0 (0.9)	134.4 (1.5)	92.9 (1.1)	4.7 (0.1)
		2	155.4 (1.8)	118.0 (1.3)	137.9 (1.6)	83.5 (0.9)	4.7 (0.1)
		3	110.1 (1.2)	402.5 (4.6)	190.1 (2.2)	78.2 (0.9)	4.9 (0.1)
		4	–	–	461.5 (5.2)	73.5 (0.8)	4.9 (0.1)
SDMA	μmol/L (μg/dl)	1	0.9 (18.8)	0.6 (11.7)	1.9 (37.4)	0.6 (11.2)	0.0 (0.7)
		2	0.6 (12.1)	0.8 (16.5)	1.7 (33.8)	0.5 (10.5)	0.0 (0.7)
		3	0.7 (13.3)	2.1 (42.8)	2.3 (46.2)	0.6 (11.8)	0.0 (0.8)
		4	–	–	5.0 (100.9)	0.5 (11.0)	0.0 (0.8)
Inosine	Units	1	8.3	9.8	–	10.1	5.2
		2	6.7	80.0	80.0	52.6	5.2
		3	7.8	80.0	80.0	25.6	5.5
		4	–	–	80.0	19.4	5.7
BAIB	μg/dl	1	2.0	3.9	2.0	2.4	0.3
		2	3.9	4.8	2.0	2.5	0.3
		3	2.0	2.0	2.0	2.0	0.4
		4	–	–	18.1	2.9	0.4
Log_{10}uClus (uClus)	(ng/ml)	1	2.8 (694.7)	2.6 (401.0)	–	2.6 (905.9)	0.2
		2	2.7 (495.6)	2.4 (258.0)	4.1 (12,494.8)	2.4 (786.5)	0.2
		3	3.9 (8781.0)	3.9 (7777.0)	3.8 (7064.3)	3.5 (7497.2)	0.2
		4	3.5 (3434.4)	3.6 (4438.0)	3.3 (2024.7)	3.0 (2099.3)	0.2
Log_{10}uCysB (uCysB)	(ng/ml)	1	2.1 (122.0)	2.8 (573.0)	–	1.8 (161.9)	0.1
		2	2.5 (350.9)	2.8 (661.3)	2.8 (615.4)	2.3 (277.8)	0.1
		3	3.1 (1308.2)	2.8 (626.3)	2.8 (671.3)	2.6 (623.0)	0.1
		4	3.1 (1165.5)	3.0 (1107.2)	2.6 (378.6)	2.6 (579.6)	0.1
Log_{10}CRP (CRP)	(mg/l) [μg/ml]	1	0.9 (8.0)	0.9 (8.0)	1.2 (15.2)	1.1 (19.7)	0.1
		2	0.9 (8.0)	0.9 (8.0)	1.8 (69.7)	1.1 (13.9)	0.1
		3	2.1 (136.4)	2.2 (167.1)	0.9 (8.0)	1.9 (103.4)	0.1
		4	–	–	2.1 (121.5)	1.7 (63.5)	0.1

Note: Time points represent: 1, preoperative value (following induction of general anesthesia but prior to the surgery and cardiopulmonary bypass); 2, postoperative value on the day of surgery; 3, value 2 days after surgery; 4, value 4 days after surgery.

Abbreviations: BAIB, beta-aminoisobutyric acid; CRP, C-reactive protein; SDMA, symmetric dimethylarginine; uClus, urinary clusterin; uCysB, urinary cystatin B.

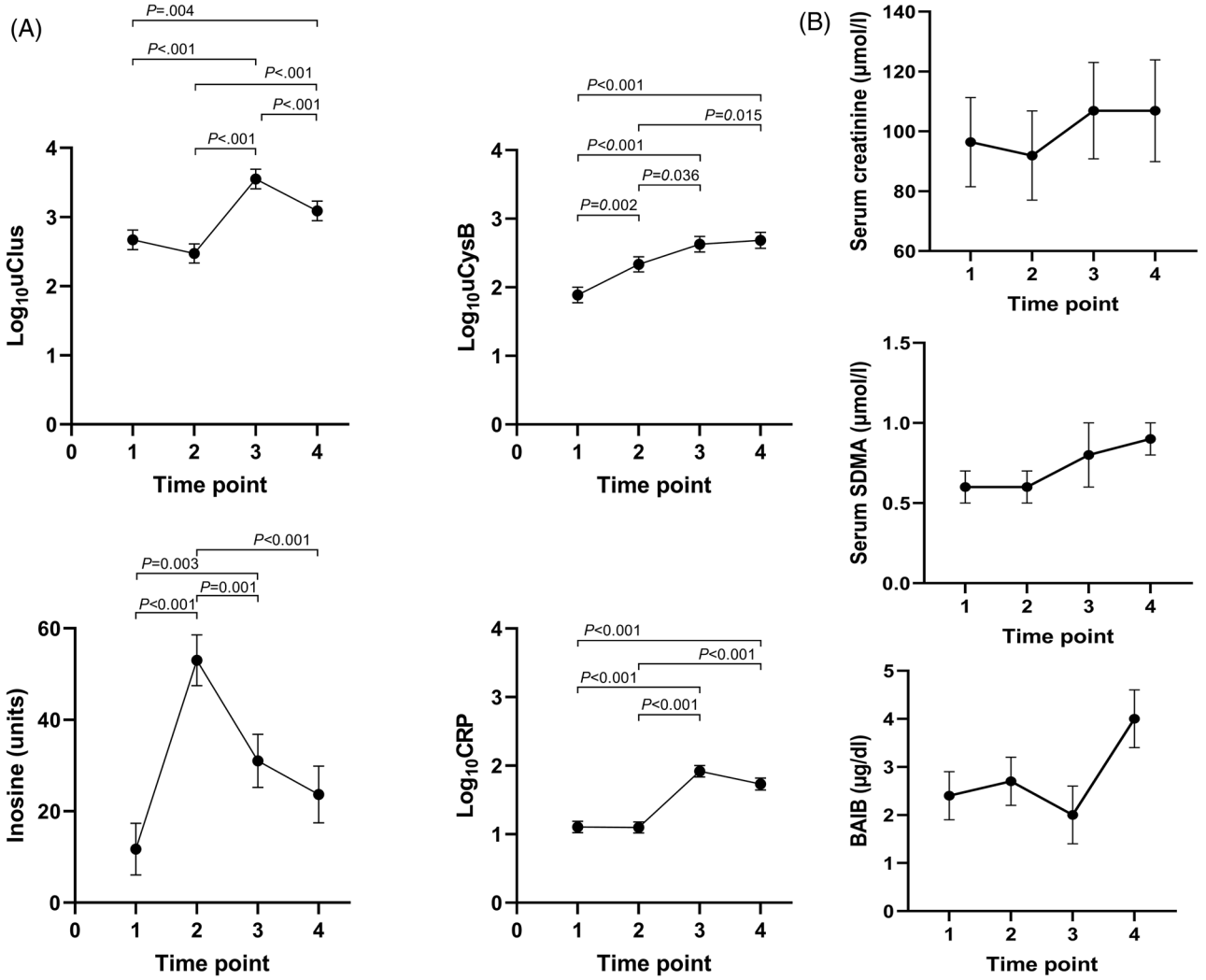

FIGURE 1 (A, B) Graphic representation of changes in biomarker values in all dogs over time. Dots represent adjusted means with their respective standard error bars. *P* denotes significant differences between adjusted means between given time points (*P* significant at <0.05). CRP, uClus, and uCysB are represented on the logarithmic scale. Time points represent: 1, preoperative value (following induction of general anesthesia but prior to the surgery and cardiopulmonary bypass); 2, postoperative value on the day of surgery; 3, value 2 days after surgery; 4, value 4 days after surgery. Abbreviations: BAIB, beta-aminoisobutyric acid; CRP, C-reactive protein; SDMA, symmetric dimethylarginine; uClus, urinary clusterin; uCysB, urinary cystatin B.

Median time for which UOP measurement values were available following surgery was 18 hours (range: 1–24). Median of average UOP values postoperatively was 3.5 ml/kg/h (range: 1.4–26.0). Out of 19 dogs, 3 developed AKI (15.8%, 95% confidence interval: 0.047–0.384), which was further classified into grade II ($n = 1$), grade III ($n = 1$), and grade IV ($n = 1$). The dog classified as grade II developed azotemia at T_2 but the serum creatinine reduced to below the value reported preoperatively by T_3. Of the remaining 2 dogs, 1 was diagnosed with AKI at T_2 and the other at T_3. Both dogs reached their respective highest grades at T_4 (Table 1).

Two dogs died following mitral valve repair and prior to discharge from hospital (mortality of 10.5%). The cause of death could not be confirmed in 1 of those dogs but differentials included a coronary event, a thromboembolic event, and an acute drug reaction. This dog did not show evidence of AKI; however, only values at T_1 and T_2 were available as the dog died within 24 hours of surgery. The second dog developed multiple organ dysfunction syndrome and died on day 8 postsurgery. The cause of death could not be confirmed either, but it was suspected to be likely due to a thromboembolic event. This dog developed AKI at T_3.

Serum CRP, uClus, and uCysB values were log-transformed prior to the analysis to their $\log_{10}$ values due to skewness of the data. No statistically significant changes in serum creatinine, SDMA, and BAIB concentrations were observed between time points. There was a significant difference between time points for inosine ($P < 0.001$), $\log_{10}$CRP ($P < 0.001$), $\log_{10}$uClus ($P < 0.001$), and $\log_{10}$uCysB ($P < 0.001$) (Figure 1A,B). Inosine increased abruptly postoperatively (T_2), and then gradually decreased. $\log_{10}$CRP values were similar at T_1 and T_2 but increased subsequently. $\log_{10}$uCysB increased at T_2 and remained high, while $\log_{10}$uClus increased significantly at T_3 and did not return

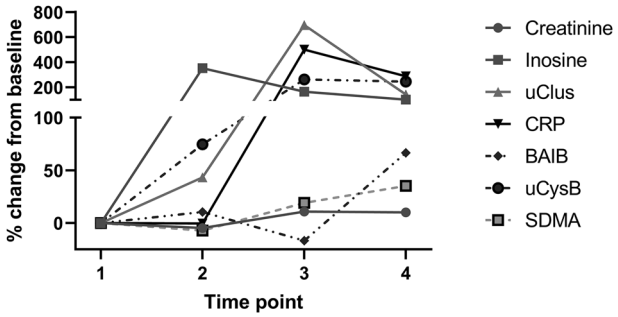

FIGURE 2 Graphic representation of manually calculated mean percentage change from baseline for each biomarker in its original units, for all dogs over time. Time points represent: 1, preoperative value (following induction of general anesthesia but prior to the surgery and cardiopulmonary bypass); 2, postoperative value on the day of surgery; 3, value 2 days after surgery; 4, value 4 days after surgery. Abbreviations: BAIB, beta-aminoisobutyric acid; CRP, C-reactive protein; SDMA, symmetric dimethylarginine; uClus, urinary clusterin; uCysB, urinary cystatin B.

to baseline (Figure 2; Tables 1 and 2). SDMA was noted to be increased at T_1 (>0.69 μmol/L [>14 μg/dl]) in 4 dogs; 2 of those dogs had SDMA > 0.89 μmol/L (>18 μg/dl) and later developed AKI (Table 1).

Initial linear univariable analysis showed potential associations (at $P < 0.1$) of serum creatinine with SDMA and BAIB. The multivariable analysis showed persistent significant association (at $P < 0.05$) between serum creatinine and SDMA ($P < 0.001$) (Table 3). Univariable analysis also indicated potential associations (at $P < 0.1$) of SDMA with serum creatinine, BAIB, and $\log_{10}$CRP. Following the multivariable analysis, only associations of SDMA with serum creatinine and SDMA with BAIB remained significant ($P < 0.001$ for each association). Univariable analysis found potential associations (at $P < 0.1$) of $\log_{10}$CRP with $\log_{10}$uClus and $\log_{10}$uCysB. The association remained significant between $\log_{10}$CRP and $\log_{10}$uClus following a multivariable analysis ($P < 0.001$) (Table 3).

4 | DISCUSSION

AKI was identified in 15.8% of dogs following cardiac surgery under CPB; however, given the small sample size, this frequency may not reflect AKI occurrence in a bigger population. This occurrence is similar to the frequency of hospital-acquired AKI documented in a general small animal ICU population that varies between 12% in dogs with abdominal sepsis and 14.6% in a general canine ICU population.[25,26] Occurrence of cardiac surgery-associated AKI in people varies greatly depending on the type of cardiac surgery performed and the definitions of AKI used. The etiology of AKI after cardiac surgery under CPB is multifactorial, including renal exposure to inflammatory mediators, ischemia reperfusion injury, oxidative stress, and neurohormonal activation leading to hypoperfusion.[27] People undergoing cardiac surgery under CPB continue to serve as clinical models of AKI due to the relatively standardized insult, the elective nature of the procedure, and the close monitoring pre- and postoperatively.[28,29] To the authors' knowledge, this is the first study investigating the use of dogs undergoing open-heart surgery as a clinical model of canine AKI. Additionally, the results of the study encourage further investigations of the use of novel biomarkers of AKI, such as inosine and uCysB, both of which changed significantly following the surgery potentially reflecting tubular injury. Use of biomarkers capable of early recognition of structural or functional renal injury in populations at increased risk of AKI, such as those undergoing surgeries under CPB, could allow prompt identification and implementation of preventative and therapeutic strategies.[4]

Several serum, plasma, and urine biomarkers have been investigated both in people and dogs as potential early biomarkers of AKI with variable results. Some of the recently investigated biomarkers include neutrophil gelatinase-associated lipocalin (NGAL), *N*-acetyl-β-D-glucosaminidase, kidney injury molecule 1, interleukins 8 and 18, cystatin C, and monocyte chemoattractant protein-1.[30–33] Some of the novel and promising biomarkers of AKI include SDMA, inosine, clusterin, cystatin B, and BAIB.[12,14] The current study showed a positive association between serum creatinine and SDMA. SDMA was additionally positively associated with BAIB. Although it has been suggested that BAIB may play a role in inhibition of renal fibrosis, other studies documented release of BAIB from myocytes during exercise. It is therefore unclear if rise in BAIB in dogs undergoing a surgery under CPB represents kidney or muscular injury.[22,34,35] Furthermore, increased SDMA (>0.69 μmol/L [>14 μg/dl]) was identified preoperatively in 4 of 19 nonazotemic dogs. Recent reports suggested a potential usefulness of SDMA in early detection of chronic kidney disease in dogs.[11,24] One study suggested that a higher cutoff point of >0.89 μmol/L (>18 μg/dl) was able to identify dogs with ≥40% decrease in glomerular filtration rate (GFR) with an improved specificity.[36] In the current study, SDMA was >0.89 μmol/L (>18 μg/dl) at T_1 without concurrent azotemia in 2 dogs and could potentially indicate decreased kidney function in those dogs despite lack of azotemia. Both of those dogs later developed AKI but further studies in larger populations would be required to evaluate if dogs with increased SDMA and normal serum creatinine are more likely to develop AKI than those with SDMA ≤ 0.89 μmol/L (≤18 μg/dl). Due to small sample size and low frequency of AKI, strength of associations between different biomarkers was not assessed separately for AKI and non-AKI dogs.

Interestingly, our analysis also documented an association between CRP and uClus. It has been previously documented that CRP, one of the major canine acute phase proteins, starts increasing at 4–6 hours following an exposure to an inflammatory trigger, peaking at 24–48 hours.[37] CRP has been shown to increase in response to a variety of inflammatory conditions and can serve as a marker of inflammation in dogs.[19,20,38,39] An increase in uClus was documented in a study performed in dogs with leishmaniosis. Authors of that study hypothesized that uClus increased in response to inflammation-associated tubular injury.[16] There is an increasing amount of evidence suggesting that inflammation plays a pivotal role in development and progression of kidney disease, and that CRP could be a mediator of AKI.[21,40–43] Although an association of CRP with uClus, a marker of tubular injury, was documented in the current study and could reflect a link between degree of inflammation and tubular injury, the

TABLE 2 Adjusted means and their respective standard errors for biomarkers, and their log-transformed (log_{10}) values when appropriate, for all cases at given time points

Biomarker	Unit of measurement	Time point	Adjusted mean	Standard error	*N*
Serum creatinine	μmol/L (mg/dl)	1	96.4 (1.1)	14.9 (0.2)	19
		2	91.9 (1.0)	14.9 (0.2)	19
		3	106.9 (1.2)	16.1 (0.2)	16
		4	106.9 (1.2)	17.0 (0.2)	14
SDMA	μmol/L (μg/dl)	1	0.6 (13.0)	0.1 (3.0)	19
		2	0.6 (12.1)	0.1 (3.0)	19
		3	0.8 (15.5)	0.2 (3.1)	16
		4	0.9 (17.7)	0.2 (3.2)	14
Inosine	Units	1	11.7	5.7	18
		2	53.0	5.6	19
		3	31.0	5.8	17
		4	23.7	6.2	14
BAIB	μg/dl	1	2.4	0.5	19
		2	2.7	0.5	19
		3	2.0	0.6	16
		4	4.0	0.6	14
Log_{10}uClus (uClus)	(ng/ml)	1	2.7 (948.5)	0.1	18
		2	2.5 (1359.6)	0.1	19
		3	3.6 (7555.4)	0.1	18
		4	3.1 (2305.5)	0.1	17
Log_{10}uCysB (uCysB)	(ng/ml)	1	1.9 (182.9)	0.1	18
		2	2.3 (319.6)	0.1	19
		3	2.6 (663.8)	0.1	18
		4	2.7 (631.6)	0.1	17
Log_{10}CRP (CRP)	(mg/L) [μg/ml]	1	1.1 (17.4)	0.1	15
		2	1.1 (17.3)	0.1	16
		3	1.9 (104.3)	0.1	16
		4	1.7 (67.4)	0.1	14

Note: Time points represent: 1, preoperative value (following induction of general anesthesia but prior to the surgery and cardiopulmonary bypass); 2, postoperative value on the day of surgery; 3, value 2 days after surgery; 4, value 4 days after surgery.
Abbreviations: BAIB, beta-aminoisobutyric acid; CRP, C-reactive protein; N, number of observations; SDMA, symmetric dimethylarginine; uClus, urinary clusterin; uCysB, urinary cystatin B.

TABLE 3 Results of the final multivariable analysis showing only significant associations

Dependent variable	Covariate	*P*-value	Estimate	Standard Error
Serum creatinine	SDMA	<0.001	0.06	0.00
SDMA	Serum creatinine	<0.001	11.11	0.83
SDMA	BAIB	<0.001	1.06	0.22
Log_{10}CRP	Log_{10}uClus	<0.001	0.35	0.08

Note: Significance for the final multivariable analysis was set at $P < 0.05$. Estimate provides the direction and magnitude of association.
Abbreviations: BAIB, beta-aminoisobutyric acid; CRP, C-reactive protein; SDMA, symmetric dimethylarginine; uClus, urinary clusterin; uCysB, urinary cystatin B.

cause–effect relationship between inflammation and AKI could not be established.

Serum inosine has been recently proposed as an early biomarker of kidney injury and recovery.[8] Serum levels of inosine, a purine metabolite, are expected to reduce (and its value reported in units increase) during renal insult as a result of exhaustion of adenosine deaminase, the enzyme converting adenine to inosine, in renal proximal tubules.[14] Inosine and uCysB, a marker of renal tubular epithelial injury, significantly changed immediately following surgery potentially reflecting recent kidney insult. This early change suggests that serum inosine and uCysB have a potential to serve as early biomarkers of kidney injury. Moreover, inosine gradually trended toward preoperative levels, likely reflecting cessation of exposure to an active kidney injury. Inosine could therefore serve not only as an early biomarker of reduced kidney function but in the future, it may also be useful in monitoring the response to preventative or therapeutic interventions.

This study had several limitations. First, increase in serum creatinine concentrations was used to identify dogs with newly developed AKI. Serum creatinine is routinely used for identification of AKI in clinical settings due to low cost, availability, ease, and speed of results acquisition. Nevertheless, serum creatinine is an insensitive marker of an early decline in GFR and is more useful in monitoring of progression of chronic kidney disease, rather than in detection of AKI. As such, direct monitoring of GFR might provide a more accurate assessment of renal function; however, currently available methods are not clinically appropriate in an acute care setting. Second, the study group was small, which may have led to type II error showing lack of association between novel biomarkers and serum creatinine, SDMA, or CRP where potentially an association existed. Equally, a type I error, indicating an association between markers where one does not exist, cannot be ruled out either. No power calculation was performed prior to commencement of the study as there were no published data available regarding the incidence of AKI in dogs undergoing open-heart surgery. As only 3 dogs developed AKI, this precluded further subanalysis of data with regard to group characteristics and AKI-specific biomarker changes. Furthermore, perioperative treatment protocol, although similar in many aspects, was not completely standardized in this population and the effect of particular medications or blood products on changes in biomarkers' concentrations could not be ascertained. Additionally, the study period was limited to the first 4 days postoperatively and the timing of sampling was dictated by the postoperative care protocol already in place in the study institution. This limited our ability to follow changes in biomarkers throughout the entire hospitalization period. We were also unable to closely track fluctuations in biomarkers levels in the first 48 hours postoperatively when concentrations of many of the biomarkers are expected to change. Similarly, monitoring of UOP was limited to the first several hours postoperatively. None of the dogs showed any evidence of oligoanuria during the initial 12 hours postoperatively. In people, UOP has been shown to be variable following cardiac surgery under CPB due to multiple factors such as prolonged hypothermia, altered renin–angiotensin–aldosterone axis, and fluid influx and efflux during the procedure.[44–46] Monitoring of UOP as a marker of kidney function in those patients is therefore often considered unhelpful. Instead, recent investigations redirect the attention to the monitoring of UOP during the actual CPB procedure. This has been shown to have a potential to identify patients at risk of developing cardiac surgery-associated AKI.[47–49]

In accordance with the ethical approval and preexisting perioperative monitoring protocol in the study institution, dogs could not be sampled specifically for the study. Therefore, some samples were missed due to an insufficient collection of blood during sampling for clinical purposes or lack of timely urine collection during voiding. Furthermore, serum creatinine value was not available for some dogs at T_3 and/or T_4. However, serum creatinine values at missing points for all dogs were obtained using a different analyzer as part of the perioperative monitoring protocol in place in study institution (data not shown). Although the direct comparison and interchange of serum creatinine values obtained by the 2 different analyzers is not appropriate, no additional cases of AKI were identified when analyzing data received from the study institution clinical laboratory.

A common practice for all the perfusionists assisting with procedures under CPB in the study institution is to ultrafiltrate all patients through the perfusion extracorporeal circuit. However, due to inconsistencies in data recording we were not able to confirm that all the dogs in the studied population underwent an ultrafiltration or determine what volume was ultrafiltrated prior to weaning from CPB. Particle size, charge, and sieving coefficient influence the effect of ultrafiltration on measurement of particles and those remain unknown for the biomarkers studied. Although the ultrafiltration may have affected the values at T_2 to an unknown degree, the effect of the ultrafiltration on biomarkers concentrations at T_3 and T_4 is less likely to be of significance. This should be particularly taken into consideration when interpreting results of inosine measurement that, when reported in units, showed an increase at T_2 but truly represented a drop in inosine concentrations.

Another potential limitation is the lack of normalization of urinary biomarkers to urinary creatinine. The concept of urinary creatinine normalization in cases of AKI is controversial. Concentrations of urinary biomarkers have been regularly normalized to urinary creatinine in chronic kidney disease to account for variability in creatinine clearance and urine flow. This practice assumes stable inter- and intraindividual urinary creatinine excretion rate, as well as a linear relationship between urinary creatinine excretion rate and urinary excretion rate of other biomarkers. However, there is lack of consensus regarding usefulness of urinary creatinine normalization of urinary biomarkers in states of acute renal impairment. While some authors found that normalization improves performance of urinary biomarkers, others argue that it may over- or underestimate the presence of AKI as the process of normalization assumes a linear relationship between urinary creatinine and biomarker excretions that may not be true in acute states.[50,51] When urinary creatinine is measured in a developing AKI, its concentration initially reduces reflecting reduction in GFR. However, with an increase in serum creatinine, the creatinine excretion rate increases paralleling the original rate, that from before the drop in GFR. Other urinary biomarkers, for example, NGAL, for which urinary excretion is sum of filtration, reabsorption, and secretion may not follow this

pattern of excretion. Consequently, normalization to urinary creatinine may lead to amplification of the urinary biomarker value immediately after GFR reduction despite constant production and excretion of the biomarker of interest. Given this lack of consensus regarding urinary normalization, future studies should consider reporting both absolute and normalized values.[51,52]

In conclusion, results of this study demonstrated that concentrations of serum inosine and uCysB changed soon after cardiac surgery under CPB and may assist in early detection of tubular injury in dogs. Future studies are needed to better elucidate the use of those novel biomarkers in a clinical setting and would benefit from use of GFR measurement as a more accurate representation of kidney function.

CONFLICT OF INTEREST

Laboratory analysis was funded and performed by IDEXX Laboratories, Inc. Sarah Peterson, Maha Yerramilli, and Murthy Yerramilli are all current or past employees of IDEXX Laboratories, Inc.

ENDNOTES

[a] Palm CA, Segev G, Cowgill LD, et al. Urinary clusterin and serum inosine: biomarkers for early identification of acute kidney injury in dogs. In: 2014 ACVIM Forum Research Abstract Program. J Vet Intern Med 2014;28(4):1367.

[b] Vetlab CombiSpin Veterinary Centrifuge, Vetlab Supplies, UK.

[c] Vetlab CombiSpin Veterinary Centrifuge, Vetlab Supplies, UK.

[d] IDEXX Laboratories, Westbrook, ME.

[e] IDEXX Laboratories, Westbrook, ME.

[f] IBM SPSS, version 26.0, SPSS Inc., Armonk, NY.

[g] Palm CA, Segev G, Cowgill LD, et al. Urinary clusterin and serum inosine: biomarkers for early identification of acute kidney injury in dogs. In: 2014 ACVIM Forum Research Abstract Program. J Vet Intern Med 2014;28(4):1367.

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