Regional Citrate Anticoagulation for Intermittent Hemodialysis in Dogs

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Background: The traditional systemic heparinization used for anticoagulation in extracorporeal therapies may cause fatal complications in animals at risk of bleeding.

Hypothesis/Objectives: To develop and validate a protocol of regional citrate anticoagulation (RCA) for intermittent hemodialysis in dogs.

Animals: A total of 172 dogs treated with hemodialysis for acute kidney injury.

Methods: In vitro titration was performed, adding trisodium citrate and calcium chloride to heparinized canine blood. A tentative protocol was used first in 66 treatments with additional heparinization and subsequently in 518 heparin-free treatments. Safety and adequacy of RCA were assessed based on clinical and laboratory monitoring, dialyzer pressure gradient, treatment completion, and visual scoring of the extracorporeal circuit.

Results: Addition of 1 mmol/L citrate to heparinized blood decreased the ionized calcium concentration by 0.23 mmol/L (95% confidence interval [CI], 0.16–0.30) and 1 mmol/L calcium increased it by 0.62 mmol/L (95% CI, 0.45–0.79). Heparin-free treatments were initiated with infusion of trisodium citrate (102 mmol/L) at 2.55 mmol/L blood and calcium chloride (340 mmol/L) at 0.85 mmol/L. Citrate and calcium administrations were adjusted in 27 and 34% of the treatments, respectively. Overall, anticoagulation was satisfactory in 92% of the treatments, with expected azotemia reduction in 95% (urea) and 86% (creatinine), stable dialyzer pressure gradient in 82%, and clean extracorporeal circuits in 92% of the treatments. Eighteen treatments (3.5%) were discontinued prematurely, 9 because of clotting and 9 for reasons unrelated to the RCA procedure.

Conclusions and Clinical Importance: Regional citrate anticoagulation allows safe and efficient heparin-free hemodialysis in dogs at risk of bleeding.

Key words: Acute kidney injury; Bleeding; Coagulation; Extracorporeal blood purification.

Extracorporeal blood purification techniques such as hemodialysis (HD) require anticoagulation of the circulating blood. The most commonly applied protocol uses systemic heparinization (SH) with unfractionated heparin administered first as a bolus of 10–50 IU/kg, followed by a constant rate infusion (CRI) of 50–100 IU/kg/h.[1,2] Heparin infusion rate is based on clinical assessment of evidence or risk of bleeding, and it is adjusted based on coagulation monitoring every 30–60 minutes during the treatment, with a goal to approximately double the coagulation time at baseline.[1] Systemic anticoagulation, however, can worsen existing bleeding lesions frequently encountered in the setting of

Abbreviations:

ACT	activated clotting time
AKI	acute kidney injury
CrRR	creatinine reduction ratio
HD	hemodialysis
iCa	ionized calcium concentration
IQR	interquartile range
Qb	blood flow
Qp	blood processed
RCA	regional citrate anticoagulation
SH	systemic heparinization
Td	time on dialysis
URR	urea reduction ratio

acute kidney injury (AKI).[3] Disseminated intravascular coagulation, gastro-intestinal ulcerations, or leptospirosis-associated pulmonary hemorrhages can deteriorate rapidly with a potentially fatal outcome.[4,5] Moreover, SH persists several hours beyond the dialysis treatment, hindering routine patient care, and potentially urgent diagnostic, medical, or surgical procedures. This risk of bleeding is amplified further in continuous or extended modes of renal replacement therapy. Although possible, reversal of heparin with protamine usually is reserved for emergencies because of the risk of anaphylactic reactions and of heparin rebound with later recurrence of bleeding.[6]

Alternatives to SH have been developed, including low-heparin or heparin-free therapies with regular saline flushes of the extracorporeal circuit, fast-short treatment protocols, use of coated dialyzer membranes, and hemodiafiltration with prefilter hemodilution.[1,7,8] The efficacy of these modified protocols in dogs, however, is

limited, resulting in increased blood losses, decreased treatment efficacy, and early failure of extracorporeal circuits.[1,8] Additionally, activation of the coagulation system in treatments with insufficient anticoagulation can potentially worsen the thrombotic risk in AKI patients who frequently are hypercoagulable.[3,9]

Regional anticoagulation techniques limited to the blood in the extracorporeal circulation have been developed for human patients treated with continuous renal replacement therapies.[1,8] In these techniques, the blood coming from the patient is anticoagulated, and this process is reversed in the returning blood, so that the blood circulating in the body has normal hemostatic characteristics. The most commonly used technique decreases the ionized calcium concentration (iCa) in the extracorporeal circuit by infusing trisodium citrate in the inflow line and restores normocalcemia with calcium chloride administration in the outflow line, before returning the blood to the patient.[10,11] Reliable and sufficient extracorporeal hypocalcemia (iCa < 0.4 mmol/L) is essential for effective anticoagulation, and dependable restoration of normocalcemia is critical for the safety of the patient, otherwise at risk of hypocalcemic or hypercalcemic complications. Protocols have been described and used widely for human patients on continuous therapies, but only rarely for intermittent HD.[12] In animals, protocols have been described for experimental hemofiltration in sheep and for apheresis in dogs.[13,14]

The aim of our prospective study therefore was to develop in vitro and validate in vivo an efficient and safe protocol of regional citrate anticoagulation (RCA) for intermittent HD in dogs with AKI.

Materials and Methods

Study Design

The study was designed to first obtain in vitro titration data for citrate and calcium in canine blood to adjust existing protocols used in humans for use in dogs needing heparin-free HD treatment. In vivo effects on calcium concentration then were evaluated in pilot dogs anticoagulated with conventional SH, enabling the use of low citrate and calcium flow rates otherwise insufficient for anticoagulation. Efficacy and safety of the established RCA protocol subsequently were evaluated in dogs using heparin-free HD treatments in which anticoagulation depended solely on the effect of citrate. Because most dogs with AKI were diagnosed with leptospirosis and active pulmonary hemorrhage, heparin-free protocols were necessary for safe HD, precluding the possibility of a comparison group under SH.

Animals

Clinically healthy blood donor dogs with unremarkable history and physical examination findings, and normal hematology and biochemistry profile results were enrolled for the in vitro experiments. For the in vivo study, client-owned dogs diagnosed with AKI and treated with intermittent HD were included. Acute kidney injury was defined by the combination of historical, clinical, laboratory, or imaging evidence, with at least 2 of the following criteria:[15,16] (1) presence of renal azotemia with a serum creatinine concentration ≥1.7 mg/dL, persisting at least 24 h after correction of prerenal factors; (2) increase in serum creatinine concentration ≥0.3 mg/dL

during a 48 h interval in the absence of prerenal factors; (3) persistent pathologic oligoanuria (<1 mL/kg/h over 6 h) after volume repletion; (4) and evidence of tubular injury with renal glucosuria or granular casts on urinalysis. Requirement for HD was based on clinical and laboratory evaluation and assessed for all dogs by 1 of the 2 authors. Dogs were not excluded if the data indicated evidence of underlying chronic kidney disease, because this would not necessarily affect their need for dialysis and anticoagulation. To truly represent the population of dogs requiring renal replacement therapies, dogs with liver injury or failure also were not excluded, despite potentially altered hepatic metabolism of citrate. All procedures were conducted in accordance with the Animal Welfare Act and subject to informed owner consent.

In vitro Titration of Citrate and Calcium

Venous blood was obtained by clean jugular venipuncture from healthy dogs and was placed in lithium heparin tubes for titration experiments. For each dog, trisodium citrate[a] was added to 5 aliquots of heparinized blood in concentrations of 1.19–3.50 mmol/L. In a second step, calcium chloride[b] was added to aliquots of each of these citrated samples in concentrations of 0.43–1.70 mmol/L. Ionized calcium concentration, other electrolyte concentrations, and venous blood gases were measured in the initial blood and in each of the citrated and recalcified samples with a clinical blood gas analyzer.[c]

Hemodialysis Procedures

Vascular access was provided by large-bore (7–14 Fr), double-lumen jugular dialysis catheters adapted to the animal size. Venovenous intermittent HD was performed with a Gambro AK200 UltraS system.[d] The dialysis prescription was tailored and adjusted to the needs of the dogs, according to recommended protocols.[1,2] The extracorporeal circuit consisted of blood tubing[e] and dialyzers[f] chosen mostly based on their priming volumes and membrane characteristics. Dialysate flow rate was 300–700 mL/min, and dialysate temperature was set at 38.5°C. The dialysate sodium and bicarbonate concentrations that would have been chosen in standard treatments with SH were decreased by 5 and 2 mmol/L, respectively, as recommended in protocols designed for humans to avoid hypernatremia and metabolic alkalosis.[11] Dialysate sodium concentration ranged from 137 to 150 mmol/L, potassium concentration from 2.0 to 3.2 mmol/L, and bicarbonate concentration from 20 to 30 mmol/L. Calcium-free dialysate[g] was used for all RCA treatments.

The duration of the dialysis procedures, the total volume of blood treated, and the blood flow rate were determined individually to reach a urea reduction ratio (URR) of approximately 35, 60, and >75% for the first, second, and later treatments, respectively, based on the expected URR modeled from 233 treatments performed previously with SH on the same dialysis platform.

RCA Protocol

The RCA protocol to be used in vivo was based on the results of the in vitro experiments for the initial flow rates and on similar protocols used in humans for monitoring and rate adjustments. Initial flow rates of trisodium citrate were calculated to decrease iCa by 0.8 mmol/L, from 1.1 mmol/L (median value in AKI dogs at the authors' institution) to a goal of 0.3 mmol/L in the circuit. Being exposed to a calcium-free dialysate, iCa is further decreased postfilter, and approximately 60% of the calcium citrate complexes are estimated to be dialyzed from the blood when using standard dialysis protocols.[17] With metabolism of citrate from the remaining complexes, calcium is freed in the circulation, and therefore,

the initial flow rate of calcium chloride infusion was calculated as 60% of the rate necessary to increase iCa by 0.8 mmol/L, from 0.2 to 1 mmol/L. Commercial solutions of trisodium citrate ($C_6H_5O_7 \cdot 3Na$, 102 mmol/L)[a] and calcium chloride ($CaCl_2$, 340 mmol/L)[b] were used as citrate and calcium sources, respectively.

A spreadsheet[h] was designed to facilitate calculation of citrate and calcium infusion rates as a function of the blood flow rate, and to compute the fluid volume to be removed by ultrafiltration to perform isovolemic HD. Monitoring of iCa was performed before treatment, after 30 minutes, and at 60–90 minutes intervals subsequently. For this monitoring, blood was sampled from the dog (S1), from the extracorporeal circuit predialyzer (S2) and post-dialyzer (S3). When adjustments of the infusion rates were required, the corresponding iCa was rechecked 30–60 minutes later. All samples were collected in lithium heparin tubes and submitted for iCa, other electrolyte concentrations, and venous blood gas measurements. The target iCa in the extracorporeal circuit (S2 and S3) was 0.2–0.4 mmol/L and following adjustments to the citrate infusion rate were planned when the target was not achieved: −10% when iCa was <0.2 mmol/L, +10% when iCa was 0.4–0.5 mmol/L, and +20% when iCa was >0.5 mmol/L. A + 10% adjustment was left to the clinician's discretion for borderline iCa (0.35–0.4 mmol/L). Target iCa in the dog (S1) was 0.8–1.2 mmol/L with following adjustments to the calcium infusion rate of +20% when iCa was <0.6 mmol/L, +10% when iCa was 0.6–0.8 mmol/L, −10% when iCa was 1.2–1.5 mmol/L, and −20% when iCa was >1.5 mmol/L. A + 10% adjustment was left to the clinician's discretion for borderline iCa (0.8–1.0 mmol/L). The last citrate and calcium flow rates used for a treatment were applied for the initiation of the next treatment in the same dog.

Evaluation of Protocol Safety and Efficacy

Initial validation of the RCA protocol was performed in pilot HD treatments with additional SH, dissociating the calcium control from the need of anticoagulation. Further treatments were performed with the final heparin-free protocol using infusions of citrate and calcium directly at the access catheter. Evaluation of the efficacy and safety of the protocol, and effects on blood biochemistry and blood gases are reported only for these treatments. Because SH has no effect on calcium, in vivo calcium changes upon citrate and calcium injections were evaluated in all treatments, including pilot treatments.

The safety of the procedure was evaluated based on clinical monitoring, including routine cardiovascular (heart rate, mucous membranes, indirect oscillometric blood pressure q15 min), respiratory (respiratory rate and type q15 min), and neurologic (mentation, pupillary light responses q30 min) variables. Particular attention was given to clinical signs potentially associated with hypocalcemia, including facial rubbing, muscle twitching, and muscle tremors. Continuous electrocardiographic (ECG) monitoring was maintained for the first HD treatment in each dog. The efficacy of anticoagulation was evaluated based on following criteria: successful completion of the procedure; change in the dialyzer pressure gradient; effective urea and creatinine reduction ratios (URR, CrRR); and, visual scoring of the extracorporeal circuit after blood rinseback (see Appendices S1 and S2). Overall, satisfaction of anticoagulation was scored as follows: very good, all efficacy variables satisfactory; minor grade complication, 1 goal not achieved; moderate grade complication, problems necessitating adjustments, but treatment performed to completion; severe grade complication, treatment aborted early due to unsatisfactory anticoagulation.

Data Analysis and Statistical Methods

Because most data sets were not normally distributed, they are presented as median (interquartile range, IQR) and analyzed using nonparametric methods. For comparison of numeric data between groups, a Wilcoxon Signed-Rank Test was used for paired data, a Mann-Whitney U-test for unpaired data, and a Kruskal-Wallis one-way analysis of variance (ANOVA) for >2 groups. A Chi-square test was used for comparison of proportions between groups. A P-value <0.05 was considered significant. Analyses were performed using commercial statistical software.[i]

Results

In vitro Citrate and Calcium Titration

The addition of trisodium citrate to heparinized whole blood decreased iCa in a linear dose-dependent manner: $\Delta iCa = -0.23 \times$ [citrate] - 0.21 ($r^2 = 0.97$, $P = 0.002$), with 1 mmol/L of citrate decreasing iCa by 0.23 mmol/L (95% CI, 0.16–0.30). The addition of calcium chloride to citrated blood increased iCa in a linear dose-dependent manner: $\Delta iCa = 0.62 \times$ [Ca] - 0.08 ($r^2 = 1.00$, $P = 0.004$), with 1 mmol/L of calcium increasing iCa by 0.62 mmol/L (95% CI, 0.45–0.79; Fig 1).
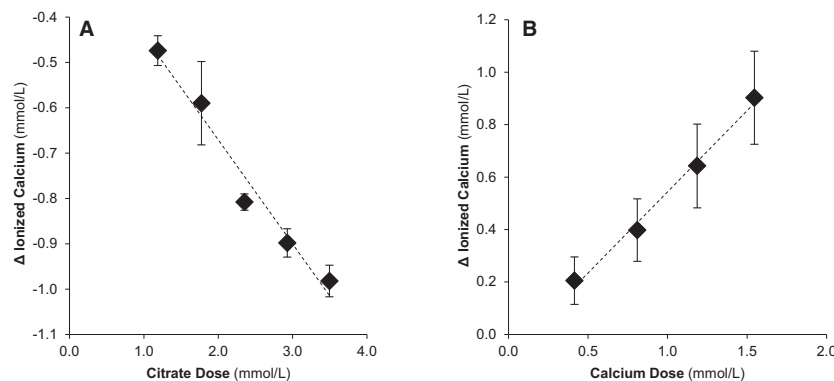
Fig 1. In vitro effect of trisodium citrate (**A**) and calcium chloride (**B**) on ionized calcium concentration in canine blood from 5 clinically healthy dogs. Data are represented as mean ± standard deviation. (A) the effect of citrate on iCa concentration can be described with the equation: change in iCa (mmol/L) = 0.23 × citrate (mmol/L) − 0.21 ($R^2 = 0.97$, $P = 0.002$). (B) The effect of calcium on iCa concentration can be described with the equation: change in iCa (mmol/L) = 0.62 × calcium (mmol/L) − 0.08 ($R^2 = 1.00$, $P = 0.004$).

Table 1. In vitro acid-base and electrolyte changes with citration and recalcification of venous blood (n = 5).

	Native blood	+ Citrate 2.93 mmol/L (Citrate)	P	+ Citrate and calcium 2.93 mmol/L (Citrate) + 0.85 mmol/L (Calcium)	P
pH	7.46 (7.45 to 7.46)	7.45 (7.44 to 7.49)	0.526	7.55 (7.50 to 7.55)	0.007
HCO_3^- (mmol/L)	21.6 (21.5 to 22.5)	19.7 (18.0 to 19.9)	0.014	21.2 (20.6 to 21.3)	0.001
BE (mmol/L)	−1.2 (−1.6 to −1.2)	−3.2 (−3.6 to −3.2)	0.003	−4.0 (−4.7 to −3.9)	0.003
pCO_2 (mmHg)	31.6 (31.1 to 34.1)	30.1 (24.3 to 30.3)	0.044	18.4 (18.2 to 18.5)	0.001
AG (mmol/L)	18.4 (17.7 to 19.3)	28.6 (27.3 to 29.0)	<0.001	24.8 (22.2 to 26.5)	0.001
Na (mmol/L)	150.9 (150.4 to 150.9)	153.0 (152.9 to 153.6)	0.006	143.0 (142.8 to 144.4)	0.005
K (mmol/L)	3.87 (3.41 to 3.99)	3.58 (3.26 to 3.61)	0.005	3.46 (3.15 to 3.50)	0.001
iCa (mmol/L)	1.30 (1.27 to 1.30)	0.41 (0.37 to 0.43)	<0.001	0.73 (0.70 to 0.73)	<0.001

HCO_3^-, bicarbonate; BE, base excess; pCO_2, CO_2 partial pressure; AG, anion gap; Na, sodium; K, potassium; iCa, ionized calcium.

The doses of citrate (2.93 mmol/L) and calcium (0.85 mmol/L) were the closest to those selected for in vivo use (2.55 and 0.85 mmol/L, respectively). Data are presented as median (IQR). P, statistical significance for paired comparisons with native blood in Wilcoxon Signed-Rank Test.

The infusion of 2.56 mmol citrate per liter of circulating blood therefore was calculated to decrease iCa by 0.8 mmol/L, according to the set goals. Considering the metabolism of the remaining calcium citrate complexes, the infusion of 0.85 mmol (60% of 1.41 mmol) calcium chloride per liter of blood was estimated to be necessary to correct iCa back to 1.0 mmol/L. Based on these data, initial citrate and calcium administration rates for in vivo treatments were set to provide concentrations of 2.55 and 0.85 mmol/L blood, respectively. For a blood flow rate of 100 mL/min, trisodium citrate 102 mmol/L therefore would be infused at a rate of 2.5 mL/min (150 mL/h) and calcium chloride 340 mmol/L at a rate of 0.25 mL/min (15 mL/h).

The main in vitro changes in electrolyte concentrations and blood gas parameters obtained with the citrate and calcium doses closest those selected for in vivo use are summarized in Table 1. The addition of trisodium citrate led to a marked increase in anion gap but only to minimal alterations in acid-base status. Recalcification with calcium chloride led to a marked decrease in sodium concentration and moderate alkalemia, mostly as a consequence of decreased pCO_2.

Animals and Diseases

One-hundred seventy-two dogs diagnosed with AKI and treated with intermittent HD were enrolled in the study, including 137 pure-breed dogs from 65 different breeds and 35 mixed-breed dogs. Sex distribution consisted of 80 intact males (47%), 40 castrated males (23%), 15 intact females (9%), and 37 spayed females (22%). Median age at presentation was 6.0 years (IQR, 2.3–8.8 years), and median body weight was 22.2 kg (IQR, 11.7–29.4 kg; range, 2.1–74.2 kg). The etiology of AKI was identified as leptospirosis in 120 dogs (70%), grape toxicity in 10 dogs (6%), other nephrotoxicoses in 8 dogs (5%), and other causes of AKI in 34 dogs (20%). The AKI grading (Appendix S3) at admission indicated 8 dogs in grade 3 (5%), 78 dogs in grade 4

(45%), and 86 dogs in grade 5 (50%). Maximal grade during hospitalization was grade 3 for 3 dogs (2%), grade 4 for 72 dogs (42%), and grade 5 for 97 dogs (56%). In 64 dogs (37%; 51 with leptospirosis and 13 with other etiologies), liver involvement was diagnosed, with a peak serum bilirubin concentration of 4.0 mg/dL (IQR, 1.3–11.7 mg/dL). Leptospirosis-associated pulmonary hemorrhages were suspected radiologically in 92 dogs (53%), and risk of bleeding associated with systemic hemostatic disorders was recognized by thrombocytopenia or prolonged coagulation times in 115 dogs (67%). Altogether, they accounted for 142 dogs (83%) in which SH was strongly contraindicated.

HD Treatments

A median of 3 (IQR, 2–4) HD treatments with RCA were performed in 172 dogs, resulting in 584 treatments included in the study. Initially, 66 pilot treatments were performed with additional SH, with heparin administered in 5 dogs using a standard protocol of initial bolus and full-dose CRI (resulting in 283 U/kg [IQR, 140–299 U/kg]) in 25 dogs with initial bolus and low-dose CRI (18 U/kg [IQR, 13–21 U/kg]), in 35 dogs with initial bolus only (IQR, 15 U/kg [7–33 U/kg]), and in 1 dog with low-dose CRI only (64 U/kg).

In the first 19 pilot treatments, citrate was injected through the heparin line of the extracorporeal circuit, but for later treatments was changed to the most proximal site accessible, the inflow connection of the dialysis catheter, because of excessive clotting proximal to the injection site. Similarly, calcium injection was moved distally from the venous drip chamber (first 8 pilot treatments) to the outflow connection of the dialysis catheter.

Evaluation of safety and efficacy of the RCA protocol was based on the last 518 treatments performed with the final heparin-free protocol. This group included 156 first dialysis treatments (30%), 129 second treatments (25%), 97 third treatments (19%), and 136

Table 2. Main characteristics of the 518 heparin-free HD treatments performed with the final RCA protocol.

Treatment	Tx 1 (n = 156)	Tx 2 (n = 129)	Tx 3 (n = 97)	Tx > 3 (n = 136)	P
Treatment duration (min)	150 (119 to 164)	188 (180 to 210)	210 (180 to 243)	240 (206 to 251)	<0.001
Blood processed (L/kg)	0.41 (0.36 to 0.45)	0.76 (0.68 to 0.85)	1.31 (1.10 to 1.47)	1.68 (1.37 to 1.99)	<0.001
Body weight (kg)					
Pre	21.7 (11.0 to 28.7)	21.3 (11.0 to 28.6)	21.9 (12.0 to 28.2)	22.9 (17.3 to 29.9)	0.47
Post	21.7 (11.0 to 28.7)	21.2 (11.0 to 28.5)	21.4 (11.9 to 28.3)	22.6 (16.6 to 29.8)	0.49
Delta	0.0 (−0.2 to 0.2)	−0.1 (−0.3 to 0.1)	0.0 (−0.3 to 0.1)	0.0 (−0.3 to 0.2)	0.58
PCV (%) [ref. 40–55]					
Pre	31.5 (26.0 to 37.0)	30.0 (25.0 to 34.0)	30.0 (24.0 to 35.5)	27.0 (22.0 to 35.8)	0.046
Post	30.0 (25.0 to 35.0)	29.0 (24.0 to 33.3)	28.0 (22.5 to 33.0)	25.0 (20.0 to 32.0)	0.004
Urea (mg/dL) [ref. 17.4–66.7]					
Pre	380 (330 to 501)	299 (235 to 359)	198 (150 to 243)	172 (129 to 216)	<0.001
Post	222 (186 to 292)	105 (78 to 148)	38 (28 to 60)	25 (15 to 37)	<0.001
URR	0.41 (0.37 to 0.47)	0.63 (0.58 to 0.69)	0.79 (0.74 to 0.83)	0.86 (0.79 to 0.90)	<0.001
Creatinine (mg/dL) [ref. 0.5–1.6]					
Pre	10.2 (8.0 to 12.3)	8.6 (7.2 to 10.5)	7.2 (6.0 to 8.3)	7.8 (6.6 to 9.2)	0.008
Post	6.4 (4.8 to 7.7)	3.8 (2.9 to 4.7)	2.2 (1.6 to 2.7)	1.8 (1.4 to 2.5)	<0.001
CrRR	0.37 (0.34 to 0.42)	0.56 (0.51 to 0.61)	0.70 (0.65 to 0.74)	0.76 (0.70 to 0.82)	<0.001

Tx, treatment; PCV, packed cell volume; URR, urea reduction ratio; CrRR, creatinine reduction ratio.

Data are presented separately for first, second, third, and later treatments, as median (IQR). P, statistical significance for comparison between groups in Kruskal–Wallis one-way ANOVA.

later treatments (26%). Neonatal extracorporeal lines were used for 157 treatments (30%), pediatric lines for 256 treatments (49%), and adult lines for 105 treatments (20%). Dialyzers Polyflux 2H were prescribed for 72 treatments (14%), Polyflux 6H for 63 treatments (12%), Polyflux 140H for 96 treatments (19%), Reva-clear for 29 treatments (6%), Evodial 1.0 for 120 treatments (23%), FSX paed for 13 treatments (3%), FX40 for 47 treatments (9%), FX50 for 40 treatments (8%), FX60 for 26 treatments (5%), and FX100 for 12 treatments (2%). The resulting extracorporeal blood volume reached 129 mL (IQR, 88–182 mL) or 8.1% (IQR, 6.7–10.2%) of the blood volume estimated at 8% of body weight.

Dialysis parameters included a treatment time [Td] of 188 minutes (IQR, 156–227 minutes), an average blood flow rate [Qb] of 97 mL/min (IQR, 55–152 mL/min), and a total volume of blood processed [Qp] of 17.2 L (IQR, 8.9–33.2 L) or 0.85 L/kg (IQR, 0.47-1.44 L/kg), with progressively increasing intensity after the initial treatments (Table 2). Resulting average extracorporeal blood residence time was 81 seconds (IQR, 63–117 seconds; range, 18–593 seconds). Median net fluid balance was neutral with a change in body weight of 0.00 kg (IQR, −0.30 to 0.12 kg) over the course of treatment.

Changes of Laboratory Variables with HD and Adjustments of Citrate and Calcium Infusion Rates

The first iCa control at 30 minutes of therapy was reached in 513/518 RCA treatments (99%). This control was not available for 5 dogs because of early discontinuation for excessive clotting (n = 2), catheter dysfunction (n = 1), or severe cardiopulmonary complication (n = 2). Twelve additional measurements were not available because of technical problems with the blood gas

analyzer. Results of iCa monitoring and adjustments of the infusion rates of citrate and calcium are summarized in Table 3 and Figure 2. Adjustments at 30 minutes of therapy were more commonly required in the first than in later RCA treatments. Whiles monitoring of iCa in the extracorporeal circuit predialyzer often led to adjustments of the citrate infusion rate, its monitoring postdialyzer was rarely useful, with 272/274 (99.3%) measurements in the target range. Of all the 864 S1 measurements (iCa in the dog) performed during these 518 treatments, only 20 (2.3%) were in a range of potential risk for hypocalcemic complication (<0.7 mmol/L), 4 of them (0.5%) being <0.6 mmol/L. Most of these hypocalcemic events were recorded at the beginning of the treatment (14/20) and during the first HD treatment for an individual dog (9/20). None was associated with any clinical manifestations of hypocalcemia, but vomiting was observed in 3 of these occurrences.

Overall, 50% of the RCA treatments (259/518) necessitated adjustments of citrate or calcium infusion rates or both. After adjustments were performed, the final citrate and calcium infusion rates for the 518 HD treatments both were slightly increased from the initial 2.55 (IQR, 2.55–2.81) and 0.85 (IQR, 0.85–0.96) mmol/L to 2.66 (IQR, 2.55–2.89) and 0.91 (IQR, 0.85–1.02) mmol/L blood, respectively.

The effects of HD with RCA on relevant biochemical and blood gas variables are summarized in Table 4. Total calcium concentration changed −0.92 mg/dL (IQR, −1.88 to +0.04 mg/dL) [−0.23 mmol/L (IQR, −0.47 to +0.01) mmol/L] (P < 0.001), decreasing the proportion of total hypercalcemia from 28 to 16% (P < 0.001) and increasing the proportion of total hypocalcemia from 28% pre-HD to 52% post-HD (P < 0.001). The decrease in total calcium concentration was mainly due to a change in the calcium gap, the

Table 3. Results of the iCa monitoring during 518 HD treatments performed with RCA and adjustments performed to the infusion rates of trisodium citrate and calcium chloride.

	First RCA Tx n = 159	Later RCA Tx n = 359	P
iCa on target at first measurement (30 min)			
Dog (S1)	122/154	300/348	0.049
	79%	86%	
Circuit, predialyzer (S2)	113/154	307/346	<0.001
	73%	89%	
Circuit, postdialyzer (S3)	76/77	196/197	0.50
	99%	99%	
Adjustments to citrate infusion rate			
Increased			
Changes (n)	64	84	
Treatments with changes (%)	57 (36%)	72 (20%)	<0.001
Decreased			
Changes (n)	3	19	
Treatments with changes (%)	3 (2%)	17 (5%)	0.12
Adjustments to calcium infusion rate			
Increased			
Changes (n)	69	128	
Treatments with changes (%)	51 (32%)	103 (29%)	0.44
Decreased			
Changes (n)	3	23	
Treatments with changes (%)	3 (2%)	22 (6%)	0.03
All adjustments			
Changes (n)	139	254	
Treatments with changes (%)	99 (62%)	160 (45%)	<0.001

RCA, regional citrate anticoagulation; Tx, treatment; iCa, ionized calcium; S1, blood sampled from the dog; S2, blood sampled from the extracorporeal circuit, predialyzer; S3, blood sampled from the extracorporeal circuit, postdialyzer.

P, statistical significance for comparison of proportions between first and later RCA treatments in Chi-Square Test.
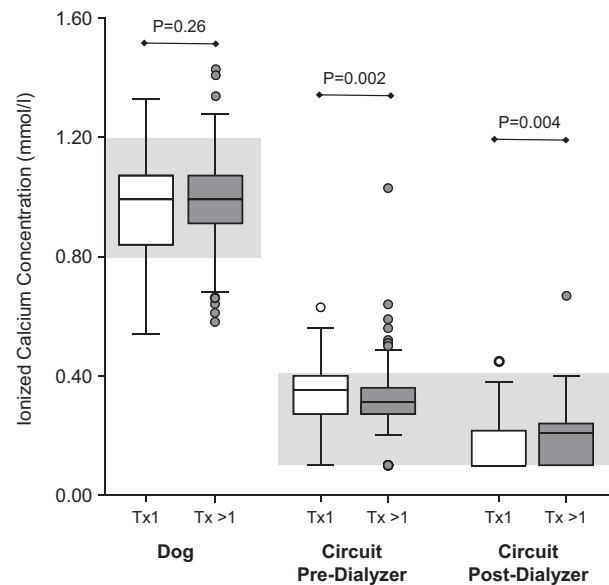

Fig 2. Box plot representation of the blood iCa concentration measured from the dogs and from the extracorporeal circuits (pre- and postdialyzer) 30 minutes after initiation of HD in 518 treatments with RCA. First treatments (white boxes, n = 159) are compared to later treatments (gray boxes, n = 359), Mann-Whitney U-test. Shaded areas represent the target iCa concentration for the corresponding samples.

Activated clotting time (ACT) was slightly lower post-HD compared to pre-HD with a change of −4 seconds (IQR, −16 to +4 seconds; $P < 0.001$), a slight but clinically irrelevant improvement in coagulation.

Safety and Efficacy of the RCA Procedure

Five hundred of 518 treatments (96.5%) could be completed successfully. Nine treatments (1.7%) had to be stopped early because of system clotting, 6 (1.2%) due to catheter-related (n = 4) or because of other technical problems (n = 2), and 3 (0.6%) as a consequence of fatal worsening of leptospirosis-associated pulmonary hemorrhages (n = 2) or cardiovascular failure (n = 1) (Table 5). Minor complications not requiring discontinuation of the procedure were observed in 19 additional treatments (3.7%). They included catheter dysfunction (n = 7, 1.4%) or other technical problems (n = 1, 0.2%), respiratory (n = 4, 0.8%) or cardiovascular (n = 4, 0.8%) complications, and 1 case each (0.2%) of fever, abdominal pain, and severe excitation. Therefore, 481 treatments (92.9%) were completed without complications.

Fatalities were observed during dialysis in 3 dogs (0.6%) and within 24 hours of treatment completion in 7 other dogs (1.4%). All fatalities were direct complications of the underlying cause of AKI, and none was directly or indirectly associated with the RCA procedure. Eight of these events were caused by worsening of leptospirosis-associated pulmonary hemorrhages, 1 by cardiovascular failure, and 1 by rupture of multiple intestinal intussusceptions. None of the classical clinical

nonionized form of Ca, of −0.24 mmol/L (IQR, −0.46 to −0.01 mmol/L; $P < 0.001$). There was no net change of iCa with a ΔiCa of 0.00 mmol/L (IQR, −0.10 to +0.13 mmol/L; $P = 0.3$). Although the proportion of ionized hypercalcemia increased from 6/501 (1.2%) pre-HD to 24/500 (4.8%) post-HD ($P < 0.001$), most of these changes remained minimal with iCa between 1.4 and 1.5 mmol/L (pre: 4/6, post: 17/24). The ratio of total to ionized calcium, typically used to indicate citrate toxicity in humans when >2.5, decreased from 2.41 (IQR, 2.18–2.73) to 2.14 (IQR, 2.00–2.34). The limit of 2.5 was exceeded in 207/501 (41.3%) treatments before and 81/493 (16.4%) treatments after therapy.

Relevant changes in blood gas parameters with the treatments were as follows: ΔpH = +0.021 (IQR, −0.024 to +0.079; $P < 0.001$), Δbicarbonate = +1.3 mmol/L (IQR, −0.5 to +3.0 mmol/L; $P < 0.001$), ΔpCO2 = +0.35 mmHg (IQR, −3.4 to +5.5 mmHg; $P < 0.001$), and Δanion gap = −8.8 mmol/L (IQR, −11.2 to −6.5 mmol/L; $P < 0.001$).

Table 4. Changes of calcium concentration, main venous blood gas parameters, and activated clotting time with HD in dogs treated with RCA (n = 518 treatments).

	Tx 1 (n = 156)	Tx 2 (n = 129)	Tx 3 (n = 97)	Tx > 3 (n = 136)	P
TCa (mg/dL) [ref. 10.0–11.7]					
Pre	10.1 (9.2–11.2)	10.2 (9.4–11.2)	10.4 (9.7–11.2)	11.4 (10.3–13.3)	<0.001*
Post	9.3 (8.5–10.4)	9.2 (8.3–10.5)	9.6 (8.6–10.4)	10.2 (9.3–11.4)	<0.001*
TCa (mmol/L) [ref. 2.50–2.93]					
Pre	2.53 (2.30–2.81)	2.55 (2.36–2.81)	2.59 (2.42–2.81)	2.86 (2.57–3.32)	<0.001*
Post	2.32 (2.12–2.61)	2.30 (2.07–2.63)	2.40 (2.15–2.60)	2.56 (2.33–2.85)	<0.001*
P (pre-post)	<0.001*	<0.001*	<0.001*	<0.001*	
iCa (mmol/L) [ref. 1.20–1.40]					
Pre	1.12 (0.96–1.21)	1.09 (0.98–1.17)	1.12 (1.03–1.21)	1.13 (1.05–1.23)	0.052
Post	1.06 (0.98–1.17)	1.10 (1.00–1.22)	1.10 (1.02–1.18)	1.14 (1.05–1.23)	0.002*
P (pre-post)	0.87	0.054	0.48	0.10	
Non-iCa (mmol/L) [ref. 1.30–1.50]					
Pre	1.46 (1.21–1.68)	1.48 (1.29–1.77)	1.50 (1.30–1.72)	1.76 (1.45–2.10)	<0.001*
Post	1.25 (1.08–1.49)	1.18 (1.02–1.46)	1.29 (1.09–1.43)	1.41 (1.21–1.68)	<0.001*
P (pre-post)	<0.001*	<0.001*	<0.001*	<0.001*	
pH [ref. 7.35–7.45]					
Pre	7.30 (7.23–7.34)	7.30 (7.27–7.34)	7.31 (7.28–7.34)	7.33 (7.28–7.40)	<0.001*
Post	7.32 (7.28–7.35)	7.33 (7.31–7.36)	7.35 (7.32–7.38)	7.36 (7.32–7.39)	<0.001*
P (pre-post)	<0.001*	<0.001*	<0.001*	0.006*	
HCO_3^- (mmol/L) [ref. 20–24]					
Pre	17.4 (14.2–19.6)	17.2 (15.5–19.7)	18.3 (16.2–20.2)	17.6 (15.8–21.0)	0.029*
Post	18.4 (16.8–20.1)	19.5 (18.0–21.2)	20.4 (18.9–21.9)	20.8 (19.5–22.4)	<0.001*
P (pre-post)	<0.001*	<0.001*	<0.001*	<0.001*	
pCO_2 (mmHg) [ref. 35–45]					
Pre	36.2 (32.6–40.3)	36.6 (34.2–39.6)	37.5 (34.3–41.9)	35.1 (30.3–39.8)	0.011*
Post	37.8 (34.7–40.2)	37.9 (34.6–40.4)	38.1 (35.6–40.6)	38.4 (34.8–41.1)	0.53
P (pre-post)	0.19	0.011*	0.88	<0.001*	
Anion Gap (mmol/L) [ref. 8–12]					
Pre	26.2 (21.8–31.4)	22.7 (20.0–26.5)	19.4 (17.1–22.5)	20.8 (18.0–24.7)	<0.001*
Post	17.8 (14.8–21.5)	13.1 (10.9–15.8)	11.7 (9.7–13.7)	10.6 (8.9–13.2)	<0.001*
P (pre-post)	<0.001*	<0.001*	<0.001*	<0.001*	
ACT(s) [ref. 60–90]					
Pre	111 (101–133)	111 (101–129)	113 (98–125)	107 (101–128)	0.96
Post	112 (99–123)	105 (98–118)	105 (94–115)	102 (92–118)	0.068
P (pre-post)	0.50	0.013*	0.011*	<0.001*	
Mg (mg/dL) [ref. 1.53–2.33]					
Pre	2.99 (2.38–3.38)	2.89 (2.45–3.23)	2.72 (2.43–3.04)	2.65 (2.36–2.96)	0.002*
Post	2.43 (2.24–2.67)	2.21 (2.07–2.36)	2.09 (1.92–2.21)	1.94 (1.82–2.19)	<0.001*
P (pre-post)	<0.001*	<0.001*	<0.001*	<0.001*	

Tx, treatment; TCa, total calcium concentration; iCa, ionized calcium concentration; HCO_3^-, serum bicarbonate concentration; pCO_2, CO_2 partial pressure; ACT, activated coagulation time; Mg, magnesium.

Data are presented separately for first, second, third, and later treatments, as median (IQR). P, statistical significance for comparison between groups in Kruskal–Wallis one-way ANOVA; P(pre-post), statistical significance for paired comparisons before and after HD in Wilcoxon Signed-Rank Test. Statistically significant differences are indicated with *.

signs associated with hypocalcemia were noted at any time during the treatments in the dogs dialyzed with RCA.

Changes in dialyzer flow characteristics were noticed by an increase in dialyzer pressure gradient of 6.5%

(IQR, −3.7 to 17.3%, $P < 0.001$) from baseline. Dialyzer filtration function was only minimally affected with an effective URR of 104% (IQR, 99–110%) of the expected URR and an effective CrRR of 91% (IQR, 82–96%) of the expected CrRR. The extracorporeal

Table 5. Efficacy of anticoagulation performed with a RCA in dogs undergoing HD (n = 518 treatments).

	Tx Completion	Dialyzer ΔP Increase < 25%	Effective URR ≥75% From Expected	Effective CrRR ≥75% From Expected	Visual Scoring Score ≤ 4
Successful	500/518 (97%)	266/323 (82%)	483/506 (95%)	435/506 (86%)	472/513 (92%)
Unsuccessful	18/518 (3%)	57/323 (18%)	23/506 (5%)	71/506 (14%)	41/513 (8%)

Tx, treatment; ΔP, hydrostatic pressure gradient; URR, urea reduction ratio; CrRR, creatinine reduction ratio.

circuits of 513 treatments could be inspected visually after blood rinseback. No visible clotting overall was observed in 96 (19%) circuits. Minimal clotting was found in 376 (73%) circuits, moderate clotting in 35 (7%) circuits, and severe clotting in 6 (1%) circuits.

Overall, anticoagulation was judged very good in 372 treatments (72%), associated with minor grade complications in 100 (19%), moderate grade complications in 26 (5%), and severe grade complications in 17 (3%) treatments. Moderate and severe grade complications more often were encountered in small dogs and during early treatments (both $P < 0.001$). Accordingly, pretreatment urea ($P < 0.001$) and creatinine ($P < 0.01$) concentrations were higher, total calcium concentration ($P = 0.04$) was lower, average blood flow ($P < 0.001$) was lower, extracorporeal blood residence time ($P < 0.001$) was longer, and URR ($P < 0.001$) and CrRR ($P < 0.001$) were lower in problem treatments.

Discussion

Our data establish for the first time a safe and efficient RCA protocol for intermittent HD developed specifically for dogs. The administration of citrate in the extracorporeal blood circuit immediately chelates divalent cations. The decrease in iCa inhibits the function of several calcium-dependent factors, disrupting important mechanisms of platelet adhesion, and activation as well as the intrinsic and extrinsic pathways of the coagulation cascade.[18] The established protocol was associated with minimal complications in a very small number of treatments. Anticoagulation was very efficacious and yielded satisfactory results in >90% of the heparin-free treatments, allowing HD treatment in dogs at risk of or with active bleeding.

The 2-stage study design with in vitro titration experiments first on normal canine blood followed by in vivo heparin-free RCA in a large group of dogs at risk of bleeding proved successful and relevant for establishing the definitive protocol to be used in dogs. The initial use of the protocol in pilot dogs under additional SH allowed us to evaluate its safety and efficacy separately. The effectively used citrate and calcium infusion rates after adjustments differed only minimally from the starting flow rates, confirming further the validity of the calculations. The monitoring protocol and the adjustments performed largely were adapted from similar protocols used in humans and proved very appropriate for use in the dog, as can be seen in the large proportion of successful treatments. The simple calculation of the initial citrate and calcium flow rates used in our study has been preferred for other slightly more complex

calculations including predialysis hematocrit, total protein concentration, and total calcium concentrations to determine the infusion rates of citrate and calcium, a decision supported by good efficacy results.[17]

One of the main practical differences between SH and RCA is the necessity to monitor iCa repeatedly during treatment. Because doing so replaces monitoring of coagulation times, it does not require removal of additional blood volume, but it may increase monitoring costs, depending on the system available. The large number of treatments (50%) requiring adjustments of flow rates of citrate, calcium or both clearly emphasizes this necessity, especially in the first treatments. The number of adjustments possibly could be decreased by increasing the starting citrate and calcium infusion rates by +4 and +7%, respectively, based on the final infusion rates achieved for the study dogs.

As opposed to SH, the infusion rates of citrate and calcium are functions of the blood flow rate and must be changed or stopped accordingly. Failure to do so may quickly result in excessive citrate infusion and cause fatal arrhythmias.[19] With insufficient calcium administration, hypocalcemia is more likely to result in neuromuscular complications before arrhythmias are observed. Insufficient citrate and excessive calcium infusion rates may result in clotting of the extracorporeal circuit and substantial blood loss from the animal. These severe and potentially fatal complications should be kept in mind, and attention should be given to the appropriate function of the whole system, particularly in very small patients with small catheter volumes and very large patients with high citrate and calcium infusion rates. Some continuous renal replacement therapy machines have incorporated automated RCA modules, eliminating the need for manual adjustments of the citrate and calcium pumps. Such systems are increasingly available on intermittent HD platforms and will represent a relevant safety advantage in the near future. However, our study clearly shows that these risks should not be overemphasized, provided appropriate monitoring is carried out, and no such complication was observed in 518 treatments on a wide range of canine sizes and blood flow rates.

Major metabolic complications resulting from absolute or relative excess in trisodium citrate administration were rarely encountered in dogs treated with the described protocol. Hypernatremia and metabolic alkalosis were never observed in the study treatments. Citrate toxicity with total hypercalcemia and ionized hypocalcemia resulting from citrate accumulation from excessive infusion rate or insufficient metabolism was never recognized clinically. Increased postdialysis calcium gap and a TCa:iCa ratio >2.5 often are used in

humans as evidence of citrate toxicity. In these dogs, both variables decreased significantly during the treatments, indicating a lack of accumulation of citrate-calcium complexes. The predialysis TCa:iCa ratio, however, was >2.5 in >40% of the treatments, limiting the use of this ratio to its relative change during the treatment. Despite a short half-life in circulation, the metabolism of citrate is located predominantly in the liver, in the skeletal muscle, and in the kidney,[20] and causes citrate anticoagulation to be relatively contraindicated in humans with renal and liver insufficiency. However, no evidence of citrate toxicity was recognized in dogs with kidney and liver disease, even in those affected with leptospirosis and combined severe renal and hepatic involvement, in which RCA was used safely. This observation probably reflects the high clearance rate of the procedure, with removal of most of the remaining citrate by the dialyzer. At the extremes of body weight, such dogs should, however, still be monitored more closely for the occurrence of citrate toxicity.

Other hematologic and metabolic effects of citrate anticoagulation were not evaluated further in our study. Considering the tight physiologic regulation of serum iCa, it is possible that the interference caused by the RCA technique may affect central metabolic pathways. For example, even a minor change in iCa is likely to alter parathyroid hormone production. In humans, a low-iCa target facilitated reaching a less positive calcium mass balance, however, at the expense of a significant increase in parathyroid hormone concentration.[21] How far such short-term alterations add to the marked dysregulation of calcium and phosphate metabolism in AKI patients remains to be evaluated.

Both heparin and citrate have been reported to affect the pro- versus anti-inflammatory balance differently, depending on the model used.[22,23] They are postulated to influence the inflammatory response either directly or through some of its key mediators. For example, RCA was shown to help decrease leukocyte and monocyte activation during passage through the extracorporeal circuit.[24] On the other hand, insufficient anticoagulation can result in activation of platelets and the coagulation cascade and thus cause additional inflammation, considering the intricate links between hemostasis and inflammation. Evaluation of these aspects was beyond the scope of our study and should be investigated in specifically designed prospective studies.

One of the main limitations of our study was the absence of a comparison group with standard SH, especially for the evaluation of safety and efficacy. Unfortunately, with 83% of dogs in which heparinization was contraindicated, a 2-group design would have resulted in a severe selection bias with marked differences in etiologies, inflammatory status, hemostatic disorders, and disease severity between the groups. We chose therefore to concentrate on the development of a safe and efficient citrate anticoagulation protocol and on a detailed description of its effects.

In conclusion, the established RCA protocol (Appendix S4) opens the possibility to safely dialyze dogs with active bleeding, at high risk of bleeding, or

in need of immediate surgery. Considering that the main indication for HD in veterinary medicine is AKI and that hemostatic disorders are frequent in this group of patients, this approach represents a major advance for blood purification techniques in critically ill dogs.

Footnotes

a Trisodium citrate 30 g/L, 102 mmol/L, Dr G. Bichsel Laboratory, Interlaken, Switzerland

b Calcium chloride 50 g/L, 340 mmol/L, Dr G. Bichsel Laboratory

c RAPIDPoint 400, Siemens Health Care Diagnostics, Zurich, Switzerland

d Gambro Hospal (Switzerland), Kilchberg, Switzerland

e Lines A-5/V-5 (priming volume, 33 mL), BL121P (100 mL), BL200 BD (151 mL); Gambro Hospal (Switzerland)

f Filters Polyflux 2H (surface area, 0.2 m²; blood priming volume, 18 mL), Polyflux 6H (0.6 m²; 52 mL) Polyflux 140H (1.4 m²; 75 mL), Revaclear (1.4 m², 84 mL), Evodial 1.0 (1.0 m²; 66 mL), Gambro Hospal (Switzerland)
Filters FX paed (0.2 m²; 18 mL), FX40 (0.6 m²; 32 mL), FX50 (1.0 m²; 53 mL), FX 60 (1.4 m²; 74 mL), FX 100 (2.2 m²; 116 mL); Fresenius Medical Care (Switzerland), Oberdorf, Switzerland
Membrane material—Polyflux filters: Polyflux membrane (polyarylether sulfone/polyvinyl pyrrolidone/polyamide); Revaclear filter: Poracton membrane (polyarylether sulfone/polyvinyl pyrrolidone); Evodial filter: HeprAN membrane (heparin-grafted acrylonitrile—sodium methallyl sulfonate copolymer/polyethyleneimine, AN69ST); Fresenius filters: Helixone membrane (Fresenius Polysulfone based)

g Dialysate A341G; Dr G. Bichsel Laboratory

h Microsoft Excel 2010, (Version 14.0.7188.5000); Microsoft Corporation, Redmond, WA

i NCSS 9.0.15; NCSS, LLC, Kaysville, UT

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Supporting Information

Additional Supporting Information may be found online in Supporting Information:

Appendix S1. Assessment criteria used for the evaluation of treatment efficacy.

Appendix S2. Modeling parameters of expected URR and CrRR using standard heparinisation in 233 HD treatments for AKI in canines.

Appendix S3. IRIS grading system for dogs with acute kidney injury (AKI).

Appendix S4. Proposed treatment protocol for regional citrate anticoagulation in dogs on intermittent hemodialysis.