Novel Methods for Damage Control of Hyperkalemia in Combat Casualties

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FINAL REPORT

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1.0 EXECUTIVE SUMMARY:

Objective: Current techniques for renal replacement therapy (RRT) require: 1) specialized equipment, 2) extensively trained personnel and 3) large logistical footprints. These constraints limit the Air Force's ability to provide life-saving treatments in forward deployed locations. Our innovations may allow for these treatments with negligible increases in equipment and logistical footprint. Furthermore, these techniques can be accomplished with minimal training for deployed providers.

Methods: For this program, three techniques for simplified RRT that require minimal training and supplies were tested. In the first aim (Phase 2), "Field expedient renal replacement therapy," we used a Belmont® rapid infuser and improvised fluids to develop an improvised method for renal replacement therapy. In the second aim (Phase 1), "Potassium binding hemoperfusion," we developed a novel hemoperfusion filter to remove potassium, leveraging the Belmont as a blood pump. Lastly, in our third aim (Phase 3), "Novel peritoneal techniques," using a potassium binder with peritoneal dialysis fluid, the fluid can be recycled to remove potassium

Results: *Phase 1:* Serum potassium concentration was significantly lower in the treatment than in the control group over time ($P = 0.02$). *Phase 2:* No difference was found in electrolyte concentrations between the commercial and improvised replacement solutions. *Phase 3:* There was no statistically significant difference in serum potassium between groups over time ($p = 0.766$).

Conclusions: In austere settings, a simplified hemoperfusion system could be used to temporize patients with hyperkalemia until evacuation to a facility with traditional RRT. The ImpRRT system achieved similar performance to CRRT and may represent a potential option for temporary RRT following disasters. Lastly, the experimental device in *Phase 3* used significantly less fluid and was able to control serum potassium levels with similar efficacy to that of conventional peritoneal dialysis.

2.0 INTRODUCTION:

Acute kidney injury (AKI), with subsequent hyperkalemia, has been recognized since World War II, when severe renal dysfunction was associated with a mortality rate of 90%.[1, 2] In the Korean War, hyperkalemia was the leading cause of death in patients with post-traumatic AKI, until the use of renal replacement therapy (RRT) reduced mortality to 53%.[3, 4] RRT remains the standard of care for the treatment of AKI and hyperkalemia that does not respond to medical management. While the occasional need for RRT in theater led to the deployment of the NxStage System One (NxStage Medical, Lawrence, MA) to Craig Joint Theater Hospital[5], rapid evacuation out of Iraq and Afghanistan ensured that most AKI with hyperkalemia occurred late in the evacuation chain (in Germany or the United States) where RRT capabilities were available. However, neither the NxStage (with its large logistical footprint requiring 50L of specialized fluid per day and need for specialized training), nor rapid evacuation out of theater are viable options in the A2/AD environment. Based on data from World War II and Korea, casualties with severe AKI will die in the A2/AD environment without access to RRT. Therefore, simplified organ support technologies are needed.

Given the ready availability of RRT in civilian care, there is little incentive for the development of novel RRT techniques that could be fielded into the austere, forward deployed setting. AFMSA must lead the way in this effort or combat casualties with severe AKI will not survive their injuries. In this work, we sought to identify the most promising of three different technologies for further development and eventual deployment. Our methods offer the potential to save the lives of critically injured war-fighters with a minimal increase in existing technologies, infrastructure and training.

For this program, three techniques for simplified RRT that require minimal training and minimal supplies were tested:

1) *Field expedient renal replacement therapy (Phase 2)*. Traditional RRT requires a dialysis catheter, tubing, a blood pump, a hemofilter and dialysate (or replacement fluid). A recent report out of Syria[6] demonstrates that clinicians with minimal training and improvised supplies can safely and effectively perform RRT under austere conditions. The largest and most complex

piece of equipment required to do RRT is the blood pump. Compared to the Syrian experience (which utilized recycled blood pumps smuggled into rebel controlled areas), the Air Force has a major advantage: the Belmont® rapid infuser. The Belmont®, which is ubiquitous in military medicine given its vital role in damage control resuscitation, is very similar to a dialysis machine. Both have a roller pump, air traps, pressure sensors, emergency shut offs and warmers. Utilizing the Belmont® and improvising dialysate (or replacement fluid) from standard intravenous fluids (both of which are available at forward deployed MTFs), an RRT capability could be added with a hemofilter, two lengths of tubing and an adapter. The total weight of these four additional components is less than half a pound. We have done model development on this system and found that the Belmont® can circulate blood from the body through a hemofilter, providing evidence for feasibility.

2) *Potassium binding hemoperfusion (Phase 1).* While a variety of toxins, metabolites and electrolytes accumulate in AKI, the primary driver of early mortality is hyperkalemia.[3, 4] Therefore, a method that focuses simply on removing potassium in the body could serve as a temporizing measure to buy time for evacuation and definitive care with RRT. To that end, we are working with CytoSorbents (Monmouth, NJ) to develop a cartridge that can remove potassium. By utilizing the principle of cation exchange, this method would not need dialysate (or replacement fluid), saving vital resources for other injured casualties. This method would also use the Belmont® to circulate blood through the filter.

3) *Novel peritoneal techniques (Phase 3).* Peritoneal dialysis (PD) is a form of RRT that utilizes the peritoneal membrane to achieve clearance via diffusion with fluid in the peritoneal space and is an accepted treatment for AKI.[7] While PD has been successfully used in the austere combat setting[8], the large amount of fluid required for exchange is a significant limitation. A standard prescription is 2L every 2 hours (for a total of 24L a day). If we again consider that the predominant killer in AKI during the acute phase is hyperkalemia, and focus clearance on that electrolyte, we propose that the amount of fluid required can be greatly reduced. By using a potassium binder (which works on the principle of cation exchange), the same 2L could be reused again and again. This technique could be modified into several different configurations (traditional PD, put in line and recirculated through a wound vac, or included in surgical

packing). The end state would be a product approximately 3-4L in volume (including the fluid, a catheter and the potassium binder) that would be all a deployed medic would need to temporize a patient with severe AKI until they could be evacuated. This method also has the added advantage that it is driven by gravity and would not require any power source. This technology is also being developed in conjunction with CytoSorbents.

3.0 METHODS, ASSUMPTIONS AND PROCEDURES:

These studies was approved by the Institutional Animal Care and Use Committee at David Grant USAF Medical Center, Travis Air Force Base, California. All animal care and use was in strict compliance with the Guide for the Care and Use of Laboratory Animals in a facility accredited by AAALAC. Animals were procured from the University of California-Davis and acclimatized for 10 days prior to the experiment. All animals were housed to provide visual, olfactory, and when possible, tactile contact with conspecifics.

Phase 1: Potassium binding hemoperfusion

General anesthesia

Ten Yorkshire-cross 5-6 month old male castrated pigs (*Sus scrofa*) weighing between 60 and 97 kg were anesthetized with an intramuscular injection of 6 mg/kg of tiletamine/zolazepam (Telazol, Zoetis, Parsippany, NJ). Following orotracheal intubation, animals were mechanically ventilated with a tidal volume of 6-10 mL/kg. Respiratory rate was adjusted to maintain end-tidal CO_2 between 35 and 45 mmHg. Anesthesia was maintained with 1.5-2.5% isoflurane (Baxter, Deerfield, IL) in 100% oxygen. Throughout the experiment, heart rate, electrocardiogram, pulse oximetry, core body temperature, and invasive arterial blood pressure were monitored. Animals received an intravenous infusion of 0.9% NaCl (Baxter, Deerfield, IL) at a rate of 5 mL/kg/hour to counteract the vasodilatory effects of anesthesia and maintain a mean arterial pressure (MAP) $\geq$ 50 mmHg. If the MAP was < 50 mmHg, animals received 1 L of 0.9% NaCl as a bolus once during the experiment. If the MAP remained < 50 mmHg despite the 0.9% NaCl bolus, animals received an intravenous infusion of phenylephrine (West Ward Pharmaceuticals, Eatontown, NJ) titrated to effect at a dose ranging from 0.1 to 3 μg/kg/minute. Intravenous dextrose (VetOne,

Boise, ID) supplementation was provided as needed to maintain serum glucose concentration > 60 mg/dL.

Vascular access

Seven French catheters (Boston Scientific Corporation, Marlborough, MA) were placed percutaneously in a femoral artery (for blood sampling and continuous arterial blood pressure monitoring) and vein (for 0.9% NaCl and drug infusions). Blood was sampled for baseline evaluation of arterial blood gases and electrolyte concentrations (Rapidlab 1200, Siemens, Malvern, PA), biochemistry panel [serum potassium, calcium (total and ionized), total magnesium, phosphorus, and creatinine concentrations] (VetScan VSpro, Abaxis, Union City, CA), and complete blood count (VetScan HM5, Abaxis, Union City, CA). A 20cm Niagara™ temporary dialysis catheter (Bard Access Systems, Salt Lake City, UT) was inserted in the right external jugular vein. The arterial and venous lumens of the hemodialysis catheter were filled with heparin (APP Pharmaceuticals, Schaumburg, IL) until initiation of extracorporeal circulation.

Surgical preparation

Bilateral nephrectomies were performed via a mid-line celiotomy. The abdomen was then closed and warming blankets were placed around the animals to prevent hypothermia.

Potassium infusion

Following bilateral nephrectomy, each animal received an intravenous bolus of potassium chloride (Hospira Inc., Wake Forest, IL) over 30 minutes according to the following formula:

$$K^+ \, dose \, (mmol) = 7.5 \times \left[\frac{Body \, weight \, (kg) \times 0.63}{3} - \left(\frac{Body \, weight \, (kg) \times 0.63}{3} \times [K^+](mmol \, / \, L) \right) \right]$$

Animals were randomized to the control or treatment group upon initiation of the potassium bolus.

Extracorporeal circuit

Animals were administered an intravenous heparin bolus followed by an infusion to maintain an activated clotting time (ACT) of at least 200% baseline value or greater than 200 seconds, whichever was the highest. The ACT was monitored hourly. The extracorporeal circuit (EC) consisted of tubing (Belmont Instrument Corporation, Billerica, MA) modified to allow connections (Perfusion adapter, Medtronic, Minneapolis, MN) between elements of the system (Figure 2). In both groups, a Belmont Rapid Infuser™ (Belmont Instrument Corporation, Billerica, MA) was used as a peristaltic pump to circulate blood. This device allows for flows up to 500 mL/minute and warms the fluids to 37.5°C. Blood was circulated from the arterial lumen of the hemodialysis catheter through a hemodialyzer (Gambro® Baxter International, Deerfield, IL) via the Belmont Rapid Infuser™ and then returned to the patient through the venous lumen of the hemodialysis catheter (Solid black lines, Figure 2). Additionally, in the treatment group, the ultrafiltrate was diverted from the hemodialyzer and circulated through two in-line cartridges, each containing novel potassium binding polymer (500 and 300 mL, respectively) (CytoSorbents Medical Inc., Monmouth Junction, NJ). The ultrafiltrate was then returned to the EC between the arterial port of the hemodialysis catheter and the intake of the Belmont Rapid Infuser™ (Grey dotted line, Figure 2B). The potassium binding cartridges (Grey dotted lines, Figure 2B) in the treatment group were further rinsed with 1 L of 0.9% NaCl, which was discarded prior to initiation of extracorporeal circulation. After assembly, the circuit was primed with 0.9% NaCl with 5000 units of heparin. After collecting baseline (T0) blood samples and removing the heparin from the hemodialysis catheter lumens, the EC was connected to the arterial lumen of the hemodialysis catheter. The Belmont Rapid Infuser™ flow was set at 250 mL/minute in the control group. For the treatment group, the Belmont Rapid Infuser™ flow was set at 400 mL/minute, and the ultrafiltrate flow through the potassium binding cartridges was maintained at 150 ± 10 mL/minute using a flow controller placed on the by-pass circuit (Figure 2B). This allowed for comparable flows through the hemodialysis catheter (venous and arterial lumens) for both groups (*i.e.*, 250 mL/minute). The heparinized 0.9% NaCl used to prime the circuit was discarded, and the EC was closed by connecting the output to the venous lumen of the hemodialysis catheter.

Data collection

Arterial blood samples were obtained every 30 minutes for 6 hours to evaluate serum potassium, total and ionized calcium, total magnesium, phosphorus, and creatinine concentrations

[3 mL of whole blood in a lithium heparin tube (Vacuette, Greiner Bio-One, Monroe, NC)], along with a complete blood count [4 mL of whole blood in a K2-EDTA anticoagulated tube (Vacuette, Greiner Bio-One, Monroe, NC)]. Animals were humanely euthanized at the end of the experiment with a lethal injection of pentobarbital (Beuthanasia D solution, Whitehouse Station, NJ).

Statistical analysis

Data were assessed for normality with analysis of skewness and kurtosis. Baseline characteristics were compared using either a t-test or Mann-Whitney rank sum test, as appropriate. For each group, changes in MAP following EC initiation were analyzed with the Wilcoxon signed rank test for paired data. Results are expressed as mean ± standard error of the mean or median [Interquartile range] for parametric and non-parametric data, respectively. Repeated measures ANOVA was used to compare parameters between the two groups and over time. If a significant difference was found, post-hoc pairwise comparisons were performed with Scheffe's adjustment. Statistical analysis was accomplished using a commercial statistics software package (Stata version 13, Stata Corp, College Station, TX).

Phase 2: Field expedient renal replacement therapy

Twelve Yorkshire-cross pigs (*Sus scrofa*), weighing 73.7 (69.5-74.6) kg, were acclimated for at least 10 days in conventional housing. After an 8 to 12 hour fast with free access to water, they were anesthetized with an intramuscular injection of 6.6 mg/kg tiletamine/zolazepam followed by isoflurane mask induction. After endotracheal intubation, animals were maintained under anesthesia with isoflurane mixed in 100% oxygen. Mechanical ventilation with tidal volumes of 6-8 mL /kg and positive end-expiratory pressure of 4 cmH_2O was regulated to maintain end-tidal CO_2 between 35-45 mmHg. Body temperature was maintained between 35-37°C using warmers. Intravenous 0.9% saline was administered at 5 mL/kg/hour throughout the experiment.

A 13.5 Fr 20cm Niagara® temporary dialysis catheter (Bard Access Systems, Salt Lake City, UT) was introduced into the right external jugular vein and bilateral nephrectomies were performed. The urinary bladder was emptied. Animals were given an intravenous bolus of heparin (100 IU/kg) followed by an infusion titrated to maintain their activated clotting time at least double baseline value or above 200 seconds, whichever was greater. A balloon-tipped catheter was

inserted in the abdominal aorta via a 12Fr femoral arterial sheath. The aortic balloon was inflated for 2 hours immediately above the iliac bifurcation. Animals were then randomized to either CRRT (NxStage System One®, NxStage Medical, Lawrence, MA) or ImpRRT.

At the end of the 2-hour occlusion period, the aortic balloon was deflated over 10 minutes and animals received 4 hours of RRT. In the ImpRRT group, the arterial line of the dialysis catheter (from the patient to the circuit) was connected to a Belmont® rapid infuser (Belmont Instrument Corporation, Billerica, MA) which was then connected to a dialysis filter (Revaclear 300, Baxter, IL), and subsequently to the venous line of the dialysis catheter (from the circuit to the patient). The Belmont® rapid infuser was used as it offers a peristaltic pump with precisely controlled flow. In addition, it has a built-in warmer to reduce iatrogenic hypothermia. The ultrafiltrate was collected from the dialysis filter into a urometer and quantified (Figure 8, 9). Improvised replacement fluid solutions were custom-made with FDA-approved stock solutions (0.45% NaCl, 3% NaCl, 10%Ca Gluconate, 50%$MgSO_4$, 8.4% $NaHCO_3$, 50% dextrose; the ratio of each component is presented in Table 3.A.) and infused into the system pre-pump (Figure 8, 9). In the CRRT group, we utilized a commercially available circuit (NxStage CAR 505 circuits, which include a Purema dialysis filter) designed for the CCRT platform (NxStage Medical, Lawrence, MA), along with commercially available replacement fluids (NxStage PureFlow RFP 402, NxStage Medical, Lawrence, MA) (Table 3.B). For both groups, we aimed to achieve an ultrafiltration rate of 25 mL/kg/hour to simulate common clinical scenarios, as recommended by the KDIGO guidelines (18). For both groups, blood flow through the circuit was set at 250 mL/min. For the CRRT group this was achieved by dialing the prescription in the machine. In the ImpRRT group, the height of the urometer was changed manually to control the pressure across the membrane of the dialyzer. Elevation of the bag was associated with a reduction in ultrafiltration and lowering was associated with an increased rate of ultrafiltrate production (Figure 8). Other methods used to titrate the ultrafiltration rate were to apply a surgical clamp to the effluent tubing or to change the rate of the blood pump by 10 mL/minute.

Throughout the rest of the experiment, animals were treated with isotonic crystalloid boluses and norepinephrine to maintain their mean arterial pressure (MAP) between 65 and 75 mmHg. If the MAP was < 65 mmHg and the central venous pressure (CVP) was < 7 mmHg, animals received 500 mL 0.9% NaCl over 10 minutes; if the MAP was < 65 mmHg and the CVP was ≥ 7 mmHg, the norepinephrine rate was increased by 0.02 mcg/kg/min increments.

Arterial blood samples were obtained at regular intervals (Figure 7) for evaluation of blood gases, white blood cell and platelet counts. Serum creatinine, potassium, calcium, magnesium, and phosphorus concentrations were also measured. Additionally, sodium, potassium, chloride, calcium, magnesium, bicarbonate, and glucose concentrations were evaluated in both the commercial and improvised replacement fluids. Animals were humanely euthanized at the end of the experiment with a lethal injection of pentobarbital.

Data were assessed for normality with analysis of skewness and kurtosis. Results are expressed as mean ± standard deviation or median [interquartile range, IQR] for parametric and non-parametric data, respectively. For parameters measured over time (creatinine, potassium, calcium, magnesium, phosphorus, and lactate concentrations) repeated measures ANOVA was used to compare parameters between the two groups and over time. If a significant difference was found, post-hoc pairwise comparisons were performed with Scheffe's adjustment. Baseline characteristics, replacement fluid and ultrafiltrate volumes, replacement fluid electrolytes and glucose concentrations, as well as total IV isotonic crystalloids and norepinephrine requirements were compared using either a t-test or Mann-Whitney rank sum test, as appropriate. Statistical analysis was accomplished using a commercial statistics software package (Stata version 13, Stata Corp, College Station, TX).

Phase 3: Novel peritoneal techniques

This technique was evaluated in an anephric model of hyperkalemia in *Sus scrofa domesticus*. Six animals were placed under general anesthesia and were instrumented to monitor vital signs and hemodynamics. Animals underwent bilateral nephrectomy and peritoneal dialysis catheters were placed bilaterally. Subjects were administered a weight-dependent dose of potassium to induce hyperkalemia and were subsequently assigned to conventional peritoneal dialysis or our experimental method. For conventional peritoneal dialysis, 2 liters of dialysate fluid was administered into the abdominal cavity and allowed to dwell for 60 minutes. Fluid was drained over 20 minutes and discarded. This was then repeated for a total of 6 exchanges. For the experimental group, 2 liters of fresh dialysate was administered into the abdominal cavity, allowed 60 minutes to dwell, and was retrieved and regenerated in the experimental device over a 20-minute period. This fluid was readministered into the peritoneal cavity and this process was repeated for a total of 6 exchanges. If <75% of potassium in the dialysate was removed after

incubation with the experimental device, the fluid was instead discarded and two liters of fresh dialysate were added. Serum biochemistry panels were conducted at baseline and hourly. Complete blood cell counts were obtained at baseline, start, and end of the experiment.

4.0 Results:

Phase 1: Potassium binding hemoperfusion

Hemodynamic parameters

After initiation of EC, there was a significant drop in MAP in both groups (Table 1). There was no significant difference in MAP between groups (P=0.17) or over time (P=0.23) (Figure 3). There was no difference in lactate concentration between groups at T0 or T360. There was no difference in fluid or phenylephrine requirement between groups (Table 2).

Potassium

There were no significant differences detected in baseline characteristics between the two groups (Table 1). Serum potassium concentration was significantly lower in the treatment group when compared to the control group, and this was consistent over time ($P < 0.001$). Pairwise analysis showed that potassium serum concentrations were significantly lower in the treatment compared to the control group at T210, T240, T270, and T300 ($P = 0.034$, $P = 0.01$, $P < 0.001$, $P = 0.004$, respectively). In the control group, serum potassium concentration at T240, T270, and T300 was significantly increased compared to T0 ($P = 0.048$, $P < 0.001$, and $P = 0.011$, respectively). In the treatment group, there was no significant difference in serum potassium over time. There was no significant difference in serum potassium between T0 and T360 for the treatment group, however, the control group had higher serum potassium at T360 compared with T0 ($p = 0.05$ for the control group, $p = 1.00$ for the treatment group) (Figure 4).

Other electrolytes

Serum ionized calcium concentration was significantly lower in treatment than control animals ($P < 0.001$). There was no difference over time for both groups ($P = 0.08$) (Figure 5A). However, there was no significant difference in total serum calcium concentration between groups or over time ($P = 0.13$ and 0.44, respectively) (Figure 5B). However, while serum total magnesium

concentration between groups was not different (P = 0.96), there was a significant increase over time for both groups (P < 0.001) with a significant increase compared to baseline at T300, T330, and T360 (P = 0.012, 0.024, and 0.005, respectively) (Figure 5C). Serum phosphorus concentration was significantly lower in the control group when compared to the treatment throughout the experiment (P < 0.001). For both groups, serum phosphorus concentrations were significantly increased over time with a significant rise compared to T0 at T270, T300, T330, and T360 (P = 0,011, 0.009, 0.004, 0.008, respectively) (Figure 5D). Serum creatinine concentration was significantly increased over time for both groups (P < 0.001) and higher in the control group (P = 0.004) (Figure 6).

Cell counts

There were no significant differences detected in platelet count between or within groups over time (P= 0.28 and 1.00, respectively) (Figure 7A). Although there was no difference between groups (P=0.93), the white blood cell count rose significantly over time (P < 0.001) (Figure 7B).

Complications

Circuit thrombosis requiring circuit exchange did not occur. In one pig, one of the customized connections failed, leading to blood leakage, which was promptly addressed by reconnecting the tubing. Two pigs (one in each group) received 1 L of 0.9% NaCl as a bolus prior to initiation of extracorporeal circulation due to hypotension (MAP < 50 mmHg). There was no significant difference in isotonic crystalloids or vasopressor requirements between the two groups (Table 2). Two of the five animals in the control group developed arrhythmias during the experiment (prolonged periods of asystole and pulseless electrical activity). However, all animals survived until the end of the experiment. No animals in the treatment arm developed significant arrhythmias.

Phase 2: Field expedient renal replacement therapy

Baseline, pre-RRT, and final characteristics are presented in Table 4. While serum creatinine concentration was significantly higher than baseline from T120 until the end of the experiment, there was no difference in serum creatinine between groups (p=0.84) (Figure 10.A.). Similarly, there were no differences in serum calcium, magnesium, or phosphorus concentrations between

groups (p=0.1, 0.68, and 0.14, respectively) (Figure 10.B.). While there was a difference between groups in serum potassium concentration over time (p=0.02), significance was lost in pairwise comparison at specific time points (Figure 10.B.). There was no difference in serum potassium concentration at the end of the experiment [Median (IQR): CRRT, 5.25 (4.96-5.41); ImpRRT, 6.0 (5.79-6.12) mmol/L; p=0.11]. Overall, serum potassium concentration was significantly higher than baseline from T120 until the end of the experiment (p<0.001 for each time point). There were no differences in replacement fluid rates [CRRT, 24.1 (23.6-24.5); ImpRRT, 24.7 (23.8-25.0) mL/kg/hour; p=0.42] or ultrafiltrate flows [CRRT, 24.1 (23.5-24.8); ImpRRT 24.0 (23.1-24.7) mL/kg/hour; p=0.75] between the CRRT and ImpRRT groups. While serum lactate increased significantly over time (p<0.001), there was no difference between groups (p=0.29) (Figure 10.C.). There were no differences in isotonic crystalloids [CRRT, 124.7 (88.6-169.4); ImpRRT, 132.3 (89.1-156.6) mL/kg; p=1.00] or norepinephrine doses [CRRT, 5.1 (3.3-15.6); ImpRRT, 10.5 (3.3-16.3) mcg/kg; p=0.81] required for resuscitation between groups. There were no differences in sodium (p=0.17), chloride (p=0.14), calcium (p=0.08), magnesium (p=0.27), bicarbonate (p=0.27), or glucose (p=0.31) concentrations between the commercially available and the improvised custom-made replacement fluid (Table 3.A. B.).

Final laboratory data and fluid balance volumes are presented in Table 4. There were no differences in laboratory results between groups. There was a slight, but significant difference in net ultrafiltration between groups.

Phase 3: Novel peritoneal techniques

Average dialysate fluid utilized in the experimental method was 3.33 L over the 6-hour period, while the conventional treatment used 12 L. There was no statistically significant difference in serum potassium between groups over time (p = 0.766). There were statistically significant differences between groups in serum calcium (p = 0.0008) but not ionized calcium (p = 0.5956) and lactate (p = 0.0004).

5.0 Discussion:

Phase 1: Potassium binding hemoperfusion

Here we have demonstrated the efficacy of a novel hemoperfusion system to decrease serum potassium levels. Most interestingly, we demonstrated stark differences in plasma potassium concentration at four hours, the typical length of a standard dialysis treatment. Furthermore, while this study was not powered to detect arrhythmias, it is notable that two animals in the control arm developed potentially fatal arrhythmias compared to none in the treatment group. The extracorporeal binding cartridge did not alter MAP, fluid, or vasopressor requirements between groups, which is substantiated by a lack of difference in lactate concentration between groups at the end of the experiment. This work serves as proof-of-concept for a novel extracorporeal method of potassium removal that could have several applications.

Phase 2: Field expedient renal replacement therapy

We established that the ImpRRT system achieved clearance equivalent to that of CRRT. Furthermore, electrolyte concentrations in the improvised custom-made replacement fluid were comparable to those of the commercially available product. This ImpRRT system could represent a compact, low-cost method to care for patients in both acute and chronic renal failure should access to conventional RRT platform be compromised and if these materials are available.

Phase 3: Novel peritoneal techniques

In this model, the experimental device used significantly less fluid and was able to control serum potassium levels with similar efficacy to that of conventional peritoneal dialysis.

6.0 Conclusions:

In Phase 1 of this research, we demonstrated the feasibility of the "bridge" dialysis concept for the management of hyperkalemia. Future work is required to better determine clearance, verify effectiveness in an injury model, and determine potential utility in conjunction with novel hemorrhage control techniques. In Phase 2, the ImpRRT system achieved similar performance to CRRT over the course of the experiment. Our ImpRRT platform shows potential promise for the care of patients with severe AKI following natural or man-made disasters, where conventional RRT is not available. Lastly, in Phase 3, we demonstrated that the experimental device used

significantly less fluid and was able to control serum potassium levels with similar efficacy to that of conventional peritoneal dialysis.

7.0 References:

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Figure 1. Phase 1 Extracorporeal circuit diagram. A. Control group. **B.** Treatment group

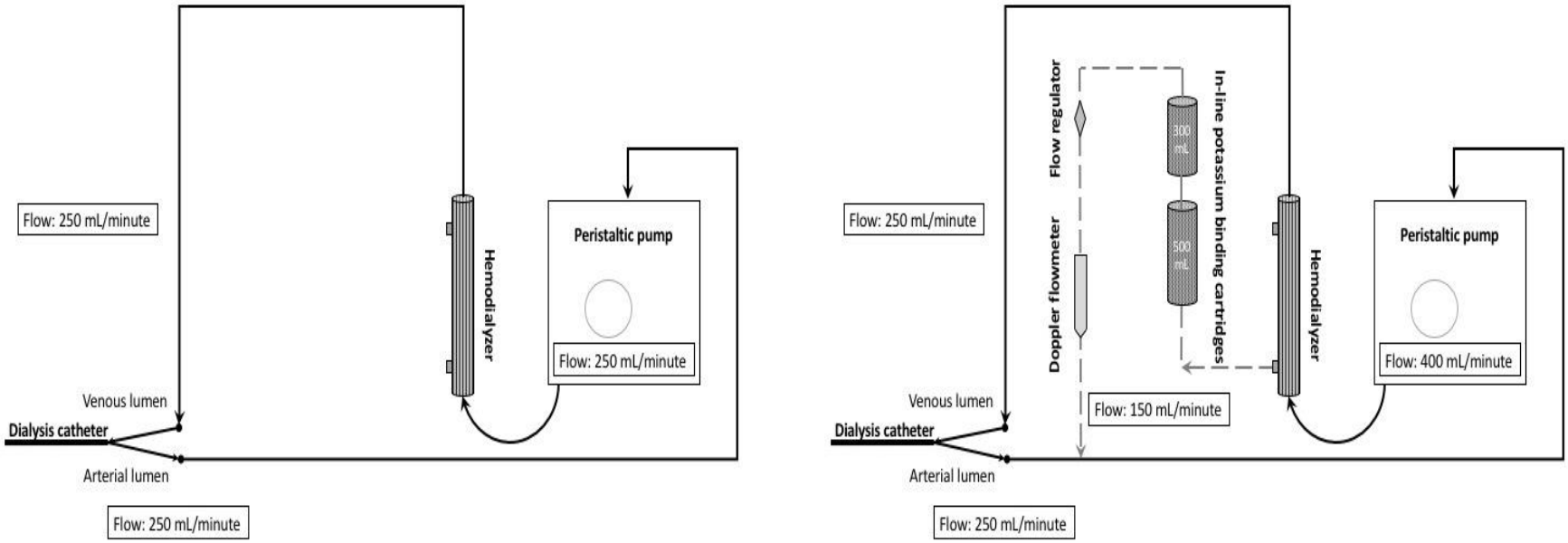
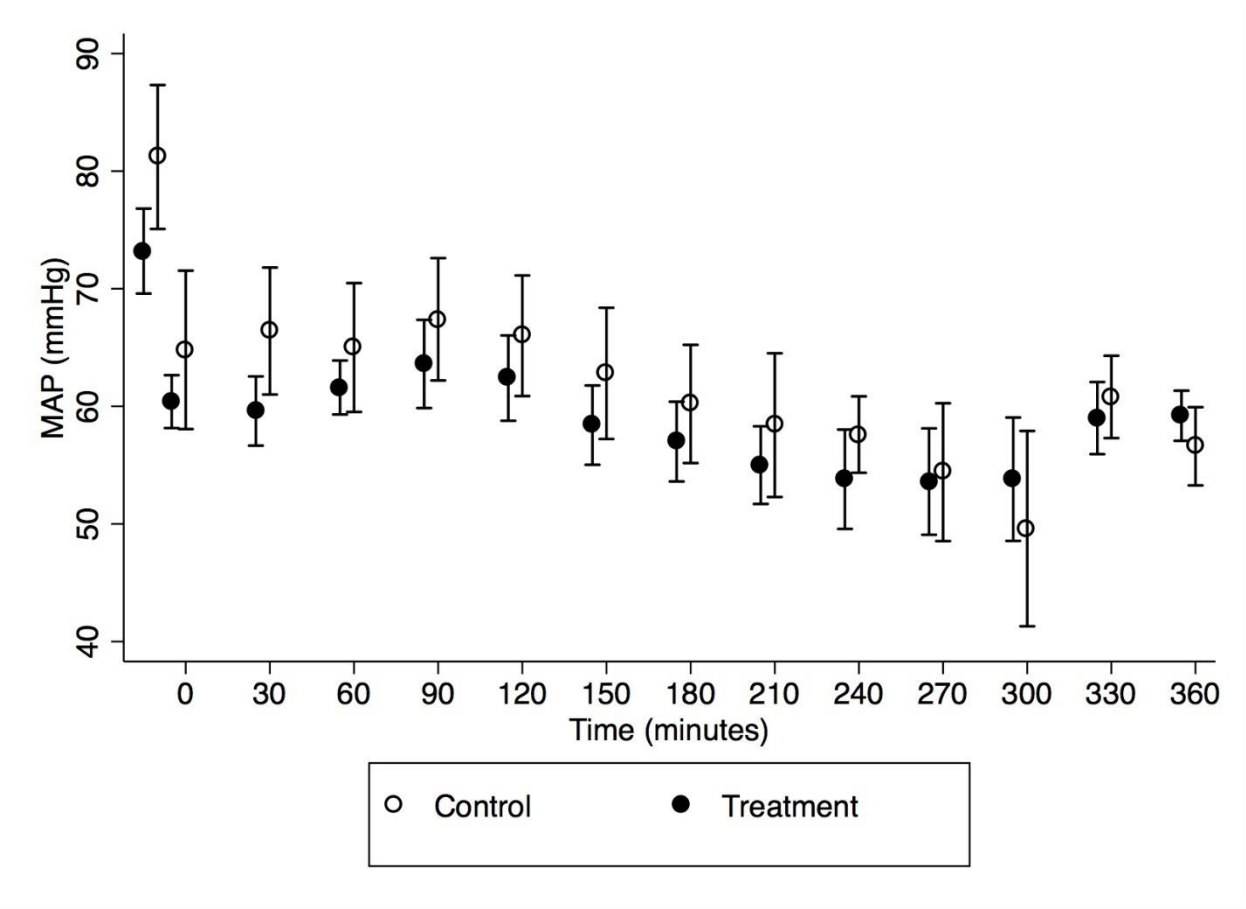
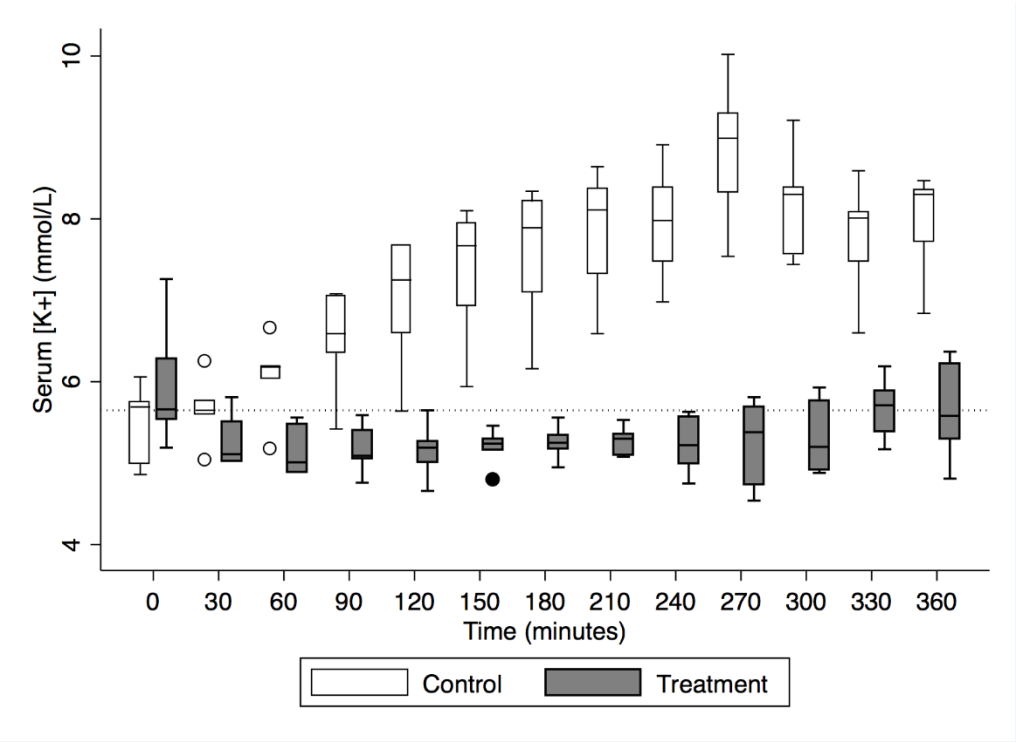


Figure 2. Phase 1 Mean arterial pressure (MAP) over time



Comparison of mean (± standard error of the mean) mean arterial pressure (MAP) over time between control and treatment groups. There was no significant difference in MAP between groups (P=0.17) or over time (P=0.23)

Figure 3. Phase 1 Median serum potassium concentration over time



Comparison of median serum potassium concentration over **time** between control and treatment groups. The horizontal line within each box defines the median value; upper and lower limits of each box denote the interquartile range. Whiskers delineate the 5–95% range. Individual data points outside of this range are plotted as individual circles.). Serum potassium concentration was significantly lower in the treatment group when compared to the control group, and this was consistent over time ($P < 0.001$). Serum concentrations were significantly lower in the treatment compared to the control group at T210, T240, T270, and T300 ($P = 0.034$, $P = 0.01$, $P < 0.001$, $P = 0.004$, respectively). In the control group, serum potassium concentration at T240, T270, and T300 was significantly increased compared to T0 ($P = 0.048$, $P < 0.001$, and $P = 0.011$, respectively). In the treatment group, there was no significant difference in serum potassium over time. There was no significant difference in serum potassium between T0 and T360 for the treatment group; the control group had higher serum potassium at T360 compared with T0 (p = 0.05 for the control group, p = 1 for the treatment group).

Figure 4. Phase 1 Mean electrolytes concentrations

Comparison of mean (± standard error of the mean) serum **A.** ionized calcium, **B.** total calcium, **C.** total magnesium, and **D.** phosphorus concentrations over time between control and treatment groups. **A.** Serum ionized calcium concentration was significantly lower in treatment than control animals ($P < 0.001$). There was no difference over time ($P = 0.08$). **B.** There was no significant difference in total serum calcium concentration between groups or over time ($P = 0.13$ and 0.44, respectively). **C.** Serum total magnesium concentration between groups was not different ($P = 0.96$), there was a significant increase over time ($P < 0.001$) with a significant increase compared to baseline at T300, T330, and T360 ($P = 0.012$, 0.024, and 0.005, respectively). **D.** Serum phosphorus concentration was significantly lower in the control group when compared to the treatment throughout the experiment ($P < 0.001$). Serum phosphorus concentrations were significantly increased over time with a significant rise compared to T0 at T300, T330, and T360 ($P = 0.009$, 0.004, 0.008, respectively).

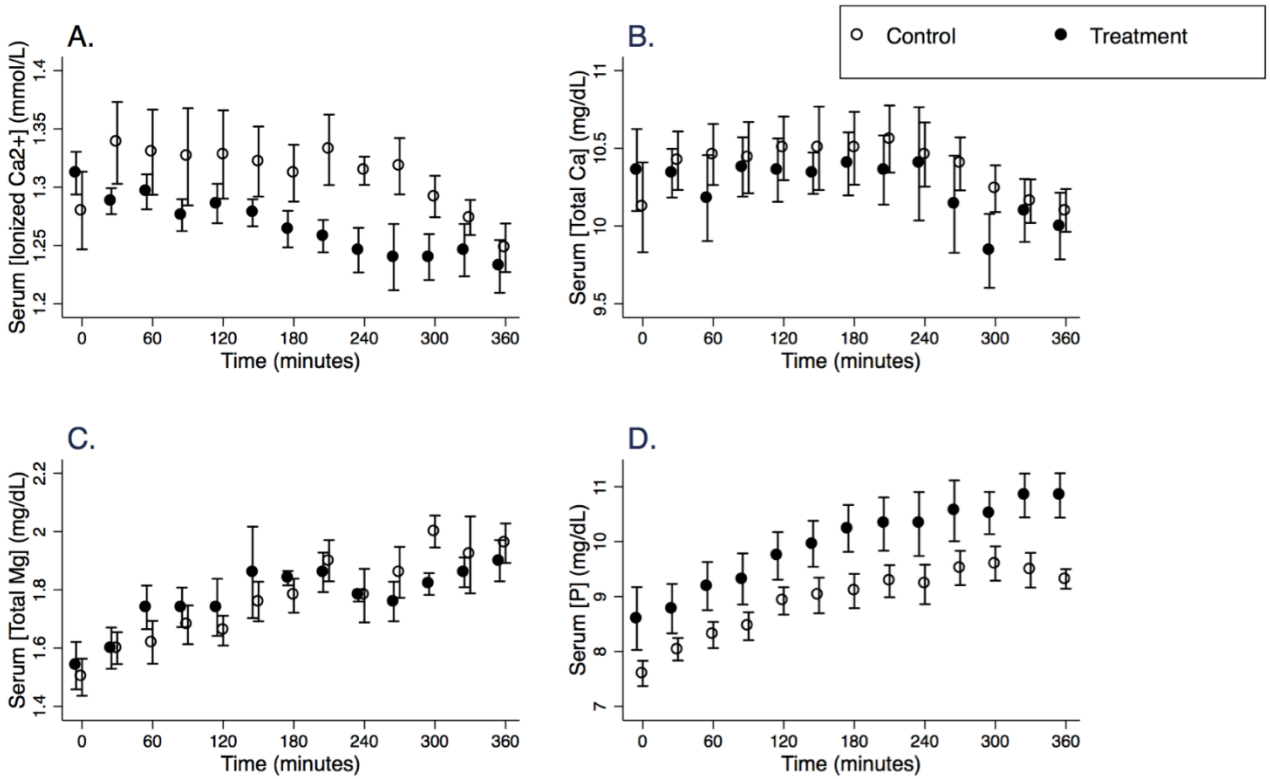
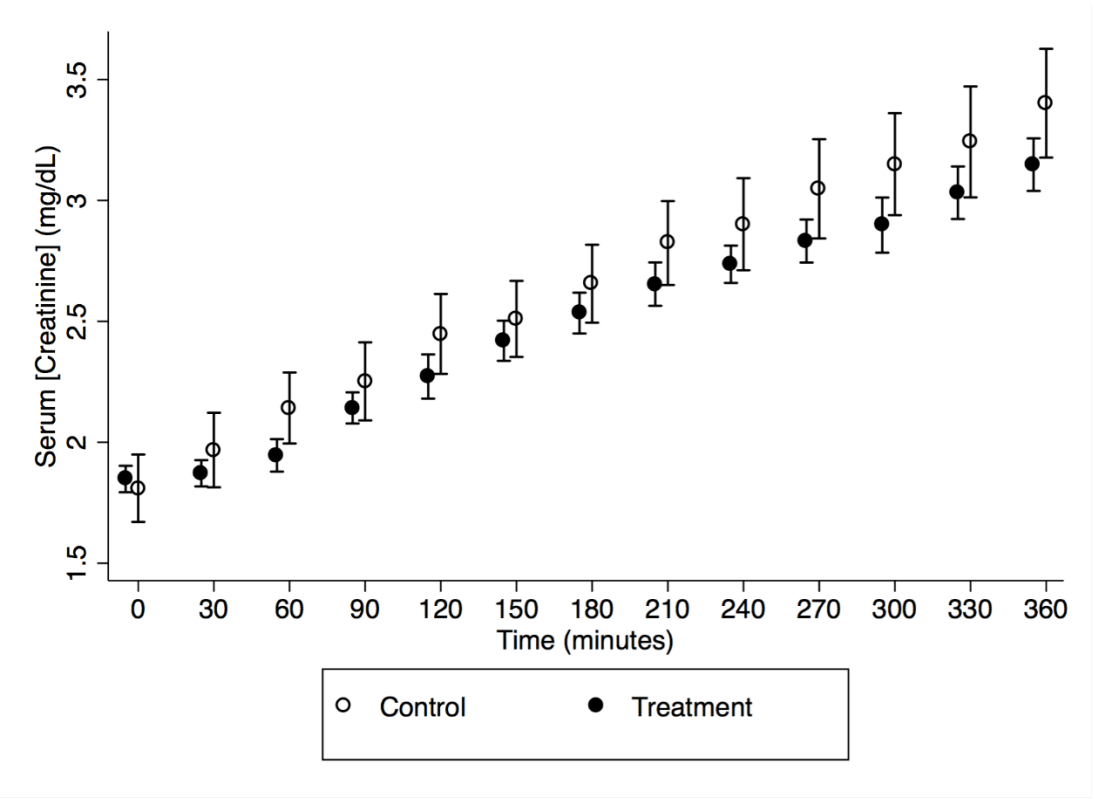
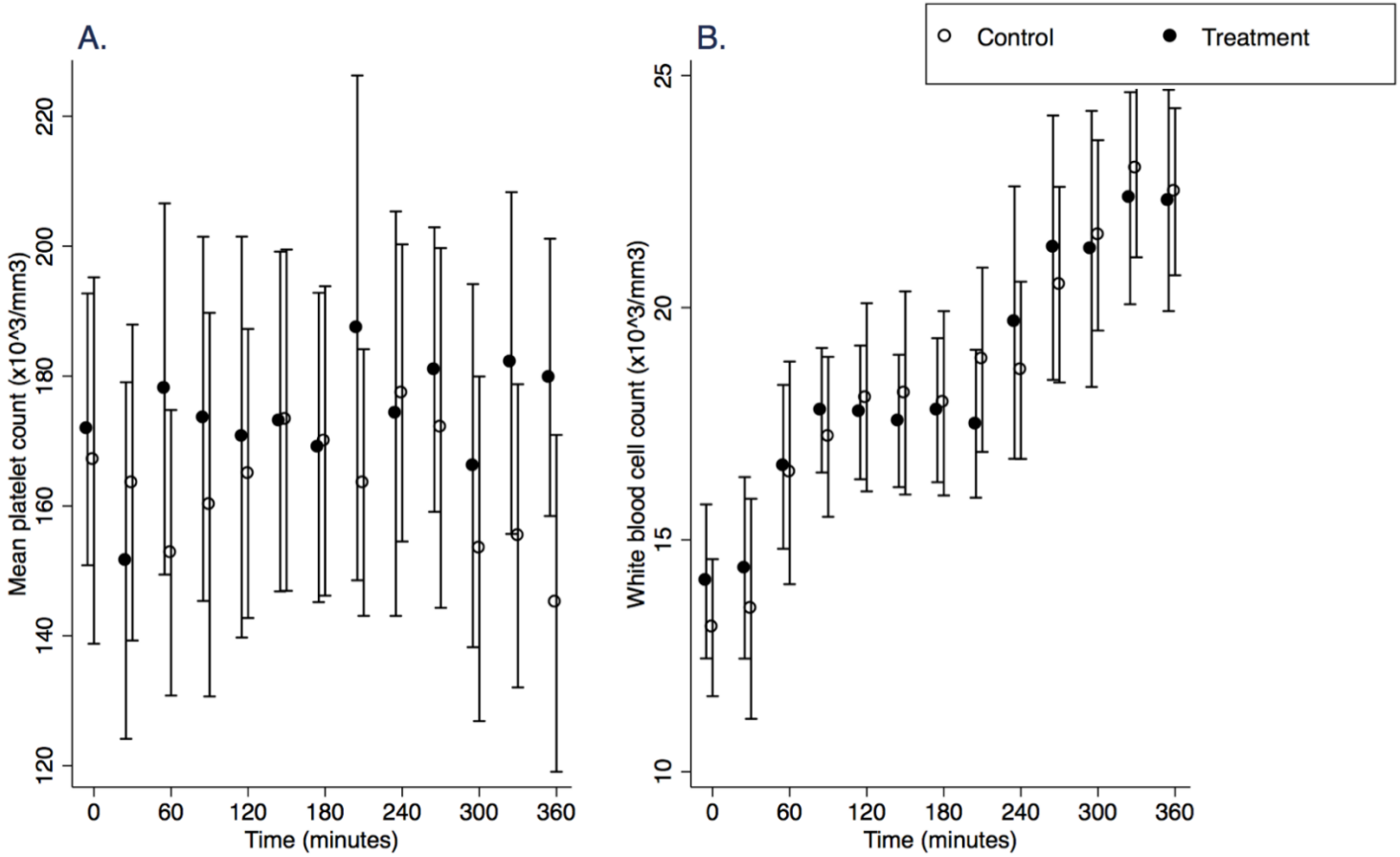


Figure 5. Phase 1 Mean serum creatinine concentrations



Comparison of mean (± standard error of the mean) serum creatinine concentrations over time between control and treatment groups. Serum creatinine concentration was significantly increased over time ($P < 0.001$) and higher in the control group ($P = 0.004$).

Figure 6. Phase 1 Complete blood count results



Comparison of mean (± standard error of the mean) **A.** platelet and **B.** white blood cell counts over time between control and treatment groups. **A.** There were no significant differences detected in platelet count between or within groups over time (P= 0.28 and 1, respectively). **B.** Although there was no difference between groups (P=0.93), the white blood cell count rose significantly over time ($P < 0.001$).

Figure 7. Phase 2

Experimental overview. Zone 3 of the aorta is between the most caudal renal artery and the iliac bifurcation. *: creatinine, electrolyte, and lactate concentrations measurement.

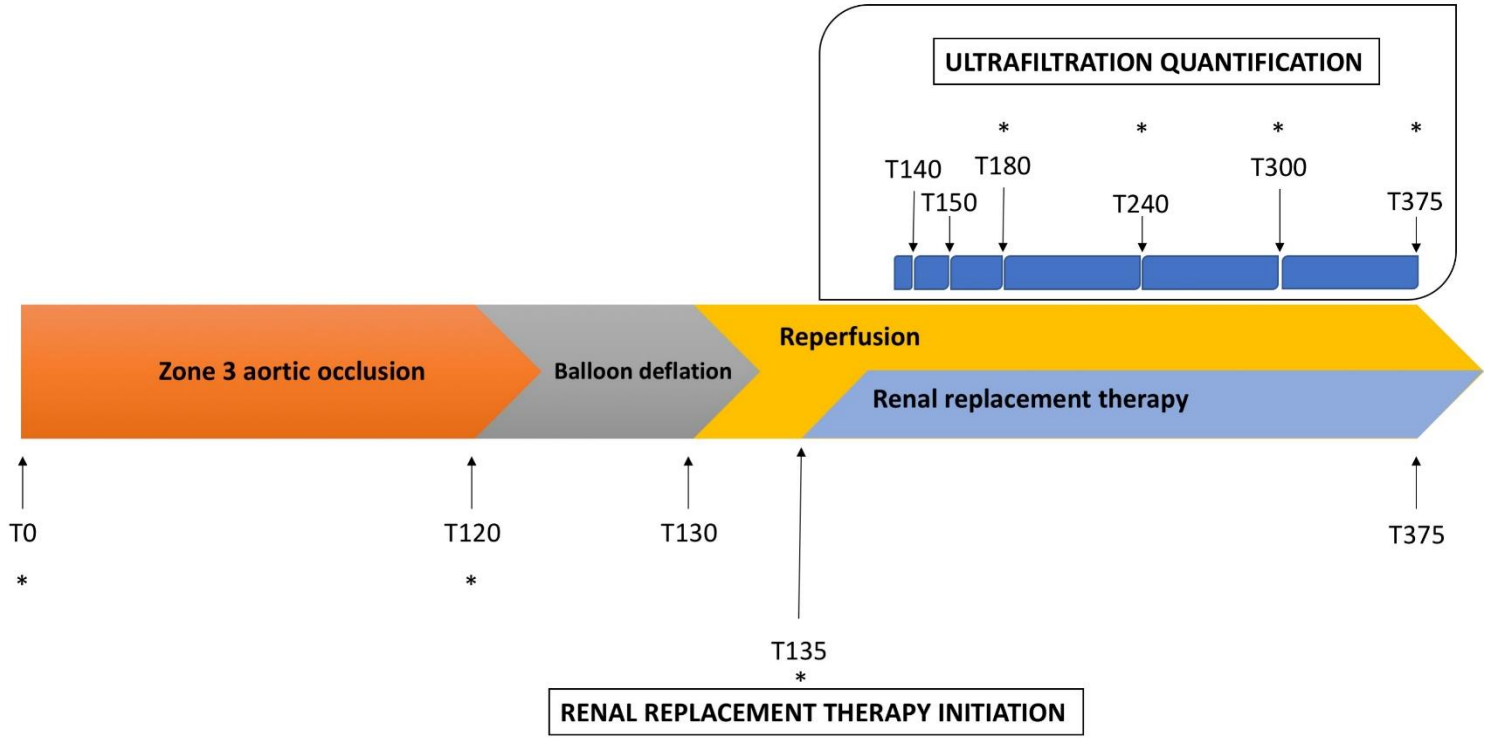
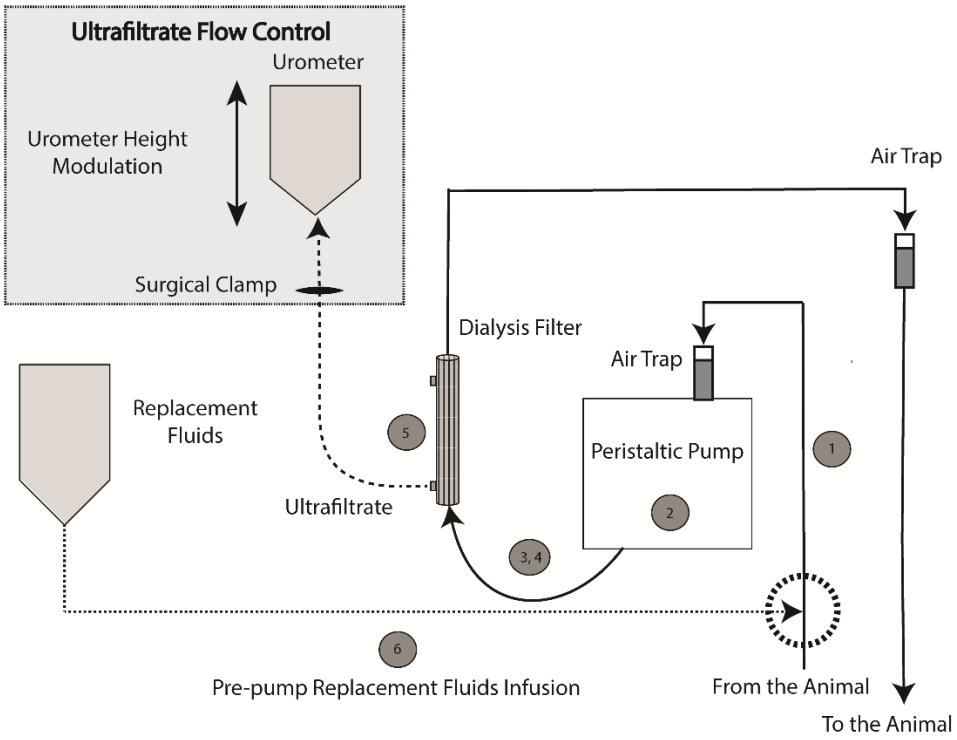
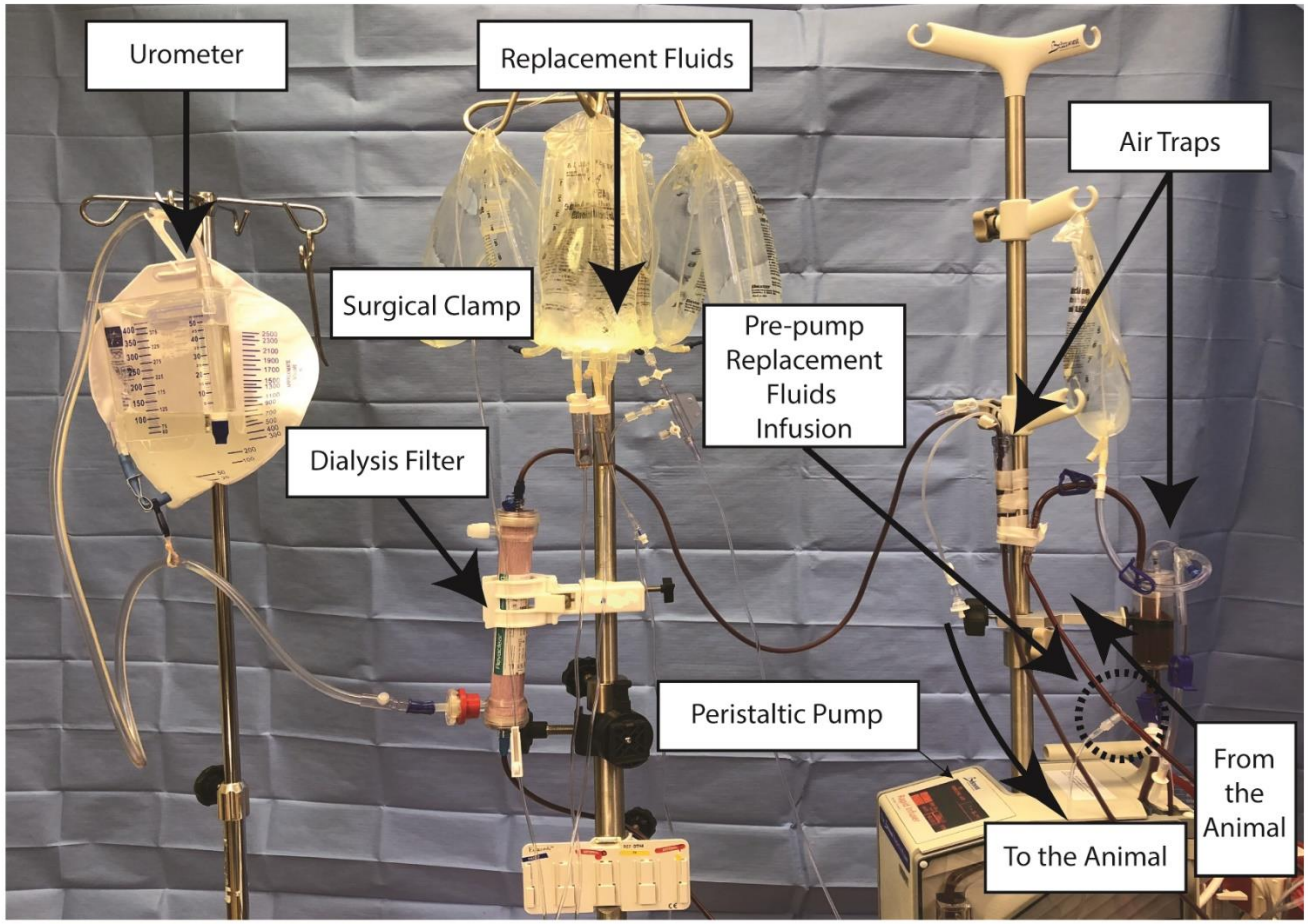


Figure 8. Phase 2



Improvised renal replacement therapy circuit overview. **1:** Belmont® 3-spike disposable set including extension tubing (Ref 903-00006); **2:** Belmont Rapid Infuser®; **3:** Molded Products Female Luer Lock Fitting to Male DIN Connector (Ref MPC-865); **4:** Molded Products DIN Connector (Ref MPC-850-16); **5:** Revaclear 300, hemodialyzer (Ref 114745/114745L); **6:** CombiSet, 2008 Series 8 mm Pre-pump Bloodline, hemodialysis blood tubing (Ref 03-2622-3).

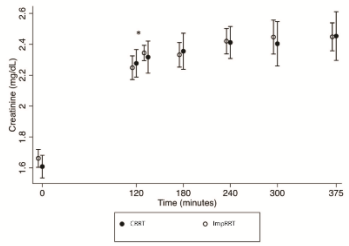
Figure 9. Phase 2



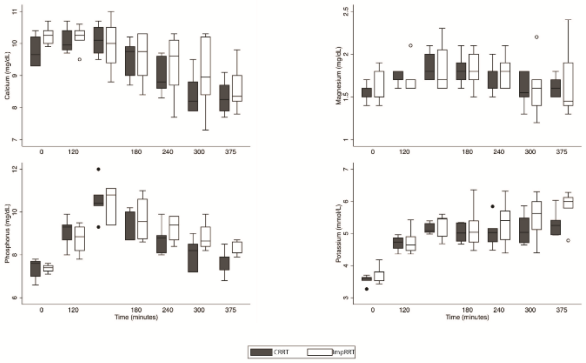
Picture of the improvised renal replacement therapy circuit.

Figure 10. Phase 2

A.



B.



C.

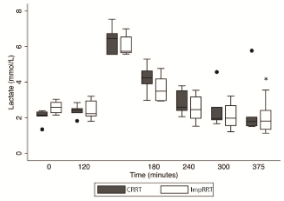


Figure 4. A. Serum creatinine concentration over time. CRRT: conventional renal replacement therapy, ImpRRT: improvised renal replacement therapy. * significant increase in creatinine concentration for both groups compared to baseline from 120 minutes until the end of the experiment.

B. Serum calcium, magnesium, phosphorus, and potassium concentrations over time. CRRT: conventional renal replacement therapy, ImpRRT: improvised renal replacement therapy.

C. Serum lactate concentration over time. CRRT: conventional renal replacement therapy, ImpRRT: improvised renal replacement therapy. * significant increase in lactate concentration for both groups over time.

Table 1. Baseline characteristics.

Variable	Treatment	Control	P-Value
Weight (kg)	74.9 ± 4.3	75.6 ± 7.1	0.93
[K^+] (mmol/L)	5.66 [5.54-6.28]	5.69 [4.99-5.75]	0.46
K^+ infusion (mmol/kg)	0.80 ± 0.01	0.81 ± 0.01	0.88
[Total Ca] (mg/dL)	10.36 ± 0.60	10.12 ± 0.65	0.56
[Total Mg] (mg/dL)	1.54 ± 0.18	1.5 ± 0.14	0.71
[Total P] (mg/dL)	8.6 ± 1.28	7.6 ± 0.51	0.14
[Creatinine] (mg/dL)	1.85 ± 0.12	1.81 ± 0.31	0.81
Platelet count (x $10^3/mm^3$)	172 ± 47	167 ± 63	0.89
White blood cell count (x $10^3/mm^3$)	14.1 ± 3.7	13.1 ± 3.3	0.67
MAP before extracorporeal circulation (T-10) (mmHg)	73.2 ± 3.61*	81.2 ± 6.1*	0.21
MAP after extracorporeal circulation (T0) (mmHg)	60.4 ± 2.25*	64.8 $\pm$ 6.73*	0.91

Data is presented as mean $\pm$ standard error of the mean and median [interquartile range] for parametric and non-parametric data, respectively. *P=0.04 for each group when comparing MAP before and after initiation of extracorporeal circulation.

Table 2. Phase 1 Isotonic crystalloids, phenylephrine requirements and lactate concentration.

Variable	Treatment	Control	P-value
Fluid administered (L)	3356 (3221-3441)	3190 (2819-331)	0.60
Phenylpinephrine (μ/kg)	2.3 (0-8.9)	4.6 (0-16)	0.75
[Lactate] T0	1.99 ± 0.28	2.23 ± 0.13	0.44
[Lactate] T360	1.84 ± 0.28	3.03 ± 0.59	0.11

Data is presented as mean ± standard error of the mean and median [interquartile range] for parametric and non-parametric data, respectively.

Table 3. Phase 2 Baseline characteristics (prior to nephrectomies)

	CRRT N=6	ImpRRT N=6	P
Animal characteristics			
Sex			
Male	5 (83%)	4 (67%)	0.50
Female	1 (17%)	2 (33%)	
Body weight (kg)	72.7 (70 – 74.4)	74.3 (69 – 74.6)	0.63
Baseline laboratory results			
BUN (mmol/L)	10.00 (9.00 – 12.00)	8.00 (7.00 – 10.00)	0.14
Creatinine (mmol/L)	1.36 (1.11 – 1.57)	1.30 (1.26 – 1.45)	0.75
Potassium (mmol/L)	3.43 (3.32 – 3.73)	3.58 (3.53 – 3.69)	0.34
Calcium (mg/dL)	10.00 (9.80 – 10.30)	10.20 (10.00 – 10.30)	0.57
Magnesium (mg/dL)	1.60 (1.40 – 1.60)	1.60 (1.40 – 1.60)	1.00
Phosphorus (mg/dL)	7.00 (6.40 – 7.20)	6.85 (6.60 – 7.00)	0.71
Lactate (mmol/L)	1.12 (0.97 – 1.43)	1.47 (1.34 – 1.69)	0.12
White blood cells ($x10^3/\mu L$)	12.14 (10.73 – 14.04)	14.25 (12.90 – 15.00)	0.26
Platelets ($x10^3/\mu L$)	225.17 ± 33.20	375.83 ± 145.48	0.34
Pre-RRT laboratory results (T135)			
BUN (mmol/L)	14.00 (12.00 – 15.00)	11.00 (10.00 – 12.00)	0.20
Creatinine (mmol/L)	2.32 ± 0.25	2.34 ± 0.11	0.83
Potassium (mmol/L)	5.09 (5.04 – 5.31)	5.45 (4.91 – 5.47)	0.58
Calcium (mg/dL)	10.10 (9.70 – 10.51)	10.00 (9.40 – 10.50)	0.78
Magnesium (mg/dL)	1.80 (1.70 – 2.00)	1.70 (1.60 – 2.05)	0.51
Phosphorus (mg/dL)	14.00 (10.30 – 10.80)	10.80 (9.40 – 11.10)	0.76
Lactate (mmol/L)	6.46 (5.55 – 6.73)	5.74 (5.66 – 6.54)	0.85
White blood cells ($x10^3/\mu L$)	15.00 (13.74 – 15.46)	15.41 (11.62 – 16.02)	0.72
Platelets ($x10^3/\mu L$)	165.00 (147.00 – 190.00)	201.00 (157.00 – 259.00)	0.20
End of experiment laboratory results			
BUN (mmol/L)	15.00 (15.00 – 17.00)	12.50 (10.00 –15.00)	0.06
% change compared to baseline	-50.00 (-66.70 – -25.00)	-48.10 (-66.70 – -42.90)	0.81

Creatinine (mmol/L)	2.45 ± 0.39	2.45 ± 0.22	0.98
% change compared to baseline	-83.26 ± 20.97	-81.84 ± 11.70	0.89
Potassium (mmol/L)	5.25 (4.96 – 5.41)	5.60 (5.79 – 6.12)	0.11
% change compared to baseline	-53.41 (-56.64 – -39.14)	-62.63 (-70.62 – -61.05)	0.15
Calcium (mg/dL)	8.25 (7.90 – 8.70)	8.35 (8.20 – 9.00)	0.57
% change compared to baseline	17.50 (15.53 – 19.39)	17.82 (11.76 – 20.19)	094
Magnesium (mg/dL)	1.60 (1.50 – 1.70)	1.45 (1.40 - 1.90)	0.42
% change compared to baseline	-6.07 (-7.14 – 0.00)	-0.00 (-26.32 – 6.25)	0.85
Phosphorus (mg/dL)	7.90 (7.30 – 7.90)	8.10 (8.10 – 8.60)	0.07
% change compared to baseline	-14.06 (-18.06 – -9.72)	-20.48 (-26.52 – -14.29)	0.33
Lactate (mmol/L)	1.78 (1.54 - 2.03)	1.81 (1.34 - 2.41)	0.87
% change compared to baseline	-58.93 (-111.46 – -4.90)	-30.19 (-53.50 – 33.14)	0.36
White blood cells ($x10^3/\mu L$)	13.98 (11.38 – 16.54)	15.30 (14.97 - 16.78)	0.36
% change compared to baseline	-17.66 (-36.33 – 0.43)	-10.75 (-20.11 – -1.93)	0.87
Platelets ($x10^3/\mu L$)	140.00 (102.00 – 152.00)	182.00 (176.00 – 248.00)	0.10
% change compared to baseline	32.98 (11.45 – 62.44)	20.47 (7.69 – 31.25)	0.34
Ultrafiltration (mL/kg)	**0.18 ± 1.77**	**-2.30 ± 1.74**	**0.04**

CRRT: conventional renal replacement therapy; IRRT: improvised renal replacement therapy; RRT: renal replacement therapy. Data is presented as mean ± standard deviation or median (interquartile range) for parametric and non-parametric data, respectively.

Table 4.

A. Volumes of stock solutions used to prepare the improvised replacement fluids.

	Lactate (mEq/L)	**HCO_3^- (mEq/L)**	**K^+ (mEq/L)**	**Na^+ (mEq/L)**	**Ca^{2+} (mEq/L)**	**Mg^{2+} (mEq/L)**	**Cl^- (mEq/L)**	**Glucose (mg/dL)**
Concentrations	0	35	0	140	3	1	109	100

B. Labelled concentrations of various solutes in the commercial replacement fluids.

	0.45% NaCl	**8.4% $NaHCO_3$**	**50% dextrose**	**3% NaCl**	**50% $MgSO_4$**	**$CaCl_2$**
Volume (mL)	1000	40	2.5	80	0.3	2.5